Biopharmaceutics of excipients in paediatric medicines

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Thesis submitted for the degree of Doctor of Philosophy

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University College London

March 2022

Declaration

I, Danielle Louise Andrews, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

March 2022

Acknowledgements

First and foremost, my thanks go to the Centre for Doctoral Training in Advanced Therapeutics and Nanomedicines for the opportunity to conduct this research with the funding provided by the EPSRC (grant: EP/L01646X/1).

I would like to express my thanks for the support afforded me by my supervisory team. In particular Professor Catherine Tuleu. Thank you for all your guidance throughout this PhD especially during the tough years brought on by COVID, the compassion you showed me as a human being, as well as a student, is greatly appreciated.

Special acknowledgment goes to my friends, in the fourth floor office and beyond, with whom I have shared ideas and laughter - you have made my time at UCL unforgettable. I am particularly thankful to Hend for all your help with my e-tongue research and to JJ, for the time spent together in the HPLC laboratory. Special thanks goes to Alex Keely for all your help and patience; without you my first few years would have seemed even more daunting.

I would like to express my gratitude to Paul Giffen for your confidence in me and for all your support when I applied to School of Pharmacy. I would also like to thank my GSK colleague and friend, Rob Ives, whose immense passion for the work rubbed off on me and first sparked the idea that I could do a PhD. You helped to instil a sense of belief in myself and I've thoroughly enjoyed collaborating over the years

Finally, thank you to Bernard and to my Mumma, the two loves of my life, who never fail to amaze me with their own achievements and strength; I hope I have made you proud, just as I am of you both.

Abstract

Children require palatable medicines. Excipients can improve the acceptability of a formulation, enabling children to take their dose as prescribed to achieve therapeutic exposure to the active pharmaceutical ingredient (API). Sweeteners have traditionally been used but there are safety concerns associated and limited success with highly aversive API. Thus there is a need to identify new approaches, such as those which steer towards a taste-neutral formulation. In order to assess the efficacy of novel taste-masking excipients, appropriate preclinical models are needed. The Brief Access Taste Aversion (BATA) model is one such method which has been used successfully for predicting the aversiveness of API but more work needs to be done to understand the translatability when excipients are present.

Some taste-masking excipients can have a negative impact on drug bioavailability and can decrease drug absorption in the gut as shown in adults. Children have a unique gut environment and therefore, there is a need to assess taste-masking excipients for such an effect in age-appropriate models.

To further understanding of excipients' impact on API bioavailability in children, this work achieved the following: 1) identified promising bitter-blockers (sodium acetate, sodium gluconate and Adenosine monophosphate sodium salt) using a novel ranking methodology. The three bitter-blockers were then assessed within liquid formulations but were unable to sufficiently taste-mask therapeutic doses of API. 2) Compared BATA and human data for taste-masking efficacy of a cyclodextrin and a maltodextrin. Differences in species tolerance to the dextrins were seen due to rat neophobia which, if not

accounted for, would prevent an accurate pre-clinical prediction of the human response. 3) Compared the effect of sorbitol, a widely used sweetener, on API blood levels in juvenile and adult rats. Sorbitol negatively impacted the bioavailability of model drug in the same way independent of age. The knowledge generated helps inform how taste-masking excipients can be used safely and efficiently in children’s medicines.

Impact statement

Children require palatable formulations for compliance. Doctors, parents and children report poor taste to be a major barrier to paediatric acceptability and therefore there is a need for effective taste-masking. Current strategies fall short and new approaches need to be explored to formulate better and safer patient-centric medicines. Further, there is a regulatory obligation to demonstrate the acceptability, and therefore the palatability, of a formulation. The BATA model is a recognised preclinical taste-assessment tool but has only be partially explored with formulated products.

The knowledge presented in this thesis is of value to formulators, developing more acceptable medicines for children, and to regulators assessing new drug applications. It provides a coherent assessment of promising bitter-blockers for their use as excipients for a taste-neutral approach which is endorsed by the European Medicines Agency (EMA). It also provides information on the potential applications and limitations of the Brief Access Taste Aversion (BATA) model for assessing dextrins. Moreover, the World Health Organisation (WHO) have outlined the importance of considering the effect of excipients on drug bioavailability in children. Some bulk excipients also used for taste-masking purposes, have been shown to impact API bioavailability in adults but no work has evaluated the effect in children. This work begins to fill this gap and highlights how sorbitol should be used with caution at high levels in liquid formulations owing to the negative effect on API bioavailability in juvenile animals. It is hoped this research will inform the choice of taste-masking excipients in children’s medicines and support formulation decisions.

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Abbreviations

3-AFC……..……..……3-alternative forced choice

ABC……..…….....ATP-binding cassette

ADI…..……..……..……acceptable daily intake

ADME……..…absorption, distribution, metabolism and excretion

ADP…..……..…… Adenosine triphosphate

AGE……..……..….advanced glycation end-products

AMP…..……..…….....adenosine monophosphate

ANOVA……..…….……analysis of variance

API ….……..……active pharmaceutical ingredient

ASPA…….....Animals Scientific Procedures Act

ATP ……..……....……adenosine triphosphate

AUC……..……..… area under the curve

BATA……..……brief access taste aversion

BCS…….……..……Biopharmaceutical Classification System

BCML……..…….*N*α,*N*α-bis (carboxymethyl)-l-lysine

BRCP……...……breast cancer resistance protein

BSA……...…….……body surface area

CML…..……..……... carboxymethyllysine

CPM……..……Chlorphenamine maleate

CTA……..……..….conditioned taste aversion

CYP……..……..… cytochrome-p450

DE……..…… Dextrose equivalent

EC50……..…..effective concentration 50 (50% reduction in human response)

EDA……..…….. Experimental Design Assistant

EFSA ……..……...…. European Food Safety Authority

EMA……..……..European Medicines Agency

EMC……..……..…. electronic medicines compendium

FDA……..……..…. Food and Drug Administration

FT-IR……..……..……..Fourier transform infra-red

GABA…….…….. Gamma Aminobutyric Acid

GI…….……..…gastrointestinal

GIT…….…….. gastrointestinal tract

GOLD…….……...…..glyoxal-derived lysine dimer

GRAS…….…… generally recognised as safe

GSTA1-1…….……..…Glutathione S-transferase alpha 1

HED…….……human equivalent dose

HMPC…….……Hydroxypropylmethyl cellulose

HP- β-CD ……..……..…Hydroxypropyl-β-cyclodextrin

HPLC……..……..High-performance liquid chromatography

IC50……..…….. Inhibitory concentration 50 (50% lick inhibition)

ICH .....International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IID……Inactive ingredients database

IP……..……Intraperitoneal

IP3…….……..…inositoltriphosphate

MDCK……..……...Madin-Darby canine kidney

mPEG-PLA……..…Monomethoxy poly(ethylene glycol)-block poly(D,L lactic acid)

MRP2……..……..Multidrug resistance associated protein 2

NACWO…….……..…named animal care and welfare officer

NC3Rs.....National Centre for the Replacement Refinement and Reduction of Animals in Research

NMR…….....…….nuclear magnetic resonance

NOAEL……..... no observed adverse effect level

NOSC…….....…….N-octyl-o-sulfate chitosan

NVS…….....……name veterinary surgeon

PBPK…….....…… physiologically based pharmacokinetic

PEG…….....……...Polyethylene glycol

P-gp…….....…….. P-glycoprotein

PK…….....…….....pharmacokinetic

PLβ2…….phospholipase C β2

PO…….per os (oral)

PPL…….project license number

PQP…….Primaquine phosphate

PTC…….phenylthiocarbamide

QHCL…….Quinine hydrochloride

QoE…….quality of evidence

QSD…….Quinine sulphate dehydrate

REC……. Research Ethics Committee

SLC…….Solute carrier

SULT…….Sulfotransferase

TAS1R ……. taste 1 receptor

TAS2R ……. taste 2 receptor

TAS3R……. taste 3 receptor

TMD…….transmembrane domain

TOF…….time of flight

TPGS…….d-α-tocopheryl-polyethylene glycol-1000-succinate

TRPM5……. transient receptor cation channel M5

USP…….United States Pharmacopeia

VGNC…….voltage gated sodium channel

WHO…….World Health Organisation

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Chapter 1

Introduction

This chapter introduces the importance of palatable medicines for children and thus the need for effective taste-masking. The ways in which taste-masking excipients can affect the bioavailability of medicines, from a paediatric angle, are discussed alongside the methods which can be used to assessed this. The widespread use of sweeteners is critically discussed and the need for new, taste-neutral, strategies is highlighted, before finally identifying research needs and overall aims of the experimental work.

1.1 The importance of excipients

All patients need medicines that are safe and efficacious which can be administered easily. To achieve this, excipients can be implemented to facilitate the safe delivery of the active pharmaceutical ingredient (API) to the site of action. Many excipients can be used in combination to support the API and usually account for the bulk of a formulation (Furrer, 2013). Excipients convey no medicinal properties but without them many active ingredients could not be made into viable drug products. One important class of excipients are taste-masking agents.

1.1.1 The need for effective taste-masking for paediatric adherence

It is estimated that only 50% of patients on long-term therapies take their medications as prescribed (Nieuwlaat *et al.*, 2014) with adherence particularly low in children and young people (Dawson, 2019). There are many factors influencing adherence in children which are summarised in figure 1.1.

Healthcare factors (medication related)

Promoting adherence

- Using once/twice daily drug options improves adherence in school age children (Dawson, 2019).
- The appearance of a medication (colour, shape, embossing), can improve patient acceptability and aid identification of the medicine (EMA, 2013).

Hindering adherence

- Poor palatability. The palatability refers to the overall appreciation of a medicine by organoleptic properties such as appearance, smell, taste, aftertaste and mouthfeel (for example texture or cooling). Formulation palatability is important to consider for dosage forms which encounter taste receptors including oral dosage forms and those administered bucally, nasally or are inhaled (Walsh *et al.*, 2014).
- Size, shape and integrity of the dosage form (e.g. film-coating). These can impact the swallowability of oral medicines. Dosage dimensions can also be a consideration for non-oral dosage forms, for example transdermal patches need to be appropriate for a child's body as to not interfere with daily routines.
- Side effects (Reed-Knight, Lewis and Blount, 2011).
- Associated pain or discomfort (Dawson, 2019).
- Difficulty with administration device/ container closure system. These should be easy to use and portable to facilitate appropriate use of the medicine independent of the environment (for example at school or at home). If a measurement tool is required, this should be supplied and accurately measure the dose (EMA, 2013).
- Frequency/ duration of treatment (Dawson, 2019).
- Required dose (tablet number or volume of liquid), the quantity needs to be appropriate for the age of the patient and small volumes/ tablet numbers are better tolerated (EMA, 2013)
- Complexity of any modification required prior to administration (for example breaking tablets using break marks) (Liu *et al.*, 2014)

Peer support

Promoting adherence

- Social acceptance of the child's condition and need for medication; the child feeling they 'fit-in' (Hanghøj and Boisen, 2014).

Hindering adherence

- Adolescents may form a new identity within friendship groups based on illness, non-adherence may result to maintain symptoms and this new identity (Hanghøj and Boisen, 2014).

Healthcare factors (related to professionals)

Promoting adherence

- Healthcare professions providing a safe environment to discuss adherence (Santer *et al.*, 2014).
- Involving older children in discussions about their medicines (Hanghøj and Boisen, 2014).

Hindering adherence

- Not allowing the family to have a sense of responsibility or play a role in decision making surrounding the medication (Bryson, 2014).

Individualistic factors (for older children, responsible for their own medication)

Promoting adherence

- A motivated child may enjoy taking responsibility for their medication (Schwartz and Axelrad, 2015).

Hindering adherence

- Lack of understanding of the benefits of their medication (Dawson, 2019).
- Forgetfulness (Dawson, 2019).
- Children displaying risk-taking behaviour may have lower adherence (Schwartz and Axelrad, 2015).

Caregiver factors

Promoting adherence

- Healthy family dynamics, good family communication and structured routine (Santer *et al.*, 2014).
- Good understanding of medication (Hanghøj and Boisen, 2014).

Hindering adherence

- Caregiver concern over side effects (Santer *et al.*, 2014)
- Forgetfulness (Dawson, 2019)
- Caregiver unwillingness to move from primary responsibility holder to facilitator as the child gets older and wants more control over their treatments; if not done well this can lead to defiance in children (Dawson, 2019)

Figure 1.1 Factors affecting adherence in children

Very young children are not responsible for their own medication schedule nor can they form an opinion on if their medication is effective. Therefore, assuming no issue with caregiver compliance, paediatric acceptability to a formulation is largely driven by medication factors.

The acceptability of a formulation is defined as the overall ability of the patient to use a medicine as intended and has a significant impact on patient compliance to the medicine (EMA, 2013). Alongside frequency of dosing and side effects, poor taste is a major barrier to paediatric patient acceptability (American Academy of Pediatrics, 2000; Venables *et al.*, 2015). Multiple sources, including doctors, parents and children themselves, report poor taste to be problematic. A survey of over 800 paediatricians found taste to be a barrier to compliance for 91% of patients (American Academy of Pediatrics, 2000) and a questionnaire of 500 parents found around half of children refuse their medicine at some time and of these 75% do so due to the taste of the drug (Milne, 2005). Responses directly from children are even more compelling with 900 European children, age between 10 and 17 years old, reporting bad taste to be the number one issue associated with taking medicines; bad taste was reported to be more problematic than feeling physical pain during administration (for example with injections) (Nordenmalm *et al.*, 2019). These responses demonstrate the importance of efficacious taste-masking approaches.

Tablets represent an attractive dosage form to deliver API to patients, including children, because they are cheap to produce and taste-masking can often be achieved using a coating (Lajoinie *et al.*, 2014). Despite this, children frequently receive liquid medicines due to the difficulty many younger patients

face swallowing capsules or tablets (Batchelor and Marriott, 2015). Liquid formulations can be challenging to taste-mask as the API is more likely to readily interact with bitter-receptors and result in poor acceptability. To address this, 'Pill Schools' are being set up within UK hospitals with the aim of training young children how to swallow tablets (Rashed *et al.*, 2021). The idea is to expand 'Pill School' services so children and their parents can access such training before being prescribed oral liquids and, where possible, solid dosage forms can be given instead. At present, liquid medications are still widely used in children (Nunn and Williams, 2005; Rashed *et al.*, 2021) so alternative taste-masking strategies are required.

1.2 Taste-masking approaches and API bioavailability

Taste-masking approaches can take a number of forms (Walsh *et al.*, 2014) and can act on, or independently from, the API (figure 1.2).

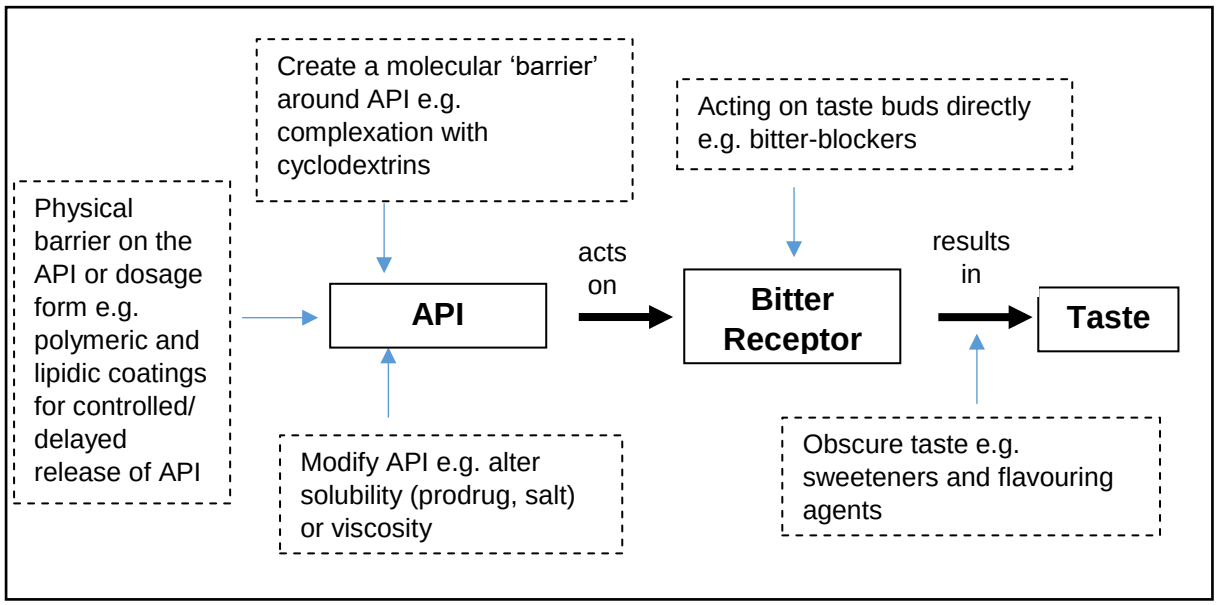


Figure 1.2 An overview of different taste-masking approaches

1.2.1 Taste-masking indirect effect on API bioavailability

An unpalatable formulation can result in abnormal API bioavailability. If a child does not successfully ingest the whole dose but coughs, dribbles or gags in response, it is impossible to know how much drug the child is actually receiving. Taste-masking excipients can be harnessed here to improve formulation palatability and thus patient acceptability and allow for ingestion of the API as intended.

1.2.2 Taste-masking direct effect on API bioavailability

Alternatively, taste-masking excipients can have a detrimental impact on drug bioavailability by acting in the gut. Excipients can alter gastrointestinal (GI) transit time and as such cast uncertainty on the amount of active drug absorbed (Koch *et al.*, 1993; D. Adkin *et al.*, 1995; Basit *et al.*, 2001; Schulze *et al.*, 2006). Excipients that speed up gut transit, reduce gut residency time and in doing so, decrease the likelihood that sufficient API will be absorbed - potentially affecting the therapeutic response.

Moreover, excipients within an orally administered formulation will encounter the enterocytes of the intestines, these cells express a number of transporter proteins which facilitate compounds crossing biological membranes. Excipients which inhibit or induce these transporters can result in changes in the amount of API efflux and thus alter exposure (Matsson *et al.*, 2009). For example, the surfactant PEG 400 inhibits the intestinal efflux transporter P-glycoprotein (P-gp) and has been shown to alter the bioavailability of Ranitidine (Afonso-Pereira *et al.*, 2016). Interestingly this effect was sex-dependent with the authors concluding this was likely due to differences in GI intestinal transporter levels between male and female rats.

The effects of taste masking excipients on drug bioavailability is summarised in figure 1.3.

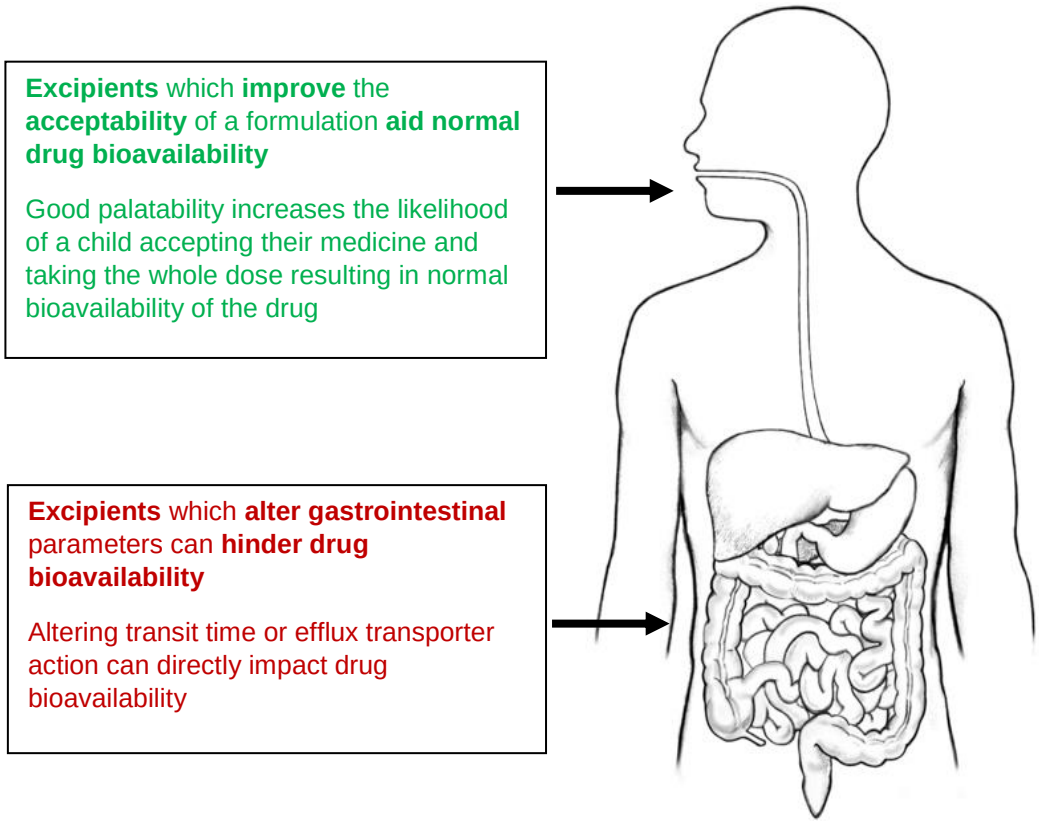


Figure 1.3 How taste-masking excipients can impact API bioavailability.

1.2.3 The need to understand the effect of age

Children have a unique gut environment depending on their developmental stage. Children mature throughout childhood and so can be sub classified according to age. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) recommends children be classified as follows; preterm newborn infants, term newborn infants (0-27 days), infants and toddlers (28 days to 23 months), children (2-11 years) and adolescents (12-16/18 years) (EMA, 2001). The purpose of this categorisation is to provide a basis for designing studies in paediatric patients

and to aid decision making on how to stratify data taking into consideration developmental biology and pharmacology (EMA, 2001).

There are a number of known anatomical and physiological differences in GI parameters between adults and young children (Khan *et al.*, 2022). Some of these differences follow a linear maturation process as a new-born becomes a toddler, and a toddler becomes a child, for example the capacities of the different GI sections and the resting volumes of fluid (Batchelor, Fotaki and Klein, 2014). Some differences do not follow a linear pattern.

1.2.3.1 The paediatric gastrointestinal tract; differences to adults

In the oral cavity, where the first stage of digestion occurs, the size of the cavity itself limits the volume (or size) of dosage forms for young children. Furthermore, the oesophagus does not reach adult length until 10 years of age, this results in changes to the transit time of a dosage reaching the stomach. The capacity of the stomach increases with age, from 10 – 20mL in new-borns, to 200mL by the age of 2 years and 1500mL by the age of 16 years (Khan *et al.*, 2022). This can impact the absorption of poorly soluble drugs as lower fluid volumes result in reduced dissolution (Nader *et al.*, 2016).

The stomach physiology of a child can have a negative impact on the absorption of API, especially for drugs which are partly absorbed in the stomach (Khan *et al.*, 2022). Gastric acid secretion begins soon after birth and increases gradually, changing gastric pH from neutral to pH 1-3 by day two of life. The pH slowly returns to neutral by day 8 and then gradually declines to adult levels over the next few years (Lu and Rosenbaum, 2014). The secretion of gastric acid in new-borns is lower than in adults which can impact

the stomach absorption of drugs such as phenytoin (a weak acid) due to increased ionisation from higher gastric pH levels (Lu and Rosenbaum, 2014; Khan *et al.*, 2022). Furthermore, gastric emptying in new-borns is reduced and variable compared with adults which can result in delayed absorption of drugs (Lu and Rosenbaum, 2014).

Although some absorption occurs within the stomach, the majority of drug absorption occurs within the small intestine (Murakami, 2017). In adults, the presence of circular folds within the small intestine slows down intestinal transit time, whilst increasing the surface area, to enable absorption of gut contents (Lander and Newman, 2013). Young children have both reduced intestine size and fewer circular folds resulting in reduced intestinal absorption. However, intestinal permeability is increased in children and, at birth, rates are three to four fold higher than adults (Ginsberg, Hattis and Sonawane, 2004). Permeability then decreases until reaching adult levels in early childhood (McOmber, Ou and Shulman, 2010). It is thought this is due to the immaturity of the intestinal mucosa resulting in a more ‘leaky’ gut (Khan *et al.*, 2022).

The composition of microbiota, which colonise the GI tract, differs in adult and children. Levels of intestinal microbiota are thought to resemble the adult environment around 4 years of age (Hollister *et al.*, 2015). Gut microbiota play a role in metabolism and GI motility and so this can potentially impact drug bioavailability (Batchelor and Marriott, 2015).

In addition to these known differences, significant knowledge gaps remain regarding the GI development of children (Batchelor, Fotaki and Klein, 2014;

Wollmer *et al.*, 2022). This makes it difficult to predict the influence of excipients on API bioavailability in children from adult studies. Fundamental gaps in knowledge of the paediatric gut preventing direct comparison include:

- Baseline GI transit times of an orally administered dosage form, gastric and intestinal fluid composition and physicochemical properties, intestinal fluid volume and pH, pressure conditions along the entire GI tract and colonic motility patterns (Wollmer *et al.*, 2022).
- A thorough understanding of when adult gut transporter levels are reached. Active transport systems are responsible for moving substances across membranes and are immature at birth. The expression of P-gp increases with age but it is not known when adult expression is reached (Batchelor, Fotaki and Klein, 2014). Contrasting results have been published (Fakhoury *et al.*, 2005; Prasad *et al.*, 2016) but it is likely intestinal P-gp expression increases throughout the first few months and reaches adult levels by 2 years of age (Lu and Rosenbaum, 2014; Khan *et al.*, 2022).

The effect of taste-masking excipients on gut absorption of API cannot be properly understood in children until relevant models are used.

1.2.4 Direct excipient action on API bioavailability; age-appropriate modelling

Testing excipients to gain a deeper understanding on their effects in children, is endorsed by the EMA. In the ‘Guideline on pharmaceutical development of medicines for paediatric use’, emphasis is placed on the importance of choosing suitable excipients for use in a paediatric medicinal product and how special safety considerations should be given owing to potential differences

between children and adults (EMA, 2013). Recent guidance on ‘Non-clinical safety testing in support of development of paediatric pharmaceuticals’, explains that where insufficient data is available on excipient effect in the intended paediatric population, further evaluation can be warranted using juvenile animal studies where appropriate (EMA, 2018). This ensures that the core values around risk-benefit analysis of a formulation are not limited to therapeutically active ingredients but extend to excipients.

Every effort should be made to use in vitro tools and avoid the use of animals where possible. However, this is not always appropriate if the tools are not representative of the target population.

1.2.4.1 In vitro models of excipient effect

Cell based methods exist which can model intestinal absorption and permeability of drugs and therefore how excipients influence this. These cell lines can differentiate into a polarised epithelial monolayer which express many of the functional characteristics of a mature enterocyte such as apical brush border microvilli and tight intercellular junctions (Volpe, 2008). Examples include assays using Caco-2 or Madin-Darby canine kidney (MDCK) cell lines (de Angelis and Turco, 2011). However neither of these are specific to the paediatric population and would not accurately model the effect of age.

1.2.4.2 Ex-vivo models of excipient effect

Ex vivo models provide a more representative way of understanding permeation effects of excipients on API. For example, the everted sac model or the use of the Ussing chamber allows for the study of drug absorption over

time and how excipients affect this (Alam, Al-Jenoobi and Al-mohizea, 2012). Such models could be used to explore the effect of age by using juvenile animal intestine sections (Koldovsky, Johnson and Koldovsky, 1988). Ex-vivo models require the sacrifice of animals. However, it has been reported that rapid deterioration of intestinal function after tissue removal, and limited viability of the intestine, is seen (Nicolas *et al.*, 2017).

1.2.4.3 In vivo models; ethical considerations and regulatory requirement

An in vivo model is the best way to study oral drug delivery and intestinal drug absorption. This is because it utilises the whole intact organism allowing for the complex interplay of different bodily systems to continue undisturbed under physiological conditions (Xu *et al.*, 2021). Furthermore, the age of the animal can be matched to represent the intended paediatric population of interest.

To carry out an in vivo study there are a number of ethical and legal steps to consider. In the United Kingdom, three licenses are required by the Animals (Scientific Procedures) Act 1986 (ASPA) before testing on animals, 1) a personal license for each person who carries out any procedure on the animal 2) a project license detailing the work 3) an establishment license for the place of work. These licenses are approved and regulated by the home office (Home office, 2013).

The National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) provides a framework which must be followed in the United Kingdom, amongst many other countries, when animal research is taking place. In accordance with their principles, a study must be

appropriately designed so that the fewest number of animals is used to still achieve a robust data set (du Sert *et al.*, 2020). They also highlight the importance of maximising the information gathered per animal with the aim of reducing further animal use. With this in mind, ex-vivo studies would not offer the most useful information per animal. A bioavailability study would be more representative than an ex-vivo approach which can encounter tissue viability limitations and utilises an artificial set up (Alam, Al-Jenoobi and Al-mohizea, 2012). A bioavailability study gives more insight into excipient effect; blood concentration of a drug can be measured over the course of a number of hours under normal biological rhythms and juvenile and adult animals can be compared directly.

To better understand how taste-masking excipients can affect API bioavailability in children it is first important to understand which excipients they are exposed to.

1.3 Sweeteners; the traditional taste-masking excipients

Traditional taste-masking strategies act to overpower the bad taste of an API. Sweeteners have been the excipients of choice as they offer an intuitive means of obscuring bad taste whilst being versatile enough to incorporate into many formulation types. However, this approach has limited success for highly bitter and highly water soluble drugs (Sohi, Sultana and Khar, 2004; Walsh *et al.*, 2014).

1.3.1 Sweetener types

Sweeteners can be categorised into two groups 1) nutritive, for example fructose and sucrose and 2) non-nutritive such as artificial sweeteners (e.g. sucralose and aspartame) or polyols (e.g. sorbitol and mannitol). Sweet solutions are known to be appealing to children (Drewnowski *et al.*, 2012) and artificial sweeteners have been a popular choice for formulators as they provide non-caloric, intense sweetness (Grembecka, 2015).

The sweetness profile (intensity, onset and duration) of the sweetening excipient needs to be considered (table 1.1) and combinations of sweeteners can be employed depending on what is required for the bitter drug. This can result in formulations containing high levels of excipients and a bulky drug product.

Table 1.1 The sweetness profile of nutritive and non-nutritive sweeteners

Sweetener type		Sweetness profile
Nutritive		Rapid sweetness onset (maximum sweetness reached within 8-10 seconds) followed by sweetness decay and a short duration of perceived sweetness No associated negative taste attributes such as bitterness of metallic taste (Tan *et al.*, 2019)
Non- nutritive	Artificial sweeteners	Sweetness onset similar to sucrose but variable peak sweetness and duration (Tan *et al.*, 2019). For example, compared to sucrose (10% w/v) - sucralose (0.0387% w/v) and aspartame (0.0827% w/v) have similar peak sweetness but have a longer duration of sweetness - acesulfame-K (0.0832% w/v) has significantly lower peak sweetness but has a similar sweetness decay Some artificial sweeteners are perceived as bitter themselves (Kuhn *et al.*, 2004) or have a bitter aftertaste (EMA, 2006) and so can be used in combination to negate the bitter profile of the other. - Acesulfame-K and sucralose in particular have significant bitterness associated with concentrations necessary for sweetness. Acesulfame-K has a shown to be perceived as just as bitter as it is sweet. - Acesulfame-K and sucralose also have a metallic aspect to their taste (Tan *et al.*, 2019).
	Polyols	Sweetness onset similar to sucrose but sweetness is less intense (Tan *et al.*, 2019). Due to their low relative sweetness, polyols are frequently employed as bulking agents and used alongside artificial sweeteners (Radeloff and Beck, 2013). Compared with sucrose (10% w/v), polyols have low peak sweetness. High concentrations are required for comparable sucrose sweetness. For example - mannitol (14.63% w/v) and xylitol (9.85%) have similar peak sweetness to sucrose (10% w/v) but limited duration of sweetness - sorbitol (13.74% w/v) and erythritol (13.33% w/v) have lower peak sweetness compared to sucrose (10% w/v) but retain their limited sweetness for longer time periods (Tan *et al.*, 2019). Some polyols have other negative taste attributes associated with their use for example sorbitol (13.74% w/v) has a bitter/ metallic off-taste and erythritol (13.33% w/v) has a chemical off-taste reported (Tan *et al.*, 2019).

1.3.2 Risk-benefit analysis

The inclusion of any excipient, including taste-masking agents, within a paediatric formulation needs to be justified. At present, there is no universal process for excipients to be deemed safe or unsafe, on each occasion they are approved alongside the API they support based on that specific need. Current EMA guidelines stipulate the need to review the safety and necessity of each excipient in the formulation (quantity and types) relative to the intended patient population including exposure and age (EMA, 2013).

Using sweeteners within formulations is commonplace and therefore the need to provide a detailed safety review to regulators is deemed unnecessary unlike if an excipient is considered new/novel (International Pharmaceutical Excipients Council, 2008). Excipients are considered by regulators to be 'known' if listed in one or more of the following 1) any of the 3 major compendia, US/European/Japanese pharmacopeia 2) any of the other widely known compendia such as the 'Handbook of Pharmaceutical excipients' or 3) the Food and Drug Administration's (FDA) inactive ingredient database (IID) which lists excipients with prior precedent of use in approved formulations (FDA, 2022). It is also important to know if the excipient is generally recognised as safe (GRAS). Table 1.2 summarises this information for the sweeteners most commonly used in paediatric formulations (Rouaz *et al.*, 2021)

Table 1.2 Sweetener regulatory status. GRAS; generally regarded as safe, IID; inactive ingredients database (Senopsys, 2018)

	US Pharmacopeia	European Pharmacopoeia	British Pharmacopeia	Japan Pharmacopeia	GRAS	IID
Aspartame	✓	✓	✓			✓
Mannitol	✓	✓	✓	✓	✓	✓
Sorbitol	✓	✓	✓	✓	✓	✓
Sucralose	✓		✓			✓
Sucrose	✓	✓	✓	✓	✓	✓

1.3.3 Issues with sweeteners

Despite their widespread use, there are a number of health concerns regarding the use of sweeteners (table 1.3).

Table 1.3 Commonly used sweeteners and reported concerns

Sweetener type		Examples	issues
Nutritive		Sucrose, Dextrose, Fructose, High Fructose corn syrup.	- The cariogenic nature of these sugars can contribute to dental caries meaning these excipients are unfavourable for use in children especially for chronic indications (EMA, 2006) - The link between nutritive sugars in the diet and colonic cancer in adolescents has been recently demonstrated. Children self-identifying as consuming high quantities of sugars in their diet had an increased risk of early onset colorectal cancer (Joh *et al.*, 2021). To keep overall sugar consumption low, they should be avoided in medicines especially for chronic indications. - These sugars are contraindicated in patients with hypoglycaemia or hereditary fructose intolerance or with co-morbidities such as diabetes (EMA, 2006)
Non-Nutritive	**Polyols**	Mannitol, Sorbitol, Xylitol, Erythritol, Maltitol.	- Sugar alcohols can alter gut parameters which can have a negative effect on drug absorption. They do this by; 1) increasing GI fluid volume thus reducing the concentration gradient of a drug in the gastrointestinal tract (GIT) 2) increasing gut motility 3) quickening transit time and reducing the API's contact time with the gut which can negatively impact absorption (Lenhart and Chey, 2017). • Sorbitol has been demonstrated to reduce the bioavailability of Ranitidine in adults. 1.25g sorbitol began to change the PK profile of the drug (Chen *et al.*, 2007). Sorbitol has no specified acceptable daily intake and is widely used in pharmaceutical formulations for example, it is used in oral solutions at 20 -25% (Handbook of Pharmaceutical Excipients, 2006) • Mannitol has been shown to have a dose dependent effect on GI transit in humans from 0.755g (D. Adkin *et al.*, 1995). Mannitol has no limit to

			its acceptable daily intake and is used widely in formulations, it is used at high levels in tablets (10-90% w/v) and at lower levels in suspensions (7% w/v) (Handbook of Pharmaceutical Excipients, 2006) - Due to the osmotic effects of polyols, patients can experience side effects such as diarrhoea (Lenhart and Chey, 2017) - Sorbitol is contraindicated in patients with hereditary fructose intolerance (as it is metabolised to fructose) and hypoglycaemia, in severe cases this may damage the liver leading to coma or death (EMA, 2006)
	Artificial sweeteners	Sucralose, Sachharin, Acesulfame potassium, Aspartame, Sodium sacharrin, Neotame.	Over the past few decades the use of artificial sweeteners has been favoured over the use of caloric sugars however there is ongoing controversy as to the risks posed by artificial sweeteners (Tandel, 2011). - Animal studies have shown that artificial sweeteners are linked with body weight gain (Swithers and Davidson, 2008) and there is significant data to suggest this translates to a link with obesity in humans (Pearlman, Obert and Casey, 2017); the sweet taste induces an insulin response which causes blood sugar to be stored in tissues but because blood sugar does not increase with artificial sweeteners there is hypoglycaemia and increased food intake resulting in increased adiposity (Hampton, 2008) - Consumption of artificial sweeteners in the diet has been linked to increased risk of stroke and coronary heart disease in women in a dose-dependent manner (Mossavar-Rahmani *et al.*, 2019). To keep overall consumption low, their use in high levels in medicines should be viewed with caution especially for chronic indications - The carcinogenic potential of saccharin has been demonstrated in animal studies (Dwaine Reuber, 1978). It is now thought that the mechanism by which this occurs is not translatable to humans (National Cancer Institute, 2016)

			however some countries, for example Canada, have banned its use as a food additive and others have restricted the levels of its use (Tandel, 2011) - Aspartame's breakdown products are toxic in high doses; phenylalanine, is an essential amino acid at normal levels but at elevated levels in the blood can lead to brain damage (Pawar and Kumar, 2002). This is problematic for children with phenylketonuria, a condition experienced by one in 20,000, where the body cannot metabolism phenylalanine. The use of aspartame requires a product warning (Tandel, 2011). - Sucralose has been shown to have a negative impact on faecal microflora, faecal pH, expression levels of P-gp and various cytochrome-p450 enzymes from 1.1mg/kg in rats (Abou-Donia *et al.*, 2008). Sucralose is used at 0.03 - 0.24% in products for human consumption and has a WHO recommended acceptable daily intake of 15mg/kg (Handbook of Pharmaceutical Excipients, 2006). This work has been met with controversy but it is not the only study to suggest sucralose to have a negative impact on the gut (Suez *et al.*, 2014, 2015; Sylvetsky *et al.*, 2016; Ruiz-Ojeda *et al.*, 2019; Shil and Chichger, 2021) and this has led to a clinical trial (The SweetMeds study) exploring the effect 4mg/kg/day sucralose has on drug absorption and metabolism (ClinicalTrials.gov, 2018). - Saccharin has also demonstrated a negative effect on the gut microflora from consumption of 90mg of the sweetener in rats (Ruiz-Ojeda *et al.*, 2019). Saccharin is used in pharmaceutical formulations at 0.02-0.5% w/v and has a WHO recommended acceptable daily intake of 2.5mg/kg (Handbook of Pharmaceutical Excipients, 2006)

1.3.4 Quantity concerns

Despite these issues, sweeteners continue to be used as a matter of course as the first-line in taste-masking. Of course, as with everything humans consume, the risk of safety concerns is linked to quantity and is dependent on the age and condition of the patient.

1.3.4.1 Acceptable daily intake

The recommended acceptable daily intake (ADI) of excipients, including sweeteners, has historically been used as a guide of safe levels. The ADI is defined as the maximum amount of a substance that can be ingested daily with no appreciable health risk and is usually presented as mg/kg (Gilsenan, 2011). The ADI is normally derived from the lowest no observed adverse effect level (NOAEL) from long term in vivo studies. Various NOAELs may be obtained during animal studies pertaining to different risks, for example reproductive toxicity may be seen in rats at 50mg/kg whereas carcinogenicity may be seen in mice at 10mg/kg. Here, 10mg/kg represents the lowest NOAEL and is thus used to calculate the ADI. The ADI is calculated from the NOAEL using a large safety factor to account for species differences, commonly 100 (Gilsenan, 2011). In this example, the ADI would be 0.1mg/kg.

The majority of these long-term toxicology in vivo studies are carried out in adult animals, with the exception of developmental toxicity studies. However, these have traditionally focused on prenatal development, with only limited assessment of postnatal developmental effects (FDA, 2006). Therefore, the relevance of ADI as a measure of safety in children is unclear (Østergaard and Knudsen, 1998).

1.3.4.2 Cumulative effect

A recent review compiling paediatric formulations highlights the frequency of sweetener use in liquid dosage forms (Rouaz *et al.*, 2021). This can be problematic for children taking multiple medications and can result in overall doses exceeding even adult maximum exposure levels (Whittaker *et al.*, 2009). Children also ingest sweeteners as part of their diet which can add to this cumulative effect. Concern surrounding the use of sweeteners in children stems from the associated safety issues (table 1.3), the quantity children can ingest and the fact that these young patients are already in an unwell and vulnerable state.

As science progresses, it is hoped that improved medicines for children will be developed but better taste-masking agents are needed to support this. It is unrealistic to think that sweeteners could be eliminated entirely from liquid formulations, nor would this be a beneficial solution. Efforts should be placed on exploring other taste-masking approaches which could be used instead of, or in combination with, sweeteners to lower their concentrations towards a less flavoursome and leaner product. Formulators need more options available in the taste-masking toolbox and new classes of excipients need to be explored. One such class involves steering towards a taste-neutral formulation.

1.4 Taste-neutral formulations

Excipients which promote a taste-neutral formulation offer a form of taste-masking which does not seek to overwhelm the API with opposing flavours.

This taste-neutral approach may be especially attractive for very soluble and aversive API that cannot be masked by sweetening alone and that cannot be encapsulated or coated. Taste-neutral excipients also strive to create a dosage form which cannot be perceived as attractive or candy-like to children, lowering the likelihood of accidental overdose (EMA, 2013). Bitter-blockers and cyclodextrins/maltodextrins are two types of excipients which could fill this gap.

1.4.1 Bitter-blockers

Many drugs are perceived as bitter (Mennella *et al.*, 2013). Poor tasting medicines are not exclusively bitter, for example some are reported as metallic (Ranbaxy Limited, 2020), but it is bitterness that is most problematic because humans have evolved to avoid bitterness to protect themselves against ingesting a potentially toxic substance (Wooding *et al.*, 2006). As a result, bitter receptors are present throughout the body including in the gut and lungs as well as in the oral cavity (Lu *et al.*, 2017). Bitter receptor blockers, known as taste-modifiers or bitter-blockers, interact with the bitter-taste perception pathway at a pharmacological level; interfering with taste receptors or the taste-transduction mechanism.

1.4.1.1 Detecting bitterness

There are five primary tastes; sweet, sour, umami, bitter and salty. Both sour and salty are mediated by ion channels whereas sweet, umami and bitter tastes are detected by two G-protein coupled receptor (GPCR) families; taste 1 receptor (TAS1R) and taste 2 receptor (TAS2R) (Temussi, 2009). Most bitter tastants are detected by their interaction with TAS2Rs which are located in taste buds (Mennella *et al.*, 2013). TAS2Rs are a large family of around 25

members, many of which can detect a huge variety of bitter molecules. It is thought that when the receptor is stimulated, the G-protein, gustducin, is activated and stimulates phospholipase C β2 which results in inositoltriphosphate activation mediating a rise in intracellular calcium levels and thus activating transient receptor cation channel M5 (TRPM5). The result is membrane depolarisation, generation of an action potential and the release of ATP which then acts on purinergic receptors activating sensory nerve fibres which in turn activate the appropriate brain centres leading to taste perception (Depoortere, 2014). This proposed pathway is summarised in figure 1.4.

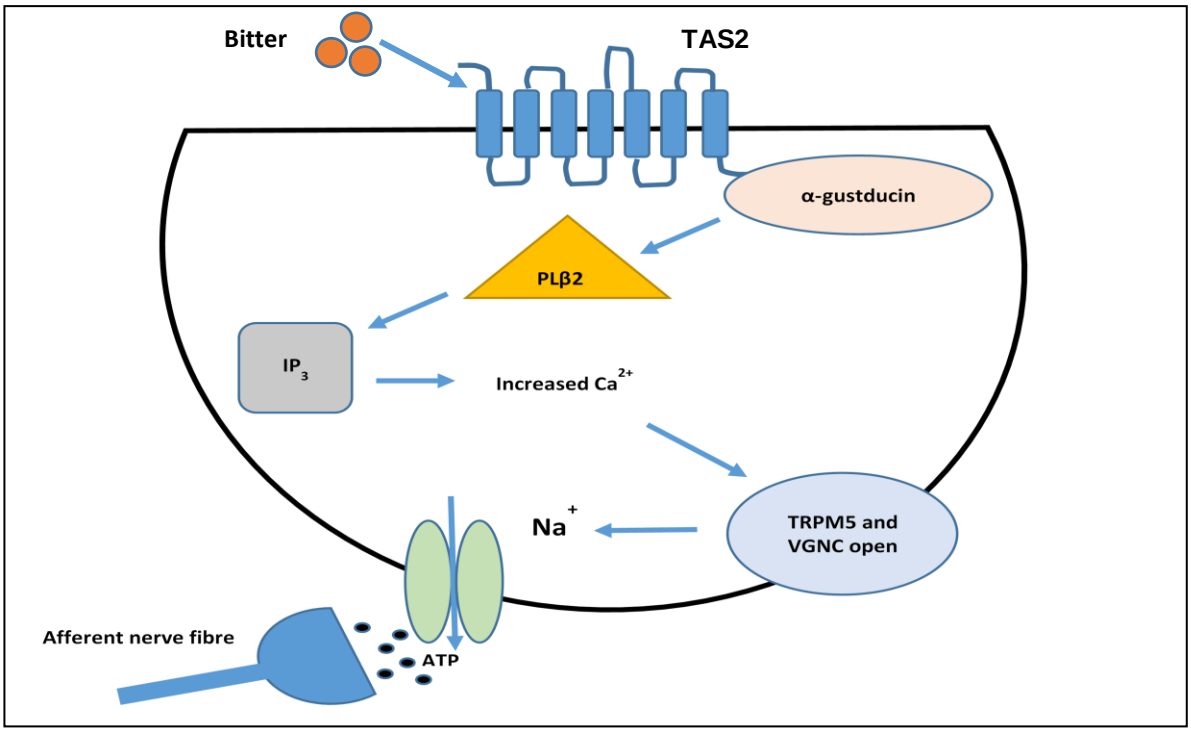


Figure 1.4 Schematic representation of the proposed human bitter taste pathway. TAS2R; taste 2 receptors, PLβ2; phospholipase C β2, IP3; inositoltriphosphate, TRPM5; transient receptor cation channel M5, VGNC; voltage gated sodium channel.

As many API interact with multiple TAS2Rs, a bitter-blocker acting as a specific bitter receptor antagonist may not mask the aversive taste entirely but is likely to dampen it. This may be sufficient for the API to become palatable enough for improved acceptability and patient compliance (Andrews *et al.*, 2021).

1.4.2 Inclusion complexes

The term dextrin applies to a range of dextrose polymers obtained by heating starch in the presence of moisture and an acid (BeMiller, 2003). Some dextrins can molecularly encapsulate drug molecules forming inclusion complexes. This is possible when dextrins form a cavity which can host guest molecules (Sohi, Sultana and Khar, 2004). This mechanism of action means they can be repurposed for use as taste-masking agents within formulations and decrease the amount of API molecules exposed to the taste buds thus reducing bitter-taste perception (Al-kasmi *et al.*, 2017). Examples include cyclodextrins and maltodextrins.

Both cyclodextrins and maltodextrins have a precedence of use in pharmaceutical formulations for other purposes (FDA, 2016; EMA, 2017). Cyclodextrins have been used to improve water-solubility and API bioavailability (EMA, 2017) and maltodextrins have been used as a carrier (Blazek-Welsh and Rhodes, 2001), as a diluent and in tablet coatings (Parikh, Agarwal and Raut, 2014). Cyclodextrins and maltodextrins are attractive as taste-masking excipients in children due to their GRAS status and safety profile (FDA, 2016; EMA, 2017) Table 1.4 lists some examples which feature in the major compendia (Handbook of Pharmaceutical Excipients, 2006).

Table 1.4 Cyclodextrins and maltodextrins with precedence of use. Ph.Eur; The European Pharmacopoeia, USP; the United States Pharmacopeia, JP; Japense Pharmacopeia

	Pharmacopoeia Monographs
Cyclodextrins	
α-cyclodextrin	Ph.Eur, USP, JP
β- cyclodextrin	Ph.Eur, USP, JP
Hydroxypropyl-β- cyclodextrin	Ph.Eur, USP
Sulfobutylether-β- cyclodextrin	USP
γ- cyclodextrin	JP, (to be included in Ph.Eur)
Maltodextrins	
C* Dry MD	Ph.Eur, USP
C*PharmDry	Ph.Eur, USP, JP
Glucidex	Ph.Eur, USP, JP
Glucodry	Ph.Eur, USP, JP
KLEPTOSE- Linecaps	Ph.Eur, USP
Lycatab	Ph.Eur, USP, JP
Maldex	Ph.Eur, USP, JP
Maltrin	Ph.Eur, USP, JP
Maltrin QD	USP
Paselli MD10 PH	Ph.Eur, USP, JP
Rice*Trin	Ph.Eur, USP, JP
Star-Dri	Ph.Eur, USP, JP
Tapi	USP

1.4.2.1 Cyclodextrins

Cyclodextrins are cyclic oligosaccharides which form a ‘bucket-like’ shape. The types of cyclodextrins, α, β and γ-cyclodextrin, differ by number of linked glycosyl units, 6, 7 and 8 respectively (Dass and Jessup, 2000). Both α and β cyclodextrin are non-sweet whilst γ-cyclodextrin has a slightly sweet taste (Zakharova *et al.*, 2016).

The number of glucose units importantly confers differences in diameter of the internal cavity because of this, α-cyclodextrin tends to complex with lower molecular weight compounds compared with γ-cyclodextrin which can house larger ones (Pavlov *et al.*, 2010). β –cyclodextrin is the most widely used as it is cheap and accessible (Del Valle, 2004) but has relatively low solubility so derivatives, such as Hydroxypropyl-β- cyclodextrins have been produced which have much improved aqueous solubility (EMA, 2014)

The outside surface of the cyclodextrins is hydrophilic due to the arrangement of hydroxyl groups and the internal, hydrophobic, core can accommodate a guest molecule forming an inclusion complex (Szejtli and Szente, 2005) (figure 1.5). These properties enable cyclodextrins to dissolve in water whilst incorporating molecules in their interior matrix (Del Valle, 2004).

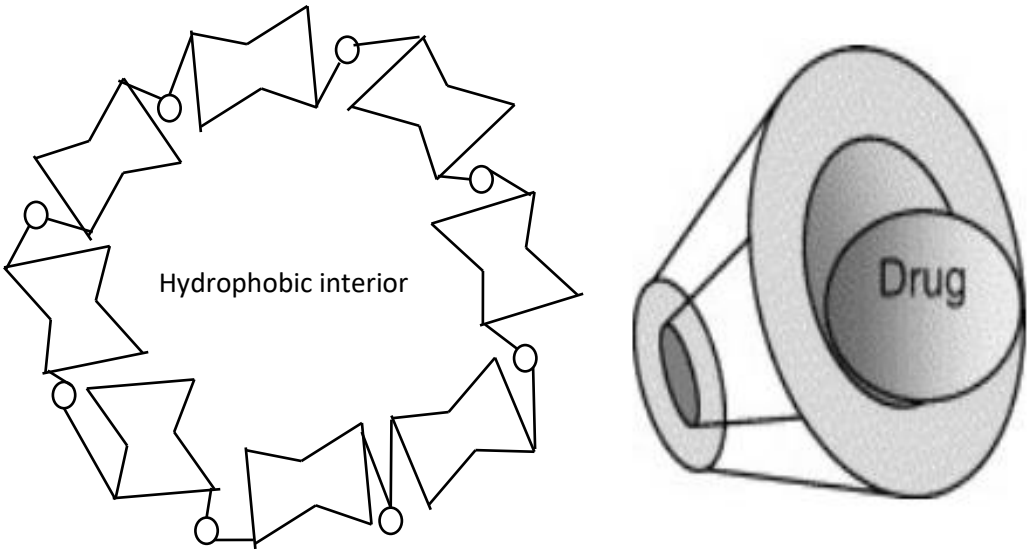


Figure 1.5 Cyclodextrin structure and depiction of an inclusion complex of a drug residing in the cavity (adapted from EMA,2017)

Theoretically, depending on size, more than one drug molecule can be housed within a cyclodextrin, or alternatively two cyclodextrins can interact with one drug molecule. However, many inclusion complexes follow a 1:1 (cyclodextrin: guest) stoichiometry (Cid-Samamed *et al.*, 2022)

1.4.2.2 Maltodextrins

Maltodextrins are non-sweet saccharide mixtures consisting of glucose units connected in dimers, oligomers and polymers which are formed under controlled enzymatic hydrolysis of starch (Preis *et al.*, 2014). Maltodextrins are classified based on their dextrose equivalent (DE) which is a measure of the reducing sugars relative to dextrose. Many of the maltodextrins shown in table 1.4 are manufactured to various DEs for different functional uses - the solubility and compressibility of maltodextrins increases with increasing DE (Handbook of Pharmaceutical Excipients, 2006). Maltodextrins usually have a DE of around 3-20 (Klinkesorn *et al.*, 2004). The amylose within the maltodextrin can form flexible helices with a hydrophobic internal surface and hydrophilic external surface enabling encapsulation of drug molecules (Kaushik and Dureja, 2014) (figure 1.6).

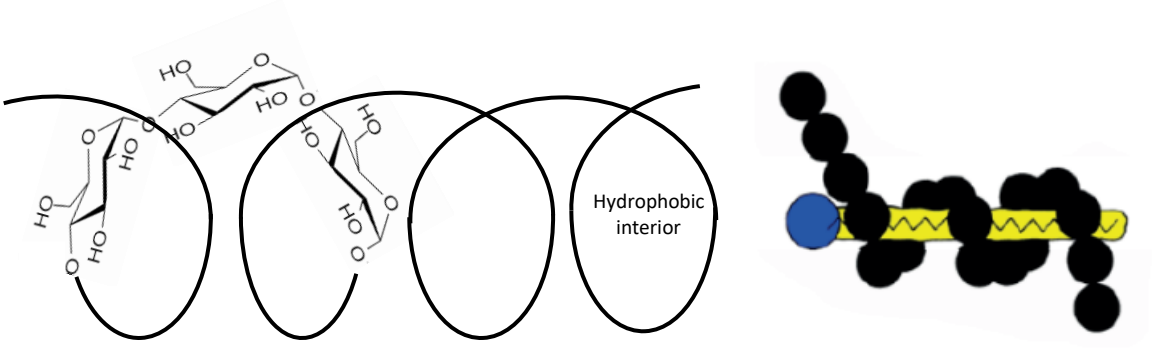


Figure 1.6 Maltodextrin structure and depiction of an inclusion complex of a drug residing in the cavity (adapted from Popescu et al, 2012)

1.4.2.3 Inclusion complexes limitations

These inclusion complex approaches also encounter limitations as some compounds will not form interactions with cyclodextrins or maltodextrins (Szejtli and Szente, 2005) for example if an API and dextrin are not a compatible size (Fifere *et al.*, 2012). Alternatively, interactions may occur but either the encapsulated portion of the API is not the part that confers bitter taste or the complex is unstable due to insufficient binding strength (Abou-Hamdan *et al.*, 2000).The stability of dextrin-drug complexes are reliant on non-covalent interactions which is important to facilitate the taste-masking function of dextrins whilst not altering the pharmacodynamic profile of the drug itself (Davis and Phipps, 2017).

1.5 Assessing the efficacy of taste-neutral excipients

In order to understand the efficacy of novel taste-masking approaches a reliable means of palatability assessment is needed; there is a regulatory obligation to demonstrate patient acceptability of a formulation (EMA, 2013).

Using children to assess the aversiveness of paediatric formulations would be the most definitive way to evaluate the efficacy of a taste-masking strategy as they represent the intended recipients. However it would be unethical and unsafe to use humans, of any age, in the early stages of drug development when there is limited safety data available on the API (Cram *et al.*, 2009). Formulation acceptability in children has to be demonstrated but this can only happen during clinical testing. Palatability assessment cannot wait until the late stages of development (when sufficient safety data is available) but

should be carried out, not as an after-though but as early as possible, when the formulation is still being optimised. Therefore, there is a need for pre-clinical models to enable this (Cram *et al.*, 2009).

1.5.1 Pre-clinical models

There are a range of non-human assessment techniques available to understand taste-masking (Mohamed-Ahmed *et al.*, 2016). A recent publication (Clapham *et al.*, 2021) has generated a tool which reviews the various palatability models available and has allocated a score to each based on various criteria. This score reflects attributes of the model such as; the overall rationale, repeatability, reproducibility, robustness, range, reliable quantitative measurement, ability to be readily interpreted, throughput (set up, duration of test, data analysis), availability, regulatory awareness, research activity (understanding within the research community) and finally response kinetics (if the tool can indicate the presence of aftertaste). Using this tool, the electronic-tongue (e-tongue) and the brief access taste aversion (BATA) model were found to be the most recognised non-human models of preclinical assessment (Clapham *et al.*, 2021).

1.5.2 e-tongue

The e-tongue is a robotic tool which uses various sensors to identify different tastes in a sample by translating the taste detected into an analytical signal. E-tongues can evaluate many solutions over a single run, although this does take time it is an automatic process. E-tongues have shown good repeatability if sensors are maintained and calibration protocols are followed and data generated from e-tongues has been used in regulatory submissions (Clapham *et al.*, 2021). There are a handful of systems available with the two most

widely used being the Astree and the Insent TS-5000Z (Mohamed-Ahmed *et al.*, 2016). The Astree uses polymeric sensors and measures initial taste whereas the Insent TS-5000z, used in this work, uses lipid-membrane sensors and also gives a reading of after-taste (Podrażka *et al.*, 2017).

1.5.2.1 Overview of the Insent TS-5000z e-tongue

The lipid in the sensor can form electrostatic and hydrophobic interactions with taste materials in the sample causing a change in the potential of the lipid membrane which is detected by a computer (Latha and Lakshmi, 2012). The taste sensors (figure 1.7) are placed into the liquid samples. The sensors represent various taste sensations and have very low thresholds of detection, for example $2x10^{-6}$ M to detect sucrose (sweetness) compared to $1x10^{-2}$ M detection concentration of the human tongue (Gupta *et al.*, 2010). The sensor acts as a transducer, converting the taste sensation it perceives into a readout (mV).

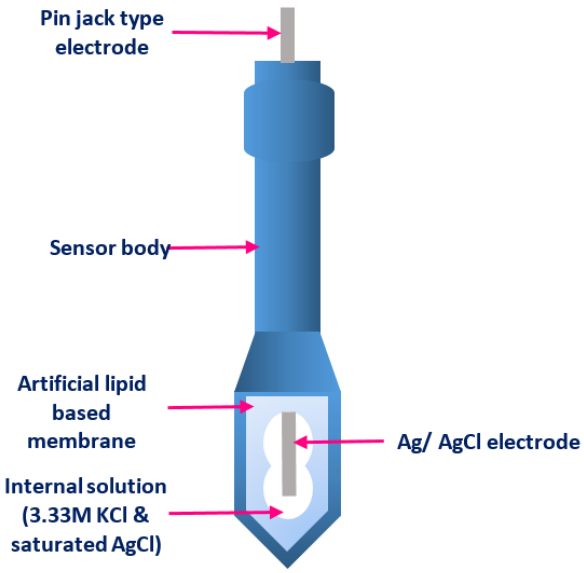


Figure 1.7 The components of a taste sensor used with the e-tongue. Image used with permission from Dr Hend Abdelhakim

After the initial reading is taken, there is a light cleaning procedure and then another measurement is taken to represent aftertaste. This aftertaste is measured by determining the change of membrane potential caused by adsorption of the substances to the lipid membrane after the cleaning procedure (Woertz *et al.*, 2011a).

1.5.2.2 e-tongue limitations

E-tongue technologies have not been fully validated against human panels yet and so data produced needs to be viewed with caution however, it is useful as a comparative tool to indicate if a taste-masking strategy is likely to have any benefit (Woertz *et al.*, 2011b). Other practical limitations include the cost of sensors and the machine itself and sensor stability; at elevated temperatures during hot weather, if the machine and any samples to be tested are not housed in a temperature controlled environment, sensor membranes can soften and become unstable. Sensors can also become saturated or damaged by some formulations so appropriate cleaning steps may be required. Furthermore, the e-tongue is not sensitive to all APIs for example, non-charged molecules are not detectable (Clapham *et al.*, 2021)

1.5.3 BATA model

The BATA model uses animals to screen formulations for aversiveness. Rats have shown excellent correlation with humans in terms of correctly identifying the rank order of bitter compounds (Clapham *et al.*, 2012; Rudnitskaya *et al.*, 2013; Cocorocchio *et al.*, 2016; Soto *et al.*, 2018). The BATA model has been shown to have good reproducibility and has excellent repeatability over the course of a typical protocol (usually between 4-5 days including training). Rats can be kept for many months during which time the animals are temporarily

water deprived whilst on study and experience minimal transient weight loss therefore the procedure is deemed ‘mild’ by governing bodies, the lowest severity category. There is also a growing regulatory awareness of the value of data generated from this model and BATA data has been included in submissions to demonstrate acceptability (Clapham *et al.*, 2021).

The rats can be presented with bitter API alone or with taste-masked formulations and water as a control in a ‘Davis Rig’ (figure 1.8). The rats are exposed to each compound for 8 seconds, followed by a 2 second water rinse, and the number of licks is recorded by the computer. The lick count for each formulation is compared to the response to water (which is set as the maximum lick number). An IC50 for a compound can be calculated as the concentration that inhibits 50% of the maximum lick number (Soto *et al.*, 2015). The effectiveness of taste-maskers can be evaluated by comparing the lick responses to the various formulations compared to the drug alone.

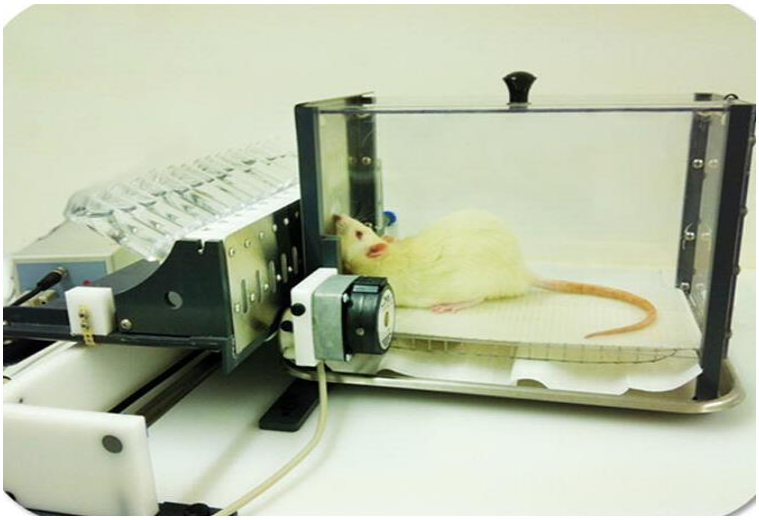


Figure 1.8 A rat in a Davis Rig being presented with different formulations

1.5.3.1 BATA model limitations

As discussed earlier in this chapter, the use of any animal model requires regulatory guidance to be followed and necessary licenses to be in place. Access to animal facilities and staff with the appropriate training can be a limitation to the widespread availability of this model. In terms of predictability, the BATA model can identify the aversiveness of a formulation but cannot provide information on specific taste-qualities. The model has been shown to be an effective assessment tool for bitter-drugs alone but work needs to further understand the predictive nature when excipients are introduced.

Cyclodextrins, maltodextrins and bitter-blockers have not been extensively assessed in the rat BATA model. These excipients are steering towards a taste-neutral formulation and so should not encounter limitations stemming from differences between human and rodent sweet taste perception (Lemon, 2015). Such issues can be experienced with sweeteners or flavouring agents making them more challenging to assess in the BATA model.

1.6 Identifying research needs

Children remain a neglected patient population when formulating and assessing medicines. Taste-masking liquid formulations remains a difficult challenge for formulators and so novel taste-masking strategies need to be sought to address this to formulate better patient-centric medicines.

The World Health Organisation (WHO), in their expert committee meeting report, ‘development of paediatric medicines: points to consider in formulation’ outlined the importance of considering if excipients affect the bioavailability of

drugs in children, naming transit time and transporters as two areas of importance (WHO, 2007). No research has investigated how commonplace taste-masking excipients affect the gut in paediatric models and so excipients continue to be used blindly.

The WHO document also highlights how taste-masking is key for children to accept their medication. This document advises against using cariogenic sweeteners. Many non-cariogenic sweeteners are known to have limited efficacy with very soluble and aversive API (Sohi, Sultana and Khar, 2004; Walsh *et al.*, 2014), have an intrinsic bitterness themselves (Kuhn *et al.*, 2004) and have a number of health concerns associated with high levels of use (Pawar and Kumar, 2002; Abou-Donia *et al.*, 2008; Hampton, 2008; Swithers and Davidson, 2008; Tandel, 2011; Suez *et al.*, 2014; Sylvetsky *et al.*, 2016; Pearlman, Obert and Casey, 2017; Mossavar-Rahmani *et al.*, 2019; Ruiz-Ojeda *et al.*, 2019; Shil and Chichger, 2021). There is a need to explore different taste-masking strategies so sweeteners no longer need to be so heavily relied upon and their quantities can be lowered towards leaner formulations.

The EMA discusses how a neutral taste to a paediatric product is desirable, citing how even acceptable flavours may become unpalatable on repeat administration (EMA, 2013). A taste-neutral approach, using cyclodextrins, maltodextrins or bitter-blockers, would circumvent the common problems faced with sweeteners and flavourings.

It is important to have predictive models of assessing the potential benefits of taste-masking strategies. The e-tongue and BATA model are two recognised

preclinical tools which can assess palatability (Clapham *et al.*, 2021) in lieu of human panels. The BATA model has shown good correlation to human data for API alone but has not been fully explored with excipients. Where data from human panels is available, output from such models should be compared to identify the predictability. Understanding the correlation and limitations of non-human tools of assessment will enable them to be used with confidence when demonstrating to regulators that product palatability has been achieved.

1.7 Thesis aims and outline

1.7.1 Covid impact on thesis aims and outline

Ongoing COVID restrictions resulted in no access to UCL laboratories from March 2020. In order to continue in vivo work, it was necessary to take the time to setup the BATA model at a new location under a new license before continuing with planned studies (chapter 4). However, pharmacokinetic work still required the university facilities to investigate gut effect of excipients. This was only able to go ahead in October 2021 due to both room capacity and study restrictions. Therefore, there was only sufficient time to evaluate one paediatric excipient for chapter 5 in vivo and further investigations into transporter action could not take place.

1.7.2 Aims

The overall aims of this thesis are:

1. To introduce the importance of effective taste-masking excipients for children and discuss the ways in which such excipients can affect the bioavailability of medicines (chapter 1).

Liquid dosage forms remain widely used in children, requiring effective taste-masking excipients. Research into the effects of such excipients on API bioavailability should be prioritised. In particular, efforts should be focused on: evaluating taste-neutral strategies for taste-masking and evaluating sweetener impact on drug absorption in children.

2. To understand if the BATA model is a predictable method to assess the taste-masking ability of a cyclodextrin and maltodextrin (Chapter 2).

   Cyclodextrins and maltodextrins such as Hydroxypropyl-β-cyclodextrin and KLEPTOSE Linecaps (a pea maltodextrin) are recognised excipients, with a good safety profile, and could be useful for taste-masking liquid formulations. They have been tested seldomly in the BATA model and little is known about the translatability of any taste-masking effects compared with human panels. This is important as it indicates whether or not the BATA model could reliably be used for preclinical assessment of formulations containing cyclodextrins/ maltodextrins as the taste-masking strategy.

3. To review the current knowledge of bitter-blockers and assess promising compounds for their utility as potential excipients in a standardised manner (Chapter 3).

   Bitter-blocking excipients have not been thoroughly explored as a viable option for oral liquid formulations which are so often prescribed to children. A number of bitter-blockers have been identified in the literature but no comprehensive systematic review of their ability to block bitterness and at what levels has been developed. These compounds have not been

assessed for their ability to be incorporated into a dosage form suitable for children in terms of safety, efficacy or usability. Such excipients have not been characterised alongside drugs to check for unwanted interaction, nor have they all been assessed for taste-masking ability in a standardised manner.

4. To assess the efficacy of promising bitter-blockers with paediatric drugs (chapter 4).

   Bitter-blockers have the potential to offer an acceptably palatable, taste-neutral, oral liquid formulation for children. In order for them to be used going forward, it is necessary to understand where they could offer benefit. This chapter assesses the bitter-blockers' ability to taste-mask two model paediatric drugs; Primaquine phosphate and Ranitidine hydrochloride, both drugs require better strategies to improve their palatability and compliance.

5. To assess the effect a taste-masking excipient has on paediatric gut permeability of model API (Chapter 5).

   Taste-masking excipients have seldom been investigated for effects on the gut which could impact API bioavailability. No taste-masking excipients have been evaluated for such effects in a paediatric model. Such knowledge can improve how the scientific community selects excipients when formulating medicines for children. This chapter focusses on sorbitol, an excipient commonly found in paediatric formulations, as a model excipient alongside Ranitidine as the model drug.

   Specific aims and objectives are outlined further in each chapter.

Chapter 2

Taste-masking with Hydroxypropyl-β-cyclodextrin and a pea maltodextrin; a comparison between a human panel and a preclinical model.

This chapter explores the suitability of the BATA model to assess the taste-masking efficacy of a cyclodextrin and a maltodextrin. Cyclodextrins and maltodextrins are attractive excipients for paediatric formulations owing to their excellent safety profile and precedence of use as excipients for other purposes. In order for them to be viable taste-masking options, it is necessary to have a reliable non-human means of assessing their performance within formulations preclinically. The BATA model offers good correlation to humans for bitterness of API but has not yet been extensively assessed for taste-masking strategies such as cyclodextrins and maltodextrins. This chapter describes the results of a human panel evaluating formulations containing Hydroxypropyl-β-cyclodextrin and a pea maltodextrin with two bitter drugs, Ranitidine hydrochloride and Sildenafil citrate, and then compares this with historical BATA data. The limitations of using the BATA model as a preclinical assessor of dextrin containing formulations are explored.

2.1 Introduction

Cyclodextrins and maltodextrins have a wide variety of uses both in the pharmaceutical and food manufacturing industries. The encapsulation properties offered by them, as introduced in chapter 1, can be harnessed to allow for controlled release of active material and to improve solubility and

bioavailability of API (Pereira *et al.*, 2021). Cyclodextrins are often used as functional excipients as carriers of sensitive ingredients to protect them from light, oxidation or decomposition (Cravotto *et al.*, 2006). Whereas maltodextrins are widely used in sports drinks, gels and foods as they are easily digested by the body and offer immediate energy (Hofman, van Buul and Brouns, 2015).

Both cyclodextrins and maltodextrins have been used in food stuffs to reduce bad taste or odours (Szente and Szejtli, 2004) as they can complex with bitter molecules and prevent their interaction with sensory receptors. In the same way, these dextrins can be utilised within medicinal formulations to prevent drug molecules from activating bitter-receptors, providing a taste-neutral medicine.

Maltodextrins and cyclodextrins are appealing as taste-masking excipients for paediatric medicines: they already have a precedence of use in medicinal formulations, they are inexpensive and have a good safety profile (BeMiller, 2003).

2.1.1 Cyclodextrin inclusion complexes

Cyclodextrins can be used as taste-masking excipients for both solid and liquid dosage forms and have been proposed to enact taste-masking in a number of ways (Arima, Higashi and Motoyama, 2012). Firstly, they can form inclusion complexes with drug molecules (figure 2.1). To form an inclusion complex, the hydrophobic moiety of the guest molecule must fit entirely, or partially, into the cavity. If a guest molecule is small, more than one molecule can be included into a cyclodextrin cavity. Alternatively, if a guest molecule is

large, more than one cyclodextrin may interact with it (Wankar *et al.*, 2020). This depends on the concentration of both the cyclodextrin and the drug (Arima, Higashi and Motoyama, 2012). Complexes are formed by hydrophobic interaction and their formation is largely governed by the structure and size of the guest molecule being compatible with the host cyclodextrin (Szejtli and Szente, 2005).

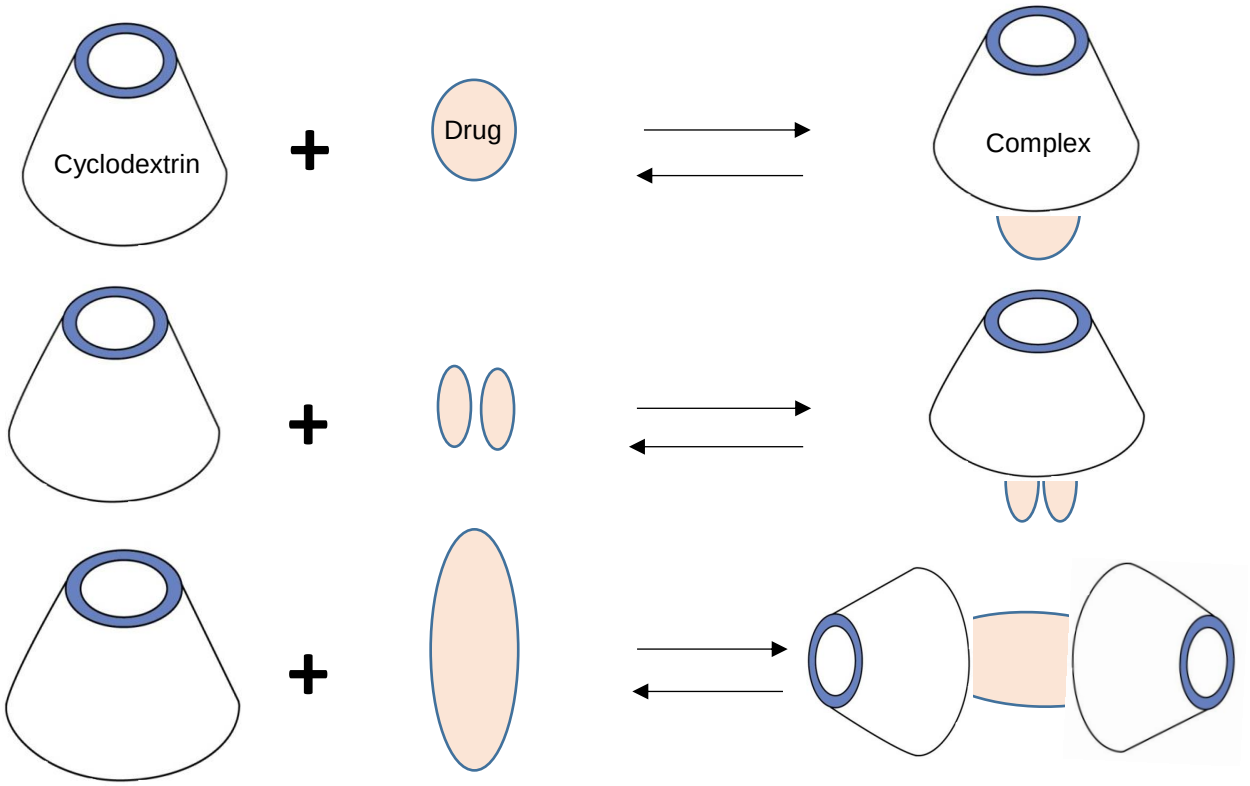


Figure 2.1 Examples of how cyclodextrin and drug molecules can form a complex.

The cavity of cyclodextrins is normally occupied by water when in solution as well as in the crystalline state (crystalline β-cyclodextrin contains approximately 13-14% (w/v) water) (Szejtli and Szente, 2005). This is energetically unfavourable as the water molecules are in contact with the

hydrophobic cavity so they are readily substituted for appropriately sized guest molecules that are less polar than water (Szejtli and Szente, 2005).

For solid preparations, inclusion complexes with API can be pre-prepared using a number of processes which involve adding moisture, mixing the drug with the cyclodextrin and then drying to form a taste-masked powder. Examples include example kneading, co-crystallisation or spray-drying (Szejtli and Szente, 2005). However, in aqueous solution no preparation is usually needed and the rate of inclusion complexation is typically a very rapid process (10^{-2}-10^{-8} s mol^{-1}) with equilibrium (between free drug, cyclodextrin and complexes) usually establishing almost instantaneously (Szejtli and Szente, 2005). Dissociation typically overrides new complex formation when there is an increase in the number of water molecules in the environment, such as in the body upon ingestion, and so the two components separate and the cyclodextrin is once again in its free form (Del Valle, 2004).

The extent of complex formation at equilibrium is determined by 1) the temperature, dissociation is increased by raising the temperature because the formation of inclusion complexes is exothermic (Arima, Higashi and Motoyama, 2012) 2) the host: guest ratio 3) the complex association constant (Szejtli and Szente, 2005). The association constant, K_{ass}, denotes the binding affinity for a cyclodextrin to a given compound. It can be determined by observed changes in physicochemical properties. For example, changes in guest solubility as a function of cyclodextrin concentration can be used to estimate the stability constant using the equation K_{ass} = slope/(S_0·[1 - slope]) where S_0 is the solubility of the drug (Arima, Higashi and Motoyama, 2012).

K_{ass} is governed by the proportion of free guest and cyclodextrin molecules compared with the complexes (Coupland and Hayes, 2014):

$$K_{ass} = \frac{[complex]}{[cyclodextrin]\,[guest]}$$

The stability of the cyclodextrin-drug complex has been theorised to be due to a number interactions including hydrophobic and Van der Waals (Abou-Hamdan *et al.*, 2000). It is known that complex stability can be aided by the release of cyclodextrin strain energy; the macrocyclic conformation of a cyclodextrin molecule, when not part of an inclusion complex, is of a higher energy state compared to when involved in an inclusion complex (Manor and Saenger, 1974). As part of this energy shift, a conformational change takes place to enable strong and specific binding to drugs (Bergeron *et al.*, 1977). It is possible that this shape change can produce an unfavourable situation where the head of the incorporated molecule slips out of the cyclodextrin. If this portion is the part which confers bitter taste then taste-masking will not be successful, no matter how strong the complex is (Abou-Hamdan *et al.*, 2000).

For taste-masking, the stability of the complex is important as free drugs in solution exert bitter taste. The larger the value of K_{ass} the more cyclodextrin that must be present to reduce bitterness of the drug. The association constant for most complexable drugs is between 0.01-10,000 M^{-1} (Szejtli and Szente, 2005) but even when K_{ass} is low (i.e. a strong binding affinity) there must be an excess of cyclodextrin to ensure they are not saturated (Coupland and Hayes, 2014).

Ideally, the binding affinity of the drug to the cyclodextrin would be higher than the affinity of the drug for the bitter-receptor (Arima, Higashi and Motoyama, 2012). If the affinity is not high enough for drug: cyclodextrin complexes to form, then free bitter molecules will endure to produce bitter taste (Coupland and Hayes, 2014)

2.1.2 Other proposed mechanism of cyclodextrin taste-masking

Cyclodextrins have also been shown to act on bitter receptors in the taste buds. Cyclodextrins are strongly hydrated on their outer surface and so do not get attached to the TAS2Rs, as hydrophobic bitter drugs do, instead they physically prevent activation by bitter API (Arima, Higashi and Motoyama, 2012).

Cyclodextrins are also thought to inhibit the function of bitter-receptors. Cyclodextrins have previously been shown to impair the function of P-gp in cell membranes by interacting with biomembrane constituents; in particular cholesterol and phospholipids (Arima *et al.*, 2001, 2004). Similarly, it is postulated that cyclodextrins can impair TAS2R function by extracting components from the adjacent membrane via their hydrophobic cavity (figure 2.2). This theory is further supported by evidence that G-protein coupled receptors, such as TAS2Rs, are located within lipid raft microdomains (Ohkubo and Nakahata, 2007) which contain high levels of cholesterol (Silvius, 2003). The interaction with membrane components is dependent on cavity size, for example β-cyclodextrins predominately releases cholesterol from the biomembrane whereas α-cyclodextrin interacts with phospholipids (Zidovetzki and Levitan, 2007).

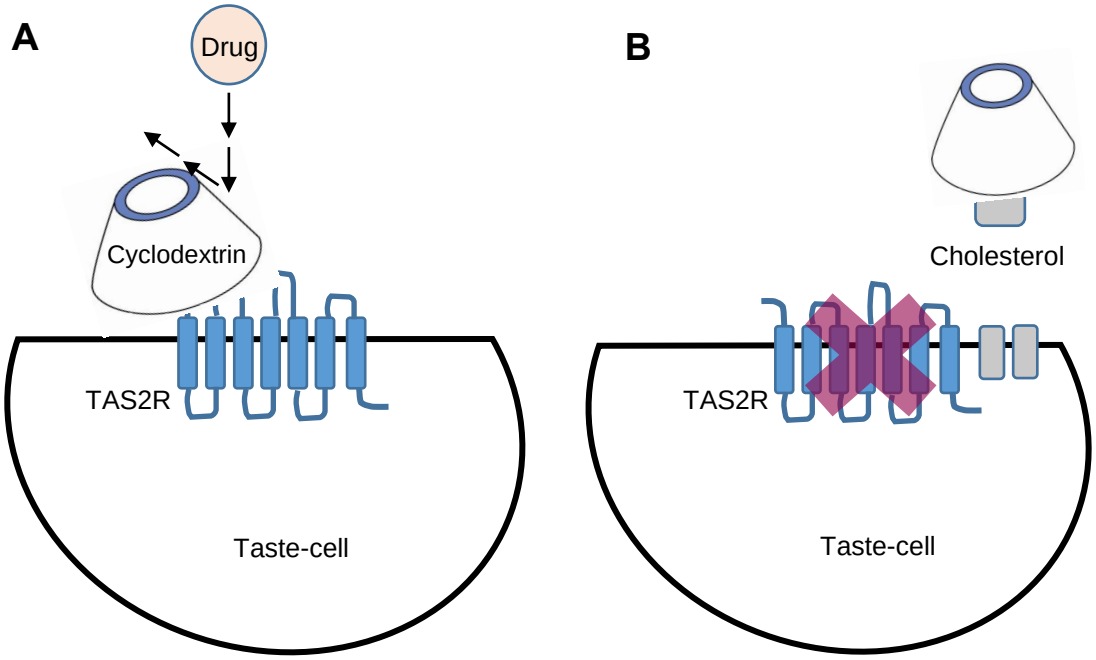


Figure 2.2 Cyclodextrins effect on bitter receptors. A) The cyclodextrin acting on the bitter receptor directly preventing activation by API. B) The cyclodextrin interacting with cholesterol from the cell membrane, disrupting bitter receptor function.

2.1.3 Maltodextrins as taste-masking excipients

Maltodextrins with high amylose content are marketed as having comparable taste-masking and solubility enhancing effects as cyclodextrins due to the amylose's ability to form inclusion complexes (Putseys, Lamberts and Delcour, 2010; Preis *et al.*, 2014). In water, amylose linear molecules easily form helical structures with an inner hydrophobic cavity (Juluri *et al.*, 2016) where a drug can reside as shown in chapter 1.

It has been demonstrated, using molecular modelling, that various complexes can form between maltodextrin helices and drug molecules. This includes interaction of drug with single, double and triple maltodextrin helices with varying degrees of stability; double helices in antiparallel formation were

found to be the most stable (Kalra, Bhat and Kaur, 2021). These interactions prevent the API from acting on TAS2Rs and eliciting bitter taste (figure 2.3).

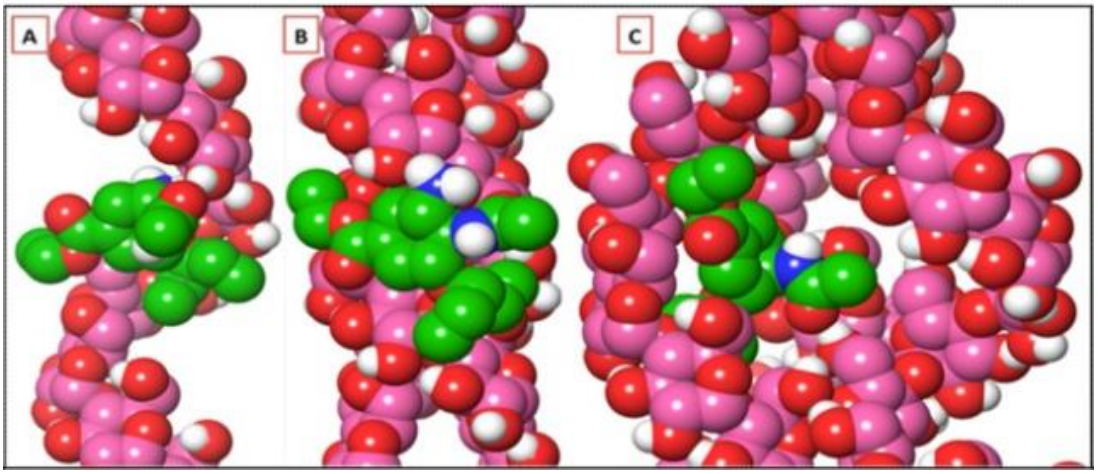


Figure 2.3 Complexes with maltodextrin helices (pink) and drug (green). A) single helix B) double helices C) triples helices. Taken from Kalra, Bhat and Kaur 2021

Maltodextrins could be advantageous for use in paediatric medicinal formulations, they are found in infant formula and have low toxicity even when consumed over long periods of time at high doses (Preis *et al.*, 2014). They are digested to simple glucose which is rapidly absorbed in the small intestine (Hofman, van Buul and Brouns, 2015), they have no limit to their ADI (EFSA, 2013) and have GRAS status (FDA, 2016).

2.1.4 KLEPTOSE Linecaps and Hydroxypropyl-β-cyclodextrin

KLEPTOSE Linecaps (DE 15-20) is a maltodextrin obtained from pea starch and is a promising maltodextrin for use in paediatric medicines (Preis *et al.*, 2014; Preis, Pein and Breitkreutz, 2021) due to the high amylose content which enable more drug interactions to occur.

The helices within KLEPTOSE Linecaps are potentially able to house more than one drug molecule depending on the length of the maltodextrin, which is variable according to the number of glucose monomers which join together.

Hydroxypropyl-β-cyclodextrin (HP- β-CD), a derivative of β-cyclodextrin has an improved safety and solubility profile compared with the parent cyclodextrin. β-cyclodextrin is GRAS with an ADI of 5mg/kg (Mortensen *et al.*, 2016) whereas 200mg/kg/day HP- β-CD has been shown to be safe even over long periods of time for children under two years old (EMA, 2017) and is metabolised by colon microflora (Szente and Szejtli, 2004).

Both HP- β-CD and KLEPTOSE Linecaps have shown taste-masking efficacy against bitter drugs in various models of palatability with examples shown in table 2.1.

Table 2.1 Examples of taste-masking by HP- β-CD and KLEPTOSE Linecaps. Molar ratios given as drug: dextrin. NSAIDs; non-steroidal anti-inflammatory, BATA; Brief Access Taste Aversion. *this work was carried out by the commercial supplier of KLEPTOSE Linecaps, Roquette.

	HP- β-CD	**KLEPTOSE Linecaps**	**Taste assessment tool**
NSAIDs*		0.009 – 0.04 % (w/v) KLEPTOSE Linecaps taste masked a range of NSAIDs when the drugs were in excess including Ketoprofen, Ibuprofen, Flurbiprofen and Naproxen. This was most effective with Ketoprofen even when the drug was in excess at 193:1 (Popescu *et al.*, 2012).	Astree E-tongue
Ranitidine	0.02 – 3.0 % (w/v) HP- β-CD taste masked from a ratio of 1:1 to 1:5 (Chay *et al.*, 2018) in a concentration dependant manner		Insent E-tongue
Dimenhydrinate	0.008 % (w/v) HP- β-CD did not improve the palatability at 1:1 within an oral dispersible film (Preis, Pein and Breitkreutz, 2021).	0.06 % (w/v) KLEPTOSE Linecaps Taste-masked at 1:1 within an oral dispersible film (Preis, Pein and Breitkreutz, 2021).	Insent E-tongue
Praziquantel	2.8 % (w/v) HP- β-CD Fully taste masked at 1:104 (Münster *et al.*, 2017).	25.3 % (w/v) KLEPTOSE Linecaps fully taste masked at 1:104 (Münster *et al.*, 2017).	BATA
Levocetrizine dihydrochloride	44.9 % (w/w) HP- β-CD improved the palatability of the drug at 1:10 within an oral dispersible tablet (Labib, 2015).		Human panel
Antihistamines	1.5 – 4.6 % (w/v) HP- β-CD dose dependently taste masked a range of antihistamines in solution including Cetirizine, Chloropheniramine, Hydroxyzine and Diphenydramine in a dose dependent manner from 1:2 (Ono *et al.*, 2011).		Human Panel

2.1.5 Limitations of using the e-tongue to assess the taste-masking of dextrins

Chay et al highlighted an important limitation of using the e-tongue to assess cyclodextrin containing formulations: anomalies were seen in bitter-sensor output when presented with control dextrin (no drug present) resulting in no clear concentration response (Chay *et al.*, 2018). The authors cited the neutral charge of HP- β-CD to be the probable cause. Perhaps also the cyclodextrin was interacting with the lipid membrane sensor of the Insent e-tongue as β-CD and its derivatives are known to interact with phospholipids and/or cholesterol (Arima, Higashi and Motoyama, 2012).

This finding casts doubt on the suitability of the e-tongue as a preclinical model for dextrin containing formulations.

2.1.6 Solubility testing as a predictor of dextrin taste-masking

The association constant for a cyclodextrin or maltodextrin with a drug is an important factor when predicting taste-masking ability (Arima, Higashi and Motoyama, 2012; Coupland and Hayes, 2014; Kalra, Bhat and Kaur, 2021). This is because it is the free drug molecules (not involved in inclusion complexes) which elicit bitter-taste. Therefore, it is logical to use phase solubility testing as a predictor of taste-masking; increased inclusion complexes results in both increased API solubility and increased shielding from bitter-receptors (Münster *et al.*, 2017). In practise however, solubility testing was not found to be fully predictive of in vivo taste-masking by HP- β-CD and KLEPTOSE Linecaps. Münster et al 2017, using Praziquantel as the model bitter drug, found in the BATA, both KLEPTOSE Linecaps and HP- β-CD significantly taste-masked the drug at 0.06mg/mL, the IC50 level (1:104 drug: dextrin molar ratio). This work also found both 2.8% (w/v) cyclodextrin

and 25% (w/v) maltodextrin to be as palatable as water. However, the phase solubility study results did not fully correlate with the in vivo findings. The solubility study showed 2.8% (w/v) HP- β-CD to increase the solubility of Praziquantel 5-fold to 1.3mg/mL but in vivo taste-masking was not achieved at this level, likely due to excess free drug molecules in solution. Also KLEPTOSE LINECAPS did not improve the solubility of the drug in the in vitro phase solubility test. Based on this, a high taste-masking ability was predicted for the cyclodextrin compared with limited taste-masking predicted for the maltodextrin, this was not the case.

It is likely that the maltodextrin was offering some taste-masking independent of complexation, the mechanisms for this are not entirely clear. Perhaps non-inclusion complexes are forming where API interacts on the surface of the maltodextrin and so are only partly shielded from receptors (Loftsson, Hreinsdóttir and Másson, 2007; Raffaini and Ganazzoli, 2020). To fully understand the taste-masking potential of these compounds it is necessary to use an in vivo whole body system.

2.1.7 The need for non-human modelling

It is necessary to evaluate formulations for their taste as early as possible during drug development to save attrition and costs later down the line. As discussed in chapter 1, it is not appropriate to use humans at this early stage due to both ethical and toxicological reasons. Therefore accurate non-human assessment tools are required (Pein *et al.*, 2014). The BATA model is a recognised preclinical tool (Clapham *et al.*, 2021) and has been used successfully to predict the palatability of API (Soto *et al.*, 2018). More work needs to be done to assess the translatability when excipients, such as

dextrins, are present in the formulation. In order to accurately use the BATA model to assess formulations containing dextrins, it must be validated for use by evaluating its translatability to humans.

2.1.8 Assessing palatability in humans

Using humans to assess the efficacy of a taste-masking strategy is gold standard and in order to assess the translatability of the BATA with cyclodextrins and maltodextrins, the human response must first be evaluated. KLEPTOSE Linecaps and Hydroxypropyl-β-cyclodextrin have a well-established safety profile so can be assessed alongside model drugs using humans. Understanding the correlation with known drugs will indicate the predictability of the BATA and therefore determine how the BATA can be used going forward to assess these dextrins within novel drug formulations.

2.1.8.1 Taste evaluation in children

It was not practical to use children in this work due to the methodological limitations that would ensue. Children have a limited attention span and show a loss of concentration and taste-fatigue which is problematic when multiple formulations need assessing (Davies and Tuleu, 2008). For this reason, it is recommended by the EMA that children only taste-test four samples as a maximum (EMA, 2006). Therefore, this work was completed in adult participants.

2.1.9 Structuring a human panel

There are a number of ways to structure a human panel depending on the desired outcome. Regardless of the method chosen to evaluate the samples,

a number of factors should be considered during study design (Lawless and Heymann, 2010) as summarised in table 2.2.

Table 2.2 Summary of factors to consider when carrying out a sensory assessment in humans. Adapted from chapter 3 of 'Sensory evaluation of Food' (Lawless and Heymann, 2010).

Local research governance policies - Research ethics committee (REC) application and approval
Test objective - Test type
Panellist - Recruitment with informed consent - Training if necessary - Incentives
Sample - Volume if liquid, size and shape if solid - Number of samples and timings for presentation - Serving temperature
Test setup - Instructions - Scoring system; types of scales and anchor words - Coding of samples and randomisation of order of samples - General items; palate cleansers, spit cups, tissues, water for rinsing
Test area - Separation of participants - Temperature - Accessibility

Before deciding on the sensory test to use to assess samples, it is necessary to know what the objectives are in the investigation. Is it important to know if there is a general difference amongst the products or is it important to assess a specific attribute and gauge the magnitude of it? The answer to this question will inform if a discrimination, affective or descriptive test is appropriate.

A discrimination test (also known as a difference test) asks 'does a sensory difference exist between my samples?'. Such tests can identify detectable differences between samples being compared in the same session. Simple

discrimination testing is useful to see if a change in a formulation leads to a perceived difference by participants (O'Mahony and Rousseau, 2003).

Affective testing asks 'what sample is most acceptable or most preferred?' and is useful for preliminary investigations. This type of testing quantifies the degree the panel likes or dislikes the product. One option here is to offer alternative products and see which is preferred by the majority. The magnitude of liking/disliking is not measured (Gillette, 1990).

Descriptive testing asks 'what is the nature of the difference between my samples?' This sensory test approach quantifies the perceived intensities of the specified attribute (e.g. bitterness) of a product (Stone, Bleibaum and Thomas, 2012). Scaling is at the core of descriptive analysis and involves sensing a product and giving a response that reflects the intensity of one or more sensations (McEwan and Lyon, 2003). If the scaling is simple and the panellists are familiar with the attribute they are asked to scale or if this attribute is easily perceived, for example bitterness, then training is not necessary. When asking for a more complex sensory attribute to be assessed, e.g. astringency, then training may be necessary and to calibrate with reference samples.

Scaling is most commonly nominal, ordinal or interval. Nominal scaling uses numbers as labels, these numbers do not reflect any particular order and the only valid comparison between individual items is to say if they belong to the same category or not. In ordinal scaling, numbers are used to rank a product with regard to some sensory attribute or preference so increasing numbers means increasing e.g. bitterness (McEwan and Lyon, 2003). In this case the

relative differences among the products are not known; it is unknown how close the product placed in second and third were in the assessed attribute. Alternatively, scaling can be used whereby the spacing of the responses is equal, so the numbers represent equal degrees of differences, this is interval-level measurement. An example of this is the 9-point hedonic scale (Peryam and Girardot, 1952) with terms from ‘like extremely’ down to ‘dislike extremely’. Labels on the scale are important as different people may interpret them differently even though they perceive the product in the same manner. This method can be used to rank variations of a product from most to least preferred. Extensions of the 9-point hedonic scale have been produced to accommodate for children (Popper and Kroll, 2011); altering the anchor words to phrases such as ‘super good’ or ‘super bad’ or for young children, anchoring with smiley faces. These can be simplified further, to basic 3-point scales for children as young as 3 years old to understand (Chen, Resurreccion and Paguio, 1996)

2.2 Aims and objectives

The aim of this work was to first identify if a cyclodextrin and a maltodextrin, namely HP-β-CD and KLEPTOSE Linecaps, could efficiently taste-mask two model bitter drugs in humans. The second aim of this work was to examine the correlation of results generated in a preclinical taste assessment model, the BATA model, and in the human panel. This would highlight if the BATA model could be used in the future to assess HP-β-CD and KLEPTOSE Linecaps as a taste-masking strategy early on in research and development.

2.3 Materials and methods

2.3.1 Materials for BATA model and human panel

Ranitidine hydrochloride and Sildenafil citrate were purchased from Fagron (Rotterdam, The Netherlands). For the human panel KLEPTOSE HPB oral grade (HP-β-CD with average degree of molecular substitution of 0.62) was obtained from Roquette (Lestrem, France), for the BATA work non-human grade 2-hydroxypropyl-beta-cyclodextrin was purchased from Sigma-Aldrich (St Louis, Missouri, USA). KLEPTOSE Linecaps 17 (DE 17) used in both the BATA and human work was purchased from Roquette (Lestrem, France).

2.3.2 Model drug choice

A number of drugs, including Sildenafil citrate and Ranitidine hydrochloride (HCL), have been previously investigated using the rat BATA model (Devantier *et al.*, 2008; Clapham *et al.*, 2012; Mohamed-Ahmed *et al.*, 2016; Tiwari *et al.*, 2017; Soto *et al.*, 2018). These drugs have different characteristics (table 2.3) which is likely to alter how effectively they can be taste-masked and so are interesting to compare.

Table 2.3 Comparing Ranitidine hydrochloride and Sildenafil citrate's characteristics. IC50 value represents the concentration which inhibits 50% of the maximum lick number in the rat BATA model. EC50 value represents the concentration that reduces the maximum human response on a visual analogue scale by half. BCS; Biopharmaceutical Classification System

	Ranitidine hydrochloride	**Sildenafil citrate**
Therapeutic strength of commercial product	15mg/mL (EMC, 2017)	10mg/mL (suspension) (EMC, 2021)
Water solubility	660mg/mL (Korteja¨rvi *et al.*, 2005)	4.1mg/mL (Ouranidis *et al.*, 2021)
BCS class	III	I/II
IC50	1.41mg/mL(Soto *et al.*, 2018)	1.06mg/mL (Soto *et al.*, 2018)
EC50	0.55mg/mL (Soto *et al.*, 2018)	1.05mg/mL (Soto *et al.*, 2018)
Therapeutic Dose (PO)	Infant (under 6 months): 1-3mg/kg three times a day Child (6 months up to 2 years): 2-4 mg/kg twice daily Child (3-11 years): 2-4mg/kg twice daily (max. per dose of 150mg) Child (12-17 years) and adult: 300mg/day	Infant (under 1 year): 0.5-1mg/kg every 4-6 hours Child (1 year and older up to 20kg): 10mg every 4-6 hours Child (over 20kg): 20mg every 4-6 hours Adult: 60mg three times a day for pulmonary arterial hypertension

2.3.3 Rationale for concentration of HP-β-CD and KLEPTOSE Linecaps

When evaluating dextrins for taste-masking, one approach is to use a specific molar ratio of drug:dextrin depending on how inclusion complexes form between them (if known). For example many inclusion complexes follow a 1:1 (cyclodextrin: guest) stoichiometry (Cid-Samamed *et al.*, 2022). However, as previously eluded to, it is possible that dextrins offer taste-masking independent of complexation – something which may only be seen with an excess of dextrin. Therefore, in this work a high, fixed, level of 25% (w/v) was chosen to ensure an excess molar ratio to drug was established

Previous work (internal, unpublished) evaluated the solubility enhancement of a range of concentrations of HP-β-CD and KLEPTOSE Linecaps, from 5 – 50 % (w/v), on a model drug (Ibuprofen). This work found 50% (w/v) was very

sticky and was difficult to mix with API and so would have limited use from a manufacturing perspective. From the other concentrations evaluated, 25% (w/v) was shown to produce the greatest solubility enhancement with both HP-β-CD and KLEPTOSE Linecaps vehicles. In order to achieve maximum solubility enhancement, and therefore potentially achieve taste-masking of other drugs, 25% (w/v) was the concentration chosen for further assessment. Furthermore, 25% (w/v) of both HP-β-CD and KLEPTOSE Linecaps enhanced the solubility of Sildenafil citrate (compared to the intrinsic water solubility), this was especially significant with the cyclodextrin. Neither vehicle improved the solubility of Ranitidine as it was already highly soluble in water. The intention here was to assess if these dextrins could improve the palatability of a highly soluble API.

2.3.4 Human panel methods

2.3.4.1 Drugs

The concentrations assessed of Ranitidine hydrochloride were based on the EC50 and the therapeutic dose. The concentrations of Sildenafil citrate were based on the EC50 and upper solubility (the therapeutic dose is a suspension and only soluble components of drug can contribute to bitter taste (Szejtli and Szente, 2005)).

2.3.4.2 Solution preparation: dextrin vehicles

A 25% (w/v) solution of HP-β-CD in aqueous vehicle was prepared in advance of test sessions by dissolving 750g of HP-β-CD in 3L of deionised water. A 25% (w/v) solution of KLEPTOSE Linecaps in aqueous vehicle was prepared in advance of test sessions by dissolving 750g of KLEPTOSE Linecaps in 3L

of deionised water. Solutions were stored in the fridge (2-8 °C) and used within 1 week.

2.3.4.3 Solution preparation: model compounds

Sildenafil citrate and Ranitidine hydrochloride solutions in dextrin vehicles were freshly prepared for each test session as in Table 2.4. The required amount of model compound was accurately weighed and added to approximately 60 % of the desired volume of vehicle, the solution was sonicated until complete dissolution of the drug, then made up to volume. Solutions were stored in the fridge (2-8 °C) and used at room temperature within 24 hours.

Table 2.4 Summary of the formulations assessed in the human panel in 25% (w/v) cyclodextrin and maltodextrin vehicles

Model compound	**25% (w/v) HP- β-CD**		**25% (w/v) KLEPTOSE Linecaps**	
	Concentration (mg/mL)		**Concentration (mg/mL)**	
Sildenafil citrate	1	3.5	1	3.5
Ranitidine hydrochloride	0.55	15	0.55	15

2.3.4.4 Participants

Twenty-four healthy volunteers gender balanced between the ages of 18 and 35 years old were enrolled in a randomised single-blind study (table 2.5). The protocol was approved by the UCL Research Ethics Committee (REC) (ID: 4612/020) and the study was conducted in a designated room with a dispensary at the UCL School of Pharmacy. Participants included in the study were able to understand and speak English and had declared they had no problems with sense of taste or smell and had not received dental care or

taken medications (other than contraceptives or over the counter medicines) for 15 days before the sessions. The also declared they had no known allergies to Sildenafil, Ranitidine or the dextrins.

Table 2.5 Summary of the human panel participants age in years

	Male (n=12)	**Female (n=12)**
Oldest	35	30
Youngest	20	18
Mean	24.4	21.8
Median	23	21.5

Each volunteer attended two sessions, one assessing Ranitidine hydrochloride and one assessing Sildenafil citrate. Each sample was given a three letter code to blind the participants to the samples. The participants received a gratification for participation of £20 per session.

2.3.4.5 Study design

The 'swirl and spit' methodology was used, with the participants assessing 10mL of each test solution. The participants were instructed to swirl it in their mouth for 10 seconds, before spitting it out. Once the participant had spat out the sample they rated the palatability using a 100mm visual analogue scale (VAS) on a computer using Qualtrics software (Provo, Utah, USA; version: November 2017). This scale ranged from 'not aversive' (our neutral target) to extremely aversive'; the higher the score the more unpalatable the formulation is (figure 2.4)

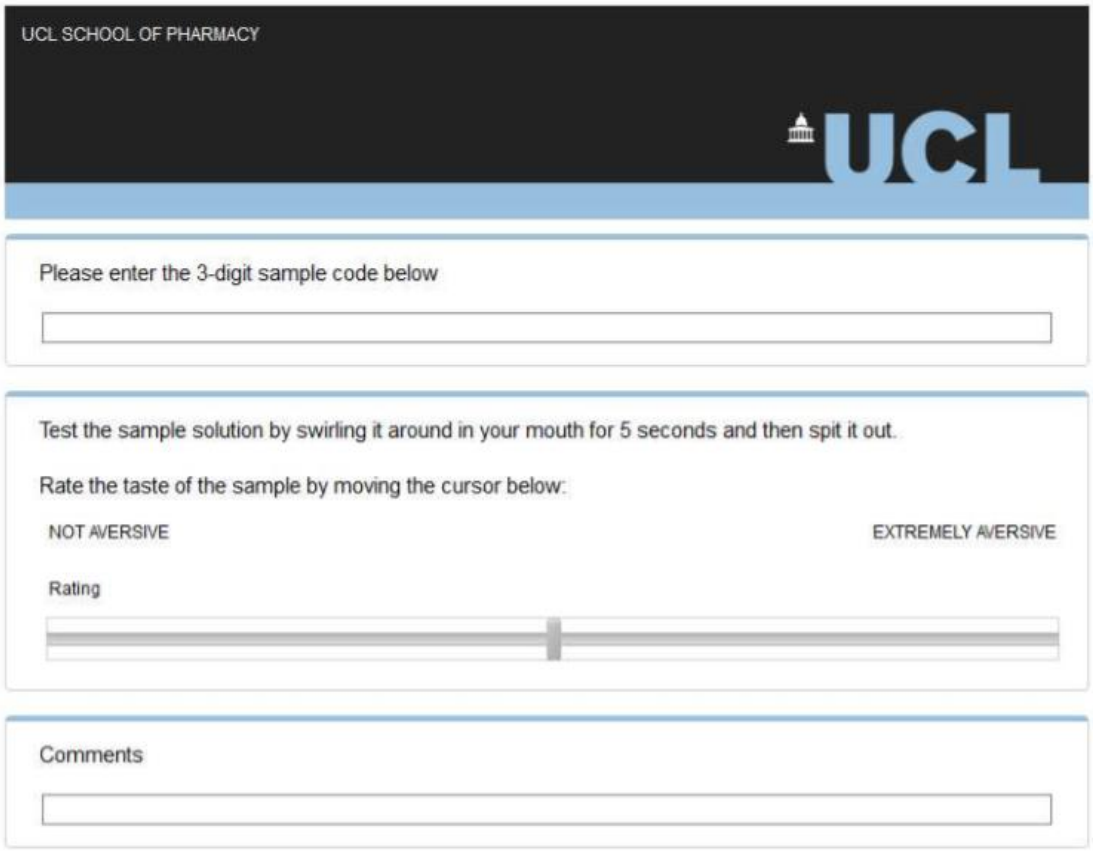


Figure 2.4 The participants entered their rating of each coded sample using the VAS

Each presentation was separated by a 10-minute washout period to ensure no after-taste was lingering. During this inter-presentation interval, participants were able to consume a plain, non-salty cracker in order to neutralise their palate.

Ranitidine and Sildenafil were assessed alongside 25% (w/v) cyclodextrin or maltodextrin. The drug solutions were tested on different days at two concentrations; 0.55 and 15mg/mL Ranitidine and 1 and 3.5mg/mL Sildenafil. In the first session for each group, both of the dextrins were given alone as a placebo, in the second session the participants did not receive maltodextrin

alone due to limited supply. The solutions were labelled with a random 3-digit code and were presented in duplicate at random.

2.3.4.6 Data analysis

All data were imported into Microsoft Excel 2016 (©Microsoft, Redmond, Washington) to arrange before transferring to R software version 3.5.0 where statistics and graphs were produced with significance set as $p<0.05$.

Results were displayed as notched box-plots showing the median and interquartile ranges, with whiskers that represent 1.5 times the 25th and 75th percentile. The notches are indicative of the 95% confidence interval of the median. Outliers are shown as circles (figure 2.5).

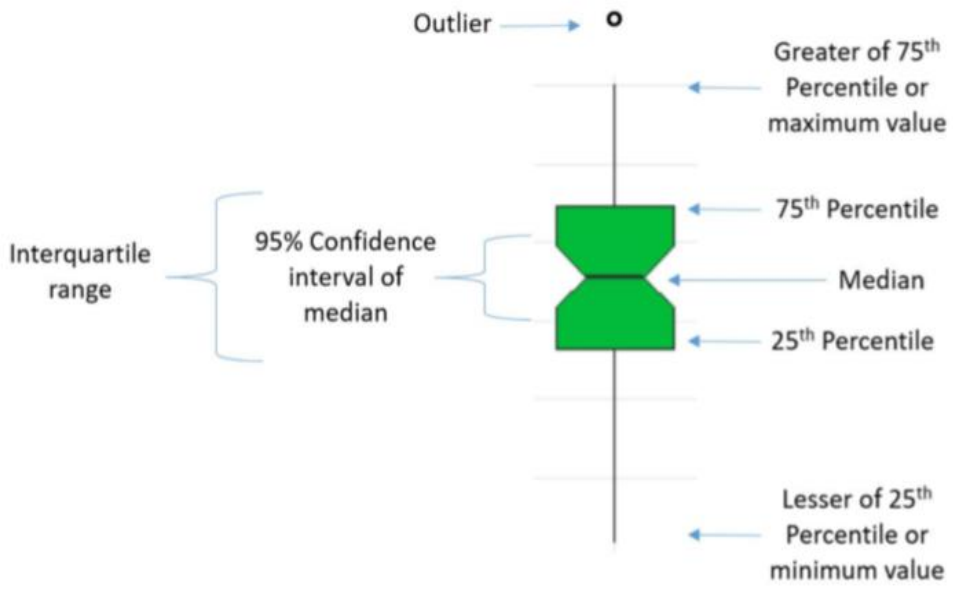


Figure 2.5 The components of a notched box-plot

The normality of the data distribution was identified using the Shapiro-Wilk test. If this test highlighted the data did not follow parametric distribution, then

Kruskal-Wallis rank sum test with Gao post-hoc analysis was used to compare differences in data sets (Gao *et al.*, 2008). If the data distribution was normal, the one-way analysis of variance was performed with Tukey's post-hoc analysis.

2.3.5 BATA model experiments

The BATA assessment was carried out and graphs were produced by colleagues at UCL.

2.3.5.1 Solution preparation: Quinine hydrochloride dihydrate

A 0.08mM solution of Quinine hydrochloride dihydrate in water was freshly prepared daily and used as a bitter control – this is the IC50 concentration. This solution was prepared by dissolving 317.53 mg of Quinine hydrochloride dihydrate (396.912 g/mol) in 1L of deionised water, then diluting by a factor of x10 with deionised water.

2.3.5.2 Solution preparation: dextrin vehicles

A 25% (w/v) solution of HP-β-CD in aqueous vehicle was prepared in advance of test sessions by dissolving 250g HP-β-CD in 1 L of deionised water. A 25% (w/v) solution of KLEPTOSE Linecaps in aqueous vehicle was prepared in advance of test sessions by dissolving 250g KLEPTOSE Linecaps in 1 L of deionised water. Solutions were stored in the fridge (2-8 °C) and used at room temperature within 1 week.

2.3.5.3 Solution preparation: model compounds

Sildenafil citrate and Ranitidine hydrochloride solutions in dextrin vehicles were freshly prepared for each test session (table 2.6). The required amount of model compound was accurately weighed and added to approximately 60%

of the desired volume of vehicle, the solution was sonicated until complete dissolution of the drug, then made up to volume. Solutions were stored in the fridge (2-8 °C) and used within 24 hours.

Table 2.6 Summary of the formulations assessed in the BATA model in 25% (w/v) cyclodextrin and maltodextrin vehicles

Model compound	**25% (w/v) HP-β-CD**			**25% (w/v) KLEPTOSE Linecaps**		
	Concentration (mg/mL)			**Concentration (mg/mL)**		
Sildenafil citrate	1	3.5	5	1	3.5	
Ranitidine hydrochloride	0.14	1.4	14	0.14	1.4	14

Sildenafil citrate in HP-β-CD or KLEPTOSE Linecaps was presented to the rats at the IC50 concentration (1mg/mL), at the upper solubility limit (3.5mg/mL) and, for HP-β-CD above the solubility limit (5mg/mL) to represent the solubility enhancement offered by the cyclodextrin.

Ranitidine hydrochloride in HP-β-CD or KLEPTOSE Linecaps was presented to the rats at the IC50 concentration (1.41mg/ml) and at a log lower (0.14mg/ml) and a log higher concentration (14mg/mL) representing the upper end of its solubility and being close to the commercial 150mg/10ml oral solution.

2.3.5.4 Animals

10 Male Sprague–Dawley rats (22 weeks old, Charles-River, Kent, UK) were housed in pairs in standard cages in a room maintained at 21±2 °C with 55±10% humidity and with a 12:12 h light/dark cycle. All training and testing occurred during the light phase. Animals had free access to chow (Harlan,

Oxon, UK) and tap water, except for training/testing periods where a water-restriction schedule occurred. Daily food and water consumption were monitored throughout the experiment and, as a welfare measure, it was checked that their weight did not drop below 85% of their free feeding weight. All procedures were carried out in accordance with Animals (Scientific Procedures) Act (ASPA) 1986 (Project Licence (PPL) number 70/7668).

2.3.5.5 BATA study design

This work was conducted over two separate BATA studies, one assessing cyclodextrin alongside the two drugs and one assessing maltodextin alongside the two drugs, with a rest week in between. The commercially available “Davis MS-160” lickometer from DiLog Instruments (Tallahassee, Florida, USA) was used for these experiments.

The first BATA week involved a four-day study, with the animals undergoing one Davis rig session each day. The first two days represent training days. For the second BATA week the animals did not repeat training day 1 as they were familiar with the equipment so this was a three-day study.

During training day one, the animals were placed into the lickometer and the shutter remained open so they had free access to one tube of deionised water over the session. For training day two, all 16 tubes contained deionised water but the shutter opened and closed and the rig moved to present different tubes to the animals. The aim of this was to familiarise them with the noise and movement of the equipment. For the two subsequent test days the sipper tubes contained either deionised water, Quinine control or one of the test solutions. During the second training day and both test days, every tube was

presented for 8-seconds after the animal takes his first lick. Then the shutter closed and the rig moved and a different tube, containing water, was presented – this remained for two seconds to act as a rinse in between solutions. The shutter then closed and another solution was presented.

For test days, solutions were allocated to bottles at random, except for deionised water as the negative control which was always in bottle 3 and presented first so the rats were motivated to continue, and the water rinse which was always in bottle 8. Each concentration was presented 4 times per session (2 bottles per test solution, 2 presentations per bottle).

Each rat was water-deprived for 22 hours before each session and was then placed in the lickometer for a maximum of 40 minutes. After each session, the rodents received tap water for rehydration for 1hr 20minutes before the next water deprivation, if any.

2.3.5.6 Data analysis

Results were displayed as before, using notched box-plots with the same post-hoc analysis.

2.4 Results and discussion

2.4.1 Human panel results: Ranitidine hydrochloride

The panel assessed two concentrations of Ranitidine, 0.55mg/mL (the formerly established human EC50 (Soto *et al.*, 2018)) and 15mg/mL (commercial product strength), alongside 25% (w/v) HP-β-CD or KLEPTOSE Linecaps. There was no significant difference between 0.55mg/mL Ranitidine + HP-β-CD or 0.55mg/mL Ranitidine + KLEPTOSE Linecaps compared to the

corresponding placebo (p=0.55 and p=0.1109), suggesting both successfully taste-masked this concentration of Ranitidine. Neither HP-β-CD nor KLEPTOSE Linecaps successfully taste-masked the higher concentration of Ranitidine as these were statistically different from their corresponding placebo.

The dextrins themselves, when given alone as placebos, had statistically different acceptability (p=0.0153) with HP-β-CD achieving a mean aversiveness rating of almost 0, however, both were very well tolerated. There was a significant difference in palatability between the KLEPTOSE Linecaps and HP-β-CD containing solutions at both concentrations of Ranitidine (0.55 and 15mg/mL). In each case the HP-β-CD containing formulation was regarded as tasting better (figure 2.6)

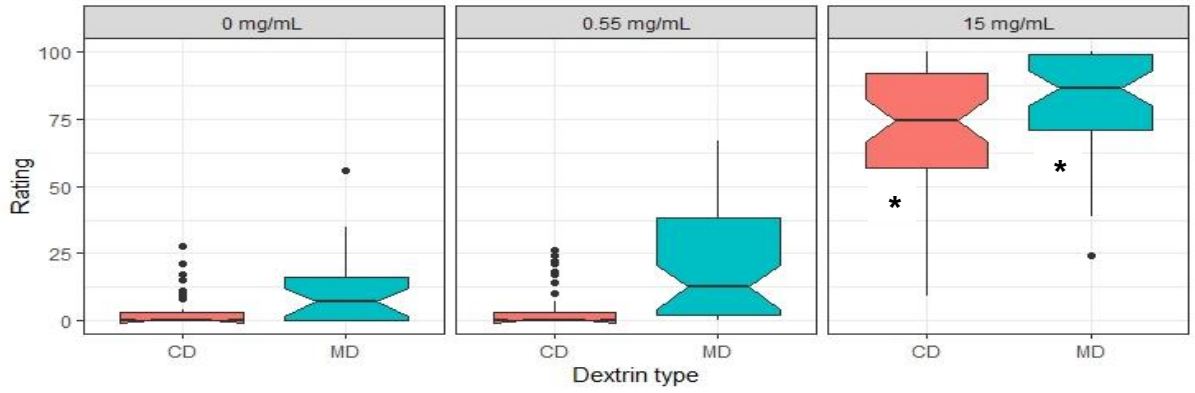


Figure 2.6 Aversiveness rating responses to 0, 0.55 and 15mg/mL Ranitidine hydrochloride by dextrin type, 25% (w/v) HP- β-CD (CD) and KLEPTOSE Linecaps (MD). * Denotes statistical difference from respective dextrin control.

The results were facetted by gender (figure 2.7). There was no gender difference in responses to any of the Ranitidine concentrations.

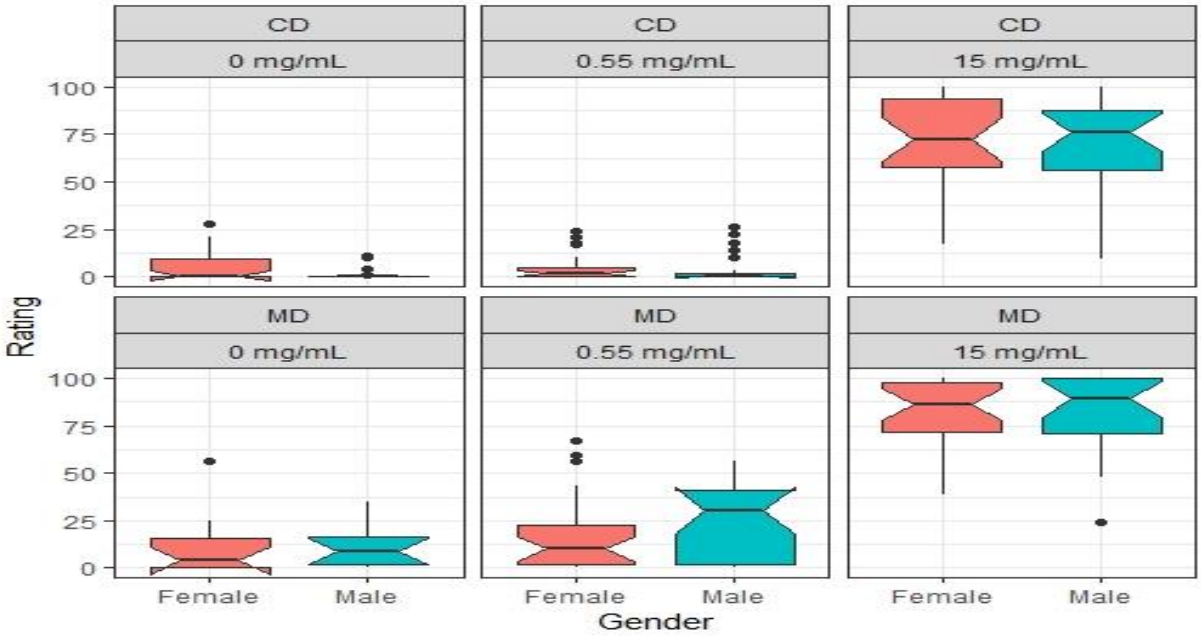


Figure 2.7 Aversiveness rating responses to 0, 0.55 and 15mg/mL Ranitidine hydrochloride by dextrin type, 25% (w/v) HP- β-CD (CD) and KLEPTOSE Linecaps (MD), according to gender.

2.4.2 Human panel results: Sildenafil citrate

The panel assessed two concentrations of Sildenafil, 1mg/mL (the human EC50) and 3.5mg/mL (upper solubility limit), alongside 25% (w/v) HP-β-CD and KLEPTOSE Linecaps (figure 2.8). Both 1mg/mL and 3.5mg/mL Sildenafil alongside HP-β-CD were significantly different from HP-β-CD alone. This was also the case with the KLEPTOSE Linecaps containing formulations when compared to KLEPTOSE Linecaps alone. This suggests taste-masking was not fully achieved by either dextrin at these concentrations. However, HP-β-CD improved the mean rating of 1mg/mL Sildenafil from approximately 50 (as this is the EC50 concentration) to around 20.2 and this was statistically comparable to the KLEPTOSE Linecaps placebo (p=0.1326). Furthermore, the rating of 3.5mg/mL Sildenafil + HP-β-CD was 48.5 which was better than expected at this saturated concentration and was statistically comparable to

1mg/mL Sildenafil + KLEPTOSE Linecaps (p=0.7042). This suggested the HP-β-CD containing formulations had a better palatability.

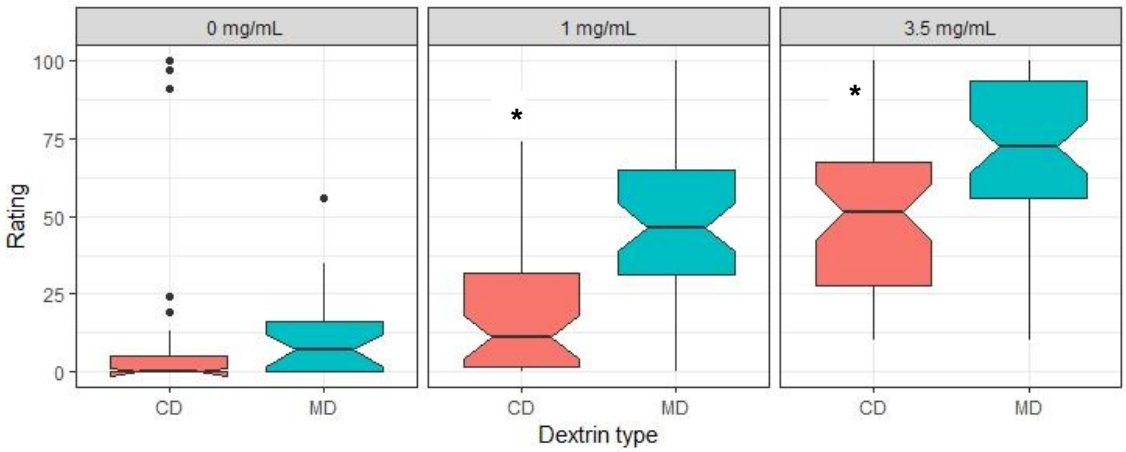


Figure 2.8 Aversiveness rating responses to 0, 1 and 3.5mg/mL Sildenafil citrate by dextrin type, 25% (w/v) HP- β-CD (CD) and KLEPTOSE Linecaps (MD). KLEPTOSE Linecaps (MD) alone data is from previous session's results. *Denotes statistical difference from alternate dextrin solution

The results were facetted by gender (figure 2.9). There was no gender difference in responses to any of the Sildenafil concentrations.

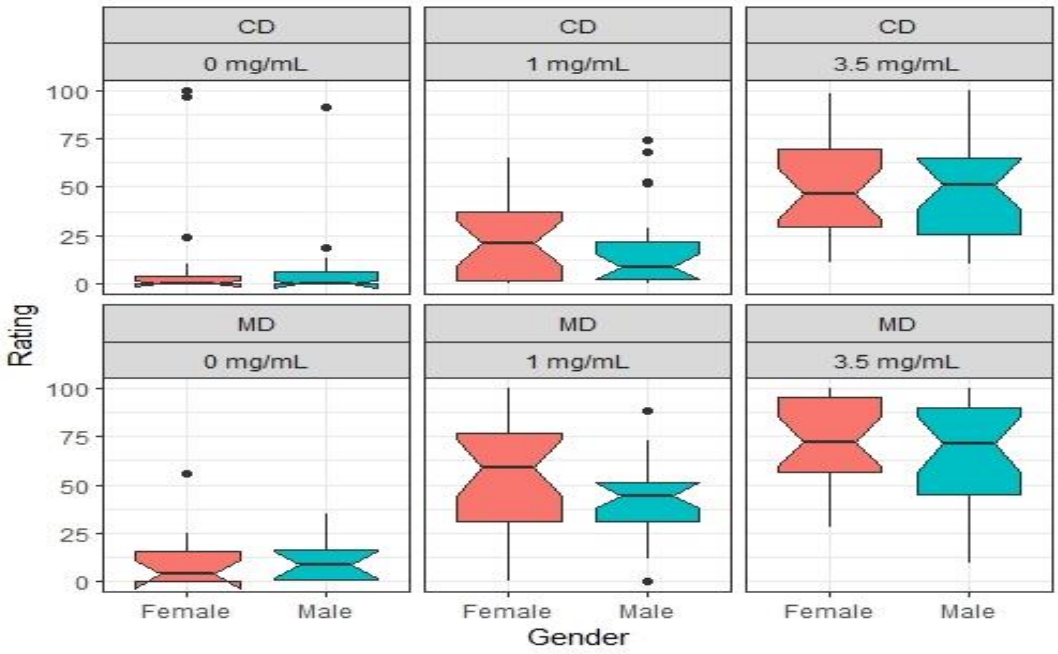


Figure 2.9 Aversiveness rating responses to 0, 1 and 3.5mg/mL Sildenafil citrate by dextrin type, 25% (w/v) HP- β-CD (CD) and KLEPTOSE Linecaps (MD) according to gender.

2.4.3 Human panel summary

The human panel showed that both 25% (w/v) HP-β-CD and 25% (w/v) KLEPTOSE Linecaps, successfully taste masked 0.55mg/mL Ranitidine HCL. At this concentration, both dextrin containing samples had no significant difference in score on the visual analogue scale compared to the respective dextrin control ($p<0.05$). Table 2.7 summarises the human panel results.

Table 2.7 Summary of human panel results, * denotes successful taste-masking

Compound	25% (w/v) HP-β-cyclodextrin		25% (w/v) KLEPTOSE LINECAPS	
Sildenafil citrate	1mg/mL	3.5 mg/mL	1 mg/mL	3.5 mg/mL
Ranitidine HCL	*0.55mg/mL	15 mg/mL	*0.55mg/mL	15 mg/mL

2.4.4 BATA results: Ranitidine hydrochloride

The BATA platform assessed three concentrations of Ranitidine, 0.14, 1.4 (IC50), 14 mg/mL (upper solubility limit) alongside 25% (w/v) HP-β-CD or KLEPTOSE Linecaps (figure 2.10). There was no significant difference between 0.14 mg/mL Ranitidine + KLEPTOSE Linecaps compared to placebo control suggesting this concentration has been successfully taste-masked. HP-β-CD did not taste-mask any concentration of Ranitidine as these were statistically different from their dextrin controls.

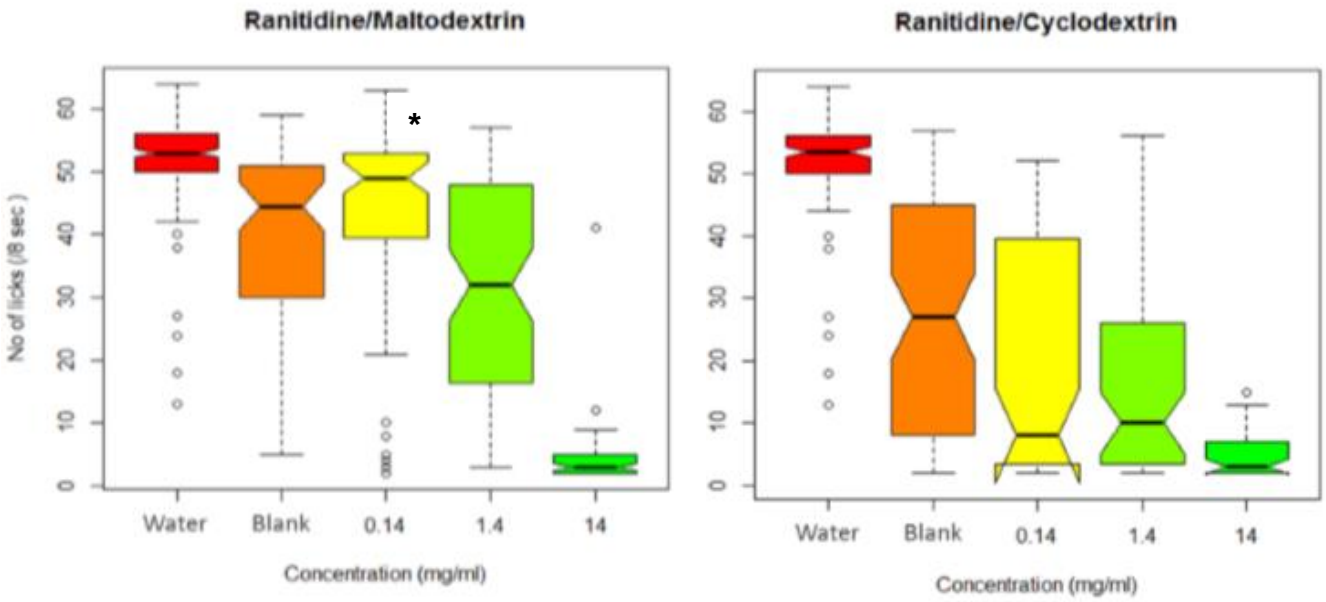


Figure 2.10 Lick responses to 0.14, 1.4 and 14 mg/mL Ranitidine hydrochloride with 25% (w/v) HP- β-CD (Cyclodextrin) and KLEPTOSE Linecaps (maltodextrin). *Denotes no statistical difference from respective dextrin control

2.4.5 BATA results; Sildenafil citrate

The BATA platform assessed three concentrations of Sildenafil citrate with 25% (w/v) HP-β-CD, 1 (IC50), 3.5 (upper solubility) and 5 mg/mL and

assessed two concentrations, 1 and 3.5 mg/mL, alongside 25% (w/v) KLEPTOSE Linecaps (figure 2.11). There was no significant difference between 1 mg/mL Sildenafil + KLEPTOSE Linecaps compared to placebo control suggesting this concentration has been successfully taste-masked. HP-β-CD did not taste-mask any concentration of Sildenafil as these were statistically different from their dextrin controls.

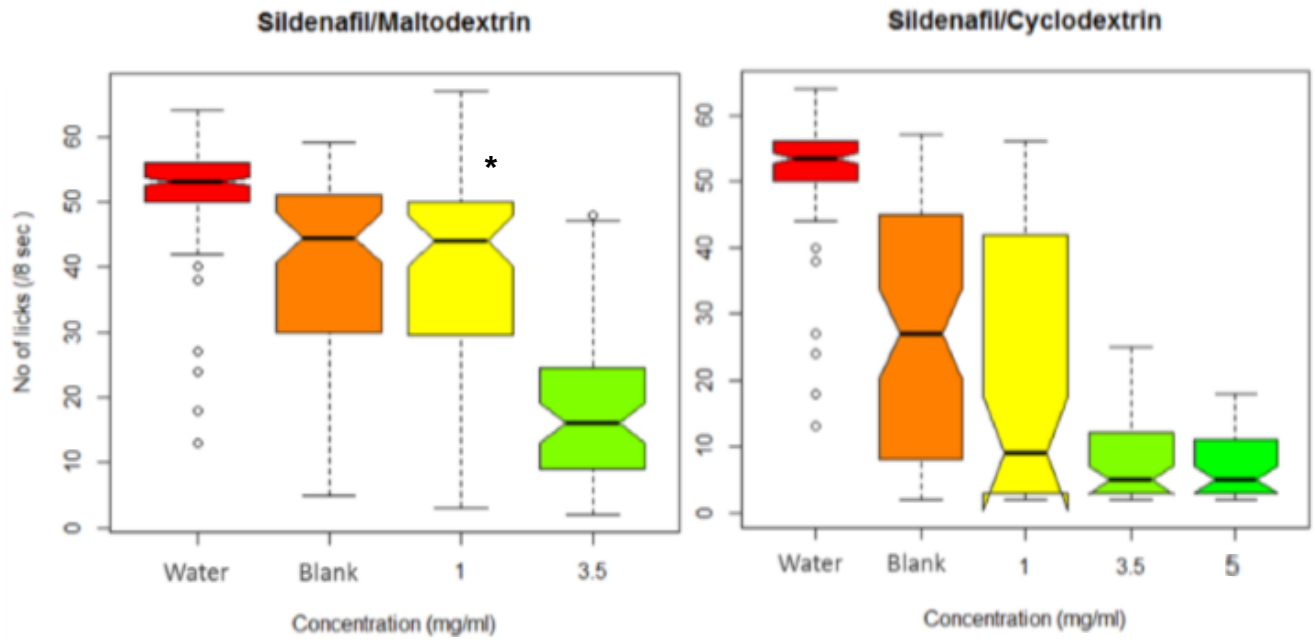


Figure 2.11 Lick responses to 1, 3.5 and 5 mg/mL Sildenafil citrate with 25% (w/v) HP- β-CD (Cyclodextrin) and 1 and 3.5 mg/mL Sildenafil citrate with 25% (w/v) KLEPTOSE Linecaps (maltodextrin) *Denotes no statistical difference from respective dextrin control

2.4.6 BATA results: dextrin placebos

The placebo formulations themselves elicited some level of aversiveness to the rats. Taking the mean number of licks over the two days, both HP-β-CD

(27.6 ± 2.1 licks) and KLEPTOSE Linecaps (39.5 ± 1.6 licks) were significantly different from water (52.1 ± 0.4 licks).

The high concentrations used, resulted in the vehicles having a very sticky texture. A low lick count may suggest the sticky solutions were difficult for the animals to lick from the sipper tubes. However, when explored further, the rats' response to the placebos was significantly different between the two testing days. The HP-β-CD placebo was very unpalatable to the rats on the first day (figure 2.12) but the number of licks was high on the second day. Similarly, the maltodextrin was highly palatable on the second day but less so on the first. This suggests there was no physical issue with licking the sticky solutions, but that the animals were exhibiting tolerance on repeat exposure.

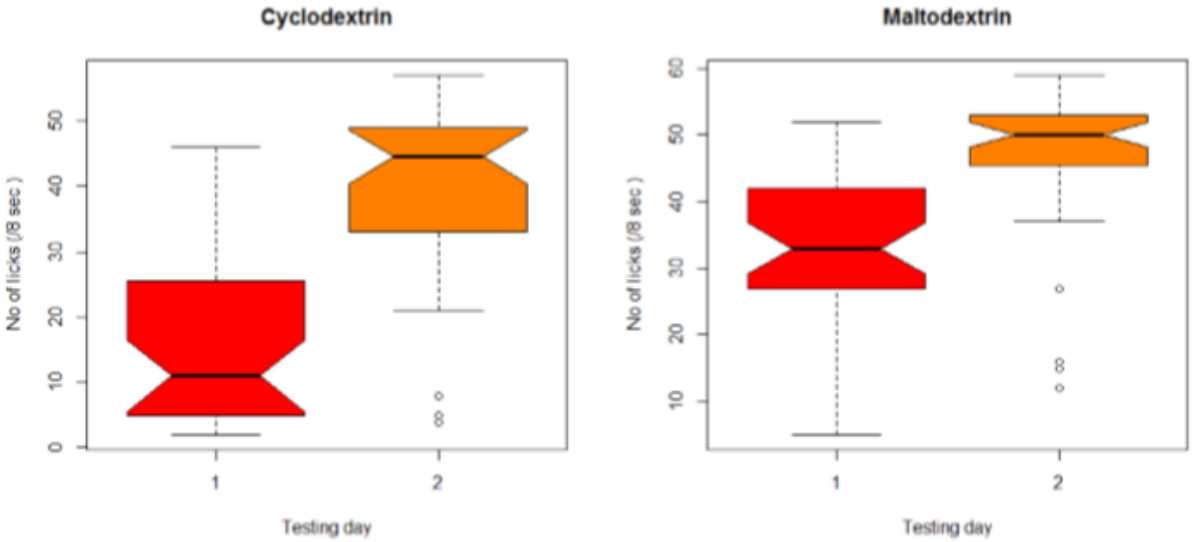


Figure 2.12 Lick responses to HP- β-CD (Cyclodextrin) and KLEPTOSE Linecaps (maltodextrin) on the two testing days.

2.4.7 BATA results summary

The BATA platform showed that 25% (w/v) KLEPTOSE Linecaps successfully taste masked both 1mg/mL Sildenafil citrate (IC50) and 0.14mg/mL Ranitidine hydrochloride. Both these concentrations had no significant lick difference compared to 25% (w/v) KLEPTOSE Linecaps control (table 2.8).

Table 2.8 Summary of BATA results, *denotes successful taste-masking

Compound	25% (w/v) HP-β-CD			25% (w/v) KLEPTOSE Linecaps		
Sildenafil citrate	1 mg/mL	3.5 mg/mL	5 mg/mL	*1 mg/mL	3.5 mg/mL	
Ranitidine HCL	0.14 mg/mL	1.4 mg/mL	14 mg/mL	*0.14 mg/mL	1.4 mg/mL	14 mg/mL

2.5 Could rats predict human aversiveness?

Since the BATA model and human panel used differing concentrations of drug in order to have a species specific IC50/ EC50, it became important to look at the molar ratio of drug: dextrin (figure 2.13).

Ranitidine hydrochloride

Human	**0.55mg/mL**	**15mg/mL**
HP- β -CD	1:103*	1: 3.8
KLEPTOSE Linecaps	1:13.8*	1:0.51

BATA	**0.14 mg/mL**	**1.4 mg/mL**	**14 mg/mL**
HP- β -CD	1:403	1:40.5	1:4
KLEPTOSE Linecaps	1:54.4*	1:5.4	1:0.54

Sildenafil citrate

Human	**1mg/mL**	**3.5mg/mL**
HP- β -CD	1:108	1:30.9
KLEPTOSE Linecaps	1:14.5	1:4.1

BATA	**1 mg/mL**	**3.5 mg/mL**	**5 mg/mL**
HP- β -CD	1:108	1:30.9	1:12.6
KLEPTOSE Linecaps	1:14.5*	1:4.1	1:2.9

Figure 2.13 Molar ratio of drug: dextrin at each concentration. *Denotes successful taste masking

2.5.1 HP-β-CD with Sildenafil citrate

The results showed HP-β-CD was unable to taste-mask Sildenafil citrate in both rats and humans even when the dextrin was in large excess. Sildenafil citrate is defined as 'slightly soluble' as it has a solubility in water of between 1-10mg/mL (Williams *et al.*, 2013). This makes it a good candidate for the hydrophobic cavity offered by HP-β-CD. Indeed, as discussed earlier in this chapter, internal work shows 25 % (w/v) HP-β-CD significantly improved the solubility of Sildenafil suggesting complexes are forming. Furthermore, the ability of Sildenafil to complex with various cyclodextrins has been demonstrated by Al Omari et al using molecular modelling, but the extent of this depended on the geometry of the dextrin. Complexation was shown to be good with 2% (w/v) β-cyclodextrin and, to a much lesser extent, achieved with 2.2% (w/v) HP-β-CD (Al Omari *et al.*, 2006). Perhaps, at the high concentration of cyclodextrin used in this work, either inclusion complexes were formed between HP-β-CD and the API but the portion of Sildenafil which confers bitterness was not wholly encapsulated within the dextrin (and still able to interact with bitter receptors), or the drug had higher affinity for bitter-receptors than it did for the cyclodextrin which resulted in no taste-masking in vivo (Arima, Higashi and Motoyama, 2012). Indeed, both these factors could play a role in HP-β-CD's inability to taste-mask Sildenafil in-vivo.

Importantly for the aims of this work, the results shown in the human panel were reflected in the BATA model.

2.5.2 HP-β-CD with Ranitidine HCL

The results showed that in humans, HP-β-CD successfully taste masked EC50 Ranitidine when in excess of 1:103. However, in the BATA model the

dextrin did not taste mask the IC50 concentration of Ranitidine even when in excess.

It is likely that HP-β-CD complexed with Ranitidine as evidenced by both NMR and molecular docking studies. Chay et al reported NMR spectra shifts consistent with significant complexation and showed, via docking studies, that the central cavity is large enough to accommodate the drug (Chay *et al.*, 2018). They also showed that the dimethylamino and the diamine groups on Ranitidine align with the oxygen atoms of the dextrin to enable the formation of direct hydrogen bonds. This group also correlated the complexation of HP-β-CD with improved taste using the e-tongue and predicted bitter reduction could be achieved in a dose dependent manner from a 1:1 – 1:4 ratio of drug: dextrin (Chay *et al.*, 2018) using similar drug concentrations as described in this chapter (0.06 – 1.5mg/mL) and using less than 3 % (w/v) cyclodextrin. They found that increasing the ratio to 1:5 offered no further benefit. This was not an accurate prediction as a large excess of dextrin was required for taste-masking in humans. This is likely due to the high solubility of Ranitidine which makes it less likely to form stable interactions with the hydrophobic cavity of the cyclodextrin. However, when there is a large excess of cyclodextrin, drug molecules bound within an inclusion complex can be readily released and almost instantaneously encounter another dextrin to form a new complex thus achieving taste-masking (Saokham *et al.*, 2018).

The probable reason for the BATA study not reflecting the human situation is likely due to the rats' aversion to HP-β-CD itself. The rats initially showed a very negative response to the placebo formulation containing HP-β-CD (figure 2.12). This improved on the second exposure. There are a few possible

explanations as to why this initial aversion was seen. Firstly, the HP-β-CD containing solutions were very sticky, a texture which was new to the animals, perhaps being off-putting and leading to a neophobic response. Secondly, although HP-β-CD is regarded as a non-sweet cyclodextrin (Zakharova *et al.*, 2016), at high concentrations it has been reported to elicit a slightly sweet taste (Ono *et al.*, 2011; Chay *et al.*, 2018). 10% (w/v) sucrose has been used as a standard to represent 'moderately sweet' taste in humans (Wee, Tan and Forde, 2018) and 25% (w/v) β-CD has been reported to have comparable sweetness to 17.1% (w/v) sucrose (Toda *et al.*, 1985). The sweetness of HP-β-CD compared with sucrose has not been published but comments received from the human panel to the cyclodextrin placebo include, 'slightly sweet' and 'pleasant' suggesting there was some level of sweetness to this high concentration. Perhaps the rats are more sensitive to the sweet flavour than humans and this is why they perceived the HP-β-CD containing solutions as initially aversive. That is not to say that rats are averse to sweetness all together – like humans, rats show preference to sweet stimuli as 'sweet' is associated with calorific content which evolution dictates us to desire (Lemon, 2015). However, for laboratory rats, fed on standard chow, sweetness is a new taste and could potentially evoke a neophobic response. Furthermore, there are some known species differences in perception of sweetness between humans and rats (Lemon, 2015). For example, glutamate is perceived as sweet to rodents (Breslin and Spector, 2008) whereas it has an umami taste in humans, moreover some sweeteners (neotame for example) can be perceived by humans but not rodents (Liu *et al.*, 2011) so it is possible

the animals are detecting a strong sweet flavour of HP-β-CD where humans are not.

Interestingly, species differences in receptors have been noted. It has been shown that rats, unlike humans, are very attracted to the taste of polysaccharides derived from starch, including maltodextrins (Sclafani, 2004). One study, evaluating rat preference to the maltodextrin Polycose, showed rats avidly drank Polycose containing solutions and preference testing showed they could detect it at very low levels (0.0001M) compared with sucrose (0.0026M) (Sclafani, 1986). This suggests rats have receptors which respond to the taste of polysaccharides (Breslin and Spector, 2008). It is therefore a possibility that HP-β-CD, was perceived as having some other flavour to the rats, which is undetectable by humans, responsible for the neophobic response.

2.5.3 KLEPTOSE Linecaps; taste-masking ability vs. human preference

The human participants seemed to prefer HP-β-CD over KLEPTOSE Linecaps. The aversiveness score for 1mg/mL Sildenafil in formulation with KLEPTOSE Linecaps was comparable to the score given to 3.5mg/mL Sildenafil with HP-β-CD. This pattern was also seen when comparing KLEPTOSE Linecaps alone with 1mg/mL Sildenafil in formulation with HP-β-CD. Similarly, there was a difference in palatability of Ranitidine at both concentrations with the two dextrins, suggesting preference for HP-β-CD. This did not translate to taste-masking ability though, as KLEPTOSE Linecaps successfully masked 3 formulations compared to HP-β-CD's 1 success (figure 2.13). Maltodextrins are not sweet-tasting (Handbook of Pharmaceutical Excipients, 2006) which was confirmed by the majority of participants in the

human panel referring to a ‘neutral’ or ‘plain’ taste of the maltodextrin placebos.

2.5.4 KLEPTOSE Linecaps with Sildenafil citrate

It is likely that Sildenafil did not form stable interactions with the maltodextrin evidenced by the fact that, in humans, the EC50 drug concentration (1mg/mL) still gave a response of 50% when in formulation with the maltodextrin suggesting the drug is not taste masked at all (figure 2.4). Further, there is no evidence in the literature to show interactions occur between the two in solution. However, the BATA model did not accurately predict this human response.

A neophobic response was seen from the rats with KLEPTOSE Linecaps but to a lesser extent than with the cyclodextrin (figure 2.12). The maltodextrin placebo was also very sticky so perhaps the initial rat aversion is owing to this characteristic. However, overall, the rodents found the maltodextrin containing formulations to be highly palatable suggesting the animals are perceiving the vehicle as having some, pleasant, taste. As aforementioned, there is evidence to show rats have receptors which can convey the taste of polysaccharides in a way humans cannot (Sclafani, 2004) and that maltodextrins are very appealing to rats. Therefore, it is likely KLEPTOSE Linecaps was offering taste-masking due to rodent preference for the vehicle rather than owing to inclusion complex formation.

2.5.5 KLEPTOSE Linecaps with Ranitidine HCL

KLEPTOSE Linecaps taste-masked Ranitidine in humans at 1:13.8 drug to dextrin molar ratio. Therefore, it is likely that the drug complexed with the

dextrin when the latter is sufficiently in excess. This was also reflected in the BATA, potentially driven by rat preference for the maltodextrin.

2.5.6 Limitations of the use of the BATA for dextrins

It is known that, as well as bitterness, rats can detect other aversive properties of a formulation; for example grittiness and viscosity (Soto *et al.*, 2018). It is likely that any novel texture or taste to the animals will be met by hesitation, even if it is a pleasant (Neath *et al.*, 2010). Therefore, when using the BATA model to assess the aversiveness of solutions which possess a novel characteristic (for example stickiness), it may be necessary to pre-exposure the animals prior to BATA experimentation. Rats can be acclimatised to vehicles; the animals normally received two bottles of water on their home cage, it is possible to replace one of these with vehicle so they can familiarise themselves with the taste and smell during non-experimental conditions. This may necessitate a license amendment and would require some safety knowledge of the vehicle to ensure it is not possible for the animals to consume quantities which may be harmful. This method can alleviate the neophobic response and allow for animals to assess novel vehicles within formulations going forward. This approach has been used successfully in the past for menthol and after a few days of exposure, the animals were consuming as much menthol solution as they were water and were then able to assess menthol in formulation with API (in house data, not published).

The assessment of maltodextrin in the BATA model may be problematic owing to the animals' apparent preference for this polysaccharide which led to a false prediction of taste-masking for Sildenafil. Conversely for cyclodextrin, although the placebo became palatable on repeat exposure, the API

containing formulations were not falsely perceived as taste-masked when compared with humans. This suggests that perhaps cyclodextrins could be used in the BATA once the neophobia was addressed (by pre-exposing new animals prior to experimentation as discussed). To understand this more fully, a range of API need to be assessed alongside HP-β-CD in both the rodents (after pre-exposure) and in humans to understand this correlation further.

2.5.7 Optimisation of dextrins as a taste-masking strategy

In this work, high concentrations of cyclodextrin and maltodextrin were used in all the test formulations to ensure an excess was available. However, more work would be necessary to optimise formulations with therapeutic strength of API, balancing the concentration required for solubility enhancement, vehicle acceptability and taste-masking potential of the dextrins. Once the levels of dextrins are better understood in humans, then further work can be undertaken to correlate BATA responses.

2.6 Conclusions

Both HP-β-CD and KLEPTOSE Linecaps successfully improved the palatability of Ranitidine at the EC50 level in the human taste-panel. However, this work demonstrates there may be differences in human and rat tolerance to such dextrins. Rats display neophobia to novel tastes and textures which was seen in this work with the placebos. Both vehicles were sticky which may have contributed to the initial hesitancy to the maltodextrin and, in particular, the cyclodextrin. Pre-exposing the animals to the vehicles prior to assessing them within formulation with a drug may improve translatability. However,

there is literature evidence to suggest rats can detect the taste of polysaccharides, in a way humans cannot, which may hinder their ability to accurately predict the human response.

Chapter 3

Evaluating the efficacy of promising bitter-blockers as a taste-masking strategy for children

This chapter describes a set of experiments investigating the efficacy of three promising bitter-blockers as identified by a novel methodology. This methodology enabled comprehensive assessment of such compounds reported in the literature by scoring them based on safety, efficacy and usability parameters for use in paediatric medicines. The bitter-blockers were assessed in the e-tongue with the aim of finding the lowest concentration required for effective bitter blocking; important for paediatric medicines where quantities of excipients should be kept to a minimum. These concentrations were then carried through to the BATA model; a more robust model of palatability. The three compounds were also tested for any covalent interaction with Quinine hydrochloride, a model bitter tastant.

3.1 Introduction

Improving the palatability of liquid formulations for children remains a challenge, with sweeteners and flavourings unable to combat particularly bitter and/or soluble API. This type of taste-masking aims to achieve an 'acceptable' taste profile which can be subjective depending on the background of the child (Mennella and Beauchamp, 2008) and may diminish with repeat administration (EMA, 2013). Furthermore, as discussed in chapter 1, sweeteners have a number of associated concerns with their clinical use. A taste-neutral outcome, using excipients which act independently of the API,

represents an updated approach to taste-masking and is endorsed by the EMA (EMA, 2013).

Bitter-blockers are compounds which act at a pharmacological level to inhibit TAS2R bitter receptors. This prevents downstream release of transmitters and so blocks the perception of bitterness and could provide a much needed alternative to taste-masking paediatric formulations. There are 25 TAS2Rs which have been identified but humans perceive many thousands of different compounds as bitter which suggests a great number of bitter tastants can be detected by a single receptor. In fact, some receptors detect only a few bitter agonists whereas others are highly promiscuous. It is likely that a number of receptors are used to perceive a particular compound as bitter (Meyerhof *et al.*, 2010) but this is not known for all drugs. A single bitter-blocking excipient will not be universally applicable as different API stimulate different TAS2Rs and to differing extents. However, it will offer an important option in the formulation toolbox which could, if not inhibit entirely, dampen down the bitterness of an API which could be enough to facilitate its ingestion in children.

Before testing a bitter-blocker's ability to supress the bitterness of standard compounds such as Quinine HCL, it is first important to understand which bitter-blockers demonstrate the other characteristics necessary to be an excipient for paediatric medicines. A number of compounds which have the ability to alter perceived bitterness in children were compiled by Walsh et al (Walsh *et al.*, 2014). Some of these compounds interact with the bitter-perception pathway as bitter-blockers but the list also included excipients which were reported to convey bitter suppression due to their sweetener

properties. This work stated the known limitations of each compound and their regulatory status but did not claim to be an exhaustive list of bitter-blockers. In fact, prior to this PhD, there was no complete review available of all known bitter-blockers nor was there a thorough risk assessment of such compounds for their use in medications.

3.1.1 Developing a novel methodology to identify promising bitter-blockers

In order to evaluate a bitter-blocker's utility as a potential excipient it was necessary to first understand the current literature. Therefore, as part of this PhD, a systematic review was undertaken and subsequently published in European Journal of Pharmaceutics and Biopharmaceutics IF ~6 (doi: 10.1016/j.ejpb.2020.10.017) entitled 'Bitter-blockers as a taste masking strategy: A systematic review towards their utility in pharmaceuticals'. The majority of work for this review took place during COVID closure of UCL.

The main objectives of this review were two-fold. Firstly, it was important to understand what compounds have been discussed within the literature as having 'bitter-blocking' action by data mining using the search terms 'taste modifier', 'bitter blocker', 'bitter antagonist' alongside either 'medicine', 'drug', 'formulation' or 'dosage form'. The papers generated were then screened for relevance and compounds acting as bitter-blockers (on bitter-receptors) were compiled. The second objective was to develop a novel methodology to score these compounds for their potential use as a taste-masking excipient. The aim here was to improve understanding of the potential benefits and limitations of these compounds. The novel scoring system assessed potential utility within a medicinal product by evaluating three factors 1) usability 2) safety and 3) efficacy/ quality of evidence (table 3.1). This structured approach began to fill

in the gaps in current knowledge around this taste-masking approach and highlighted in what way bitter-blockers could be applied.

Table 3.1 Scoring criteria for bitter-blockers. ADI; acceptable daily intake. QoE; quality of evidence

	Score of 0	**Score of 1**	**Score of 2**	**Score of 3**
Safety	Evidence of a hazardous nature in low/ efficacious concentrations OR No information available on safety	Incomplete or little information is known on the safety of the compound OR No information found but is a close structural analogue of another compound with GRAS status	The compound is deemed safe for example has GRAS status OR Has a known ADI that exceeds the efficacious dose OR Is found in the human diet with no concern highlighted on its use although it may be associated with allergies in some patients so requires strict labelling OR Is patented for human use	The compound is deemed safe (for example has GRAS status) AND Not associated with allergies in patients AND one of the following Is patented for human use OR Has a known ADI that exceeds the efficacious dose, or regulatory bodies have stated there is no limit to the ADI OR Is found in the human diet with no concern highlighted on its use OR The compound has recent precedence for use in human consumables and/or pharmaceuticals
Efficacy and QoE	No demonstrable efficacy shown as a bitter blocker OR Study demonstrating efficacy has inconclusive or unreliable results	Efficacy shown in a cell-based model expressing a limited number of receptors (which gives no context to its action) or using a sensor technology (which has limitations (Haraguchi *et al.*, 2016) and also gives limited context) OR Transient bitter blocking shown in an animal model	Demonstrates effective transient bitter blocking against one compound which has been demonstrated in a human panel of at least n=8 OR Demonstrates effective transient bitter blocking against	Demonstrates effective transient bitter blocking against more than one compound with efficacy demonstrated in human sensory panels of sufficient sample size, at least n=8 (unpublished in house data shows n=8 to be sufficient to distinguish between

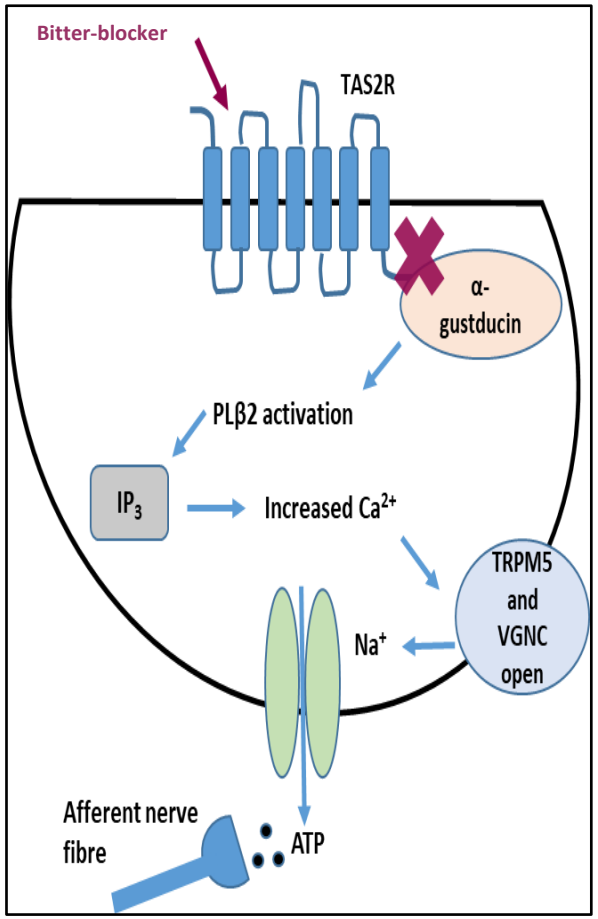
		(e.g. BATA) against one compound OR Efficacy shown against one bitter compound in a human panel of insufficient number (n<8)	more than one compound which has been demonstrated in a model that does not involve humans (n>8) or in a human panel of insufficient participant number (n<8)	low and high levels of bitterness)
Usability	No known information on stability/ solubility AND/OR Not available to purchase AND/ OR More than three limitations to its use, for example, specific storage is required/ it is only suitable for extemporaneous preparations/ it has a flavour in itself which may be aversive to some patients/ it is expensive to purchase	Poor compatibility with API identified or likely to occur AND/OR Solubility either - inappropriate (e.g. only soluble in ethanol/ DMSO) Or - only partially soluble in water Or - poor solubility; too low to convey efficacy if an efficacious concentration has been demonstrated in a human panel or, if no efficacious concentration is known, requires more than 30mL of solvent to dissolve 1g i.e. less soluble than 33.33mg/mL (Williams *et al.*, 2013) AND/OR Stability either - specific time period of stability unknown Or - not stable for at least 3 months (regardless of storage conditions required, e.g. refrigeration) AND/OR Not available to purchase readily/ requires a number of synthesising steps AND/OR This compound has up to three limitations to its use; for example, specific storage is required/ it is only suitable for extemporaneous preparations/ it has a flavour in itself which may be aversive to some patients/ it is expensive to purchase	Acceptable solubility; either exceeding that required for efficacy in humans or, if not known, above 33.33mg/mL AND Acceptable stability of at least 3 months (regardless of storage condition required e.g. refrigeration) AND No demonstrable evidence of its ease of use in humans AND Readily available to purchase AND The compound may have up to two additional requirements that limits its use in some way. For example, storage; refrigeration may be necessary, or the compound may only be appropriate for extemporaneous preparations or it may have its own flavour/taste that could be aversive to some patients (e.g. sour)	Demonstrable ease of use in humans, for example if it is required to be in solution for efficacy, publications report it solubilised in appropriate media and could be administered in sensible quantities (for example 5mL total volume if administered to children (Batchelor and Marriott, 2015) or 10mL if administered to adults (Mohamed-Ahmed *et al.*, 2016)) AND Solubility exceeding that required for efficacy in humans, or if unknown, above 33.33mg/mL and stable for at least 3 months at room temperature AND No aversive taste potential; it is either tasteless or pleasant tasting AND Readily available to purchase. AND The compound has no additional limitations to its use

This approach was designed to be readily translational, with compounds scoring highly with GRAS status, good usability, and availability off the shelf (being unencumbered by patents). This is in contrast to other approaches which may be more targeted and therefore slower, involving a number of stages such as compound screening in vitro prior to assessment (Unitaid, 2020). The aim of this review was to identify what compounds are already known to the scientific community so more immediate action could be taken to assess their potential benefit.

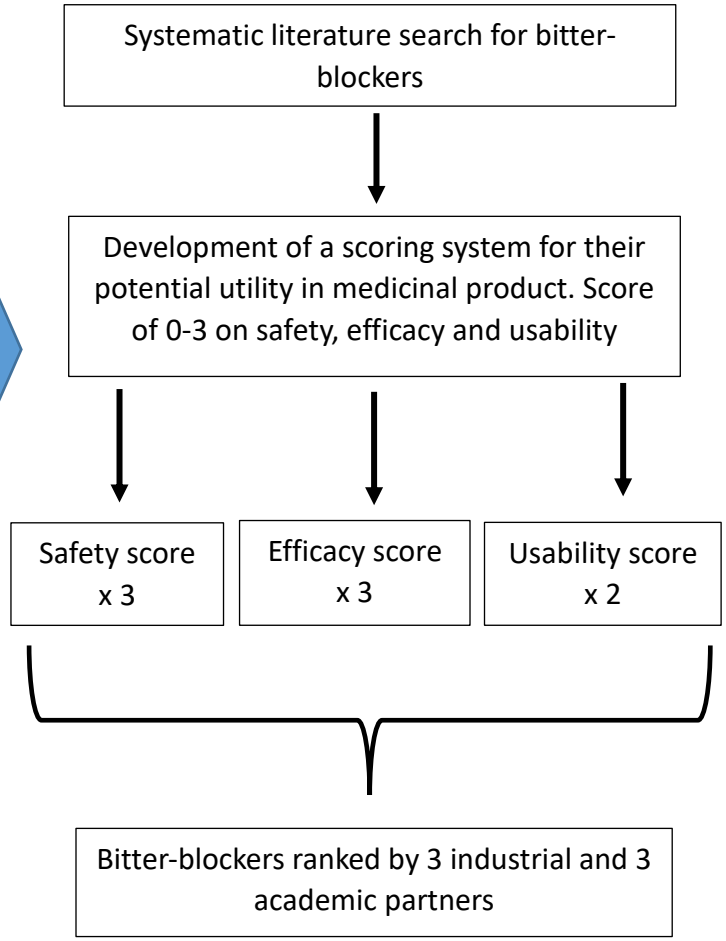
3.1.1.2 Industrial collaboration and weighted scoring

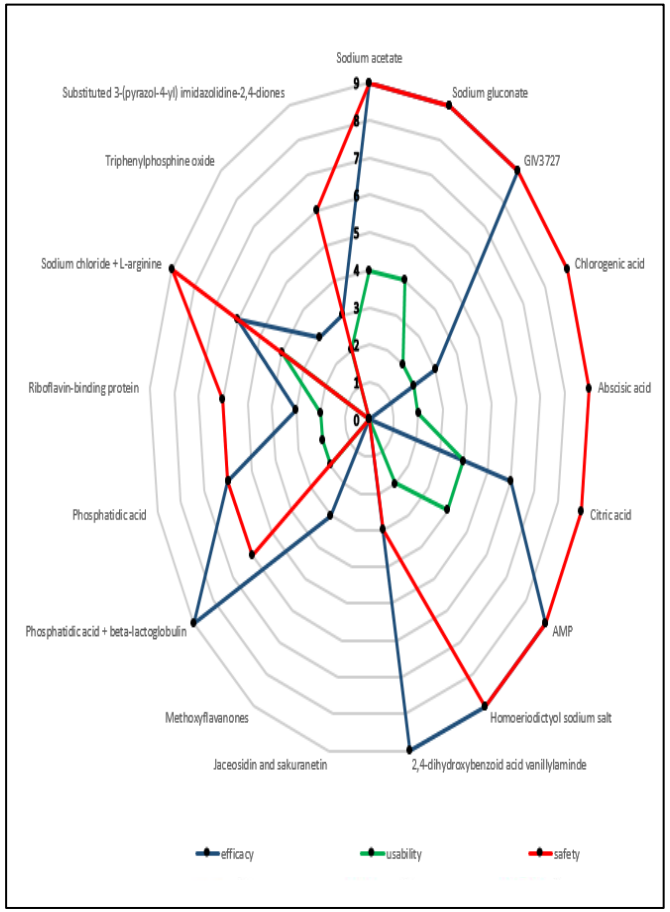
The scoring system and the literature-derived information on each bitter-blocker, was passed to three industrial and three academic partners who each, independently, scored the compounds. To reduce bias, the initial scoring system was drawn up prior to the literature search and was only updated to improve clarity if there were discrepancies in interpretation. The final score for each compound was generated using a weighted scoring system. The safety of an excipient is paramount therefore this was given a weighted score of three. Similarly, an excipient should not be used without purpose and demonstrable efficacy and so efficacy (including quality of evidence) of the bitter-blocker was considered equally important and also given a weighted score of 3. Usability was given a weighted score of 2 since the use of a bitter-blocker could be tailored to fit its characteristics, for example if it does not have long-term stability it could be used for extemporaneous preparation. Such factors may make an excipient less desirable but does not mean they cannot succeed as an excipient given the

appropriate conditions. The approach, and overall findings, of the review is summarised in figure 3.1.



Bitter-blocker antagonising TAS2R bitter receptors





Bitter-blockers overall scores by safety, efficacy and usability

Figure 3.1 Summary of the approach taken in the systematic review to highlight promising bitter-blockers

From this work three bitter-blockers were identified as being promising for further evaluation. Sodium gluconate (Mennella, Pepino and Beauchamp, 2003), sodium acetate (Keast and Breslin, 2002) and sodium adenosine monophosphate (NaAMP) (Keast and Breslin, 2002) each have an excellent safety profile and have shown efficacy in human panels at 300mM, 100mM and 20mM respectively (table 3.2)

Table 3.2 Top three bitter-blockers identified in the review and their associated scores

Bitter-blocker and level of assessment	Mechanism of Action	Safety information	Demonstration of efficacy	Usability
• **AMP (Adenosine 5' monophosphate); nucleotide found in RNA** **Assessed as NaAMP in human panel** (Keast and Breslin, 2002) **and as AMP in both *in vivo* and *ex vivo* assessment** (Ming, Ninomiya and Margolskee, 1999)	AMP acts on peripheral taste inhibition. The glossopharyngeal nerve innervates taste receptor cells in the tongue and is responsive to bitter stimulus. 0.1mM (0.035mg/mL) AMP significantly inhibited the nerve responses to bitter compounds such as quinine and denatonium benzoate. It is thought AMP may alter the receptor G-protein coupling and act as a taste modifier here (Ming, Ninomiya and Margolskee, 1999)	AMP is found in many foods and is found in breast milk (Walsh *et al.*, 2014) It has GRAS status for use in food and drinks and oral pharmaceutical dosage forms (FDA, 2004) AMP is patented for use in human consumables and pharmaceuticals (Margolskee and Ming, 2003) AMP has no precedence in pharmaceuticals	Human panel of 14 adults; 20mM NaAMP (7.4mg/mL) in pH 5 deionised water, on average, reduced the bitter perception of the following bitter compounds by 67%; 10mM pseudoephedrine, 4mM ranitidine, 50mM acetaminophen, 0.1mM quinine and 1.2M urea. This study did not give the bitter inhibition results for individual pharmaceuticals (Keast and Breslin, 2002) G-protein activation assay using bovine taste cell membranes; AMP (0.01 - 5mM) dose-dependently inhibited transducin activation by bitter compounds. 2.5 mM AMP inhibited activation of transducin by 5mM denatonium benzoate and 1 mM quinine (Ming, Ninomiya and Margolskee, 1999) Mouse two-bottle preference test; AMP had an inhibitory effect on the bitter perception of concentrations up to 5mM denatonium	Solubility in water of 100 mg/mL (Sigma-Aldrich, 2022) which exceeds efficacious concentration AMP is stable if refrigerated at 4°C; it maintains its initial concentration after 25 weeks of storage. If exposed to room temperature, AMP solution will begin to degrade after a few days (Martínez-García *et al.*, 2002) Commercially available from Sigma Has a savoury taste (Fuke and Shimizu, 1993)

			benzoate and 10 mM quinine. AMP was effective at 0.5 mM for quinine and 1 mM for denatonium benzoate(Ming, Ninomiya and Margolskee, 1999)	
Overall score = 22 Limitations; requires refrigeration and has a slight savoury flavour		**Safety score = 3** (GRAS and in the human diet)	**Efficacy/QoE score= 3**	**Usability score = 2** (requires refrigeration and may have limited use due to its umami flavour (Yamaguchi and Ninomiya, 2000))
• **Sodium gluconate** **Assessed in paediatric sensory panels** (Mennella, Pepino and Beauchamp, 2003; Mennella *et al.*, 2014)	Sodium ions are thought to act on specific bitter receptors directly. The exact mechanism is unknown, sodium may shield the receptor proteins, modulate ion channels or act on second messenger systems (Keast and Breslin, 2002). It is not a universal bitter receptor blocker or modulator as its influence on compounds differs (Kroeze and Bartoshuk, 1985; Breslin and Beauchamp, 1995)	Sodium gluconate is generally regarded as safe for use in pharmaceuticals and foods (FDA, 2018). There are some concerns about exposing patients to excess sodium but 2mL of 0.3M sodium gluconate provides approximately 14mg sodium, the daily limit for children has been recommended to be 1,500mg per day and 2,300mg for adults (Health.gov, 2015) so this quantity is unlikely to be an issue with regulatory bodies Sodium containing compounds have been patented for bitterness inhibition in pharmaceuticals (Hikaru and Akiko, 2003)	In a paediatric sensory panel of 41 children, ages 7-10, 2mL 300 mM (65.4mg/mL) sodium gluconate improved the perceived palatability of 0.5 M urea in 70% of the children. There was no difference in the palatability of urea + salt compared to salt alone. 0.3 M sodium gluconate improved the perceived bitterness of 0.08M caffeine in 68% of the children but this solution was perceived as more bitter than the salt alone. Children also ranked sodium gluconate as equally preferable to water (Mennella, Pepino and Beauchamp, 2003) In another paediatric panel of 154 children 300 mM sodium gluconate reduced bitter perception of 0.119mM quinine (Mennella *et al.*, 2014)	The solubility of sodium gluconate in water is approximately 600mg/mL (2.75M) at 25°C (Pedrosa and Serrano, 2000) Sodium gluconate is reportedly very stable, especially in water (Prescott *et al.*, 1953) and has been demonstrated to be stable for at least 3 months as part of a medicinal cream (Jungbunzlauer, 2022) Sodium gluconate is commercially available from sigma The mild salty flavour may be off-putting for some patient populations such as adults – children do not find the saltiness aversive (Mennella, Pepino and Beauchamp, 2003)
Overall score = 22 Limitations; saltiness may be aversive		**Safety score = 3**	**Efficacy/QoE score= 3**	**Usability score = 2** (salty flavour may be aversive to some patients)

• **Sodium acetate** **Assessed in human sensory trials** (Keast and Breslin, 2002; Sharafi, Hayes and Duffy, 2013)	See above	GRAS status (FDA, 2018). 10mL of 100mM sodium acetate provides approximately 23mg sodium Sodium containing compounds have been patented for bitterness inhibition in pharmaceuticals (Hikaru and Akiko, 2003)	100mM (8.2mg/mL) sodium acetate reduced the bitter perception of a range of bitter pharmaceuticals, including 0.1mM quinine and 1.2M urea, by 55% on average in a human sensory panel of 14 participants, the solutions were in 10mL (Keast and Breslin, 2002) 1.33M sodium acetate also reduced the perceived bitterness of a range of green vegetables by 42% in a human panel of 37 people (Sharafi, Hayes and Duffy, 2013)	Good solubility in water 246.1mg/mL (3M) (Chemical Book, 2019) and good stability for up to 1 year in solution (Cayman Chemical, 2022) Commercially available from sigma Sodium acetate has a mild salty flavour (Henney, Taylor and Boon, 2010)
Overall score = 22 Limitations; saltiness may be aversive		**Safety score = 3**	**Efficacy/QoE score= 3**	**Usability score = 2** (salty flavour may be aversive to some patients)

These bitter-blockers needed to be further evaluated in order to fully understand their place in the formulation toolbox. They had not been assessed in a standardised manner for taste-masking, nor had they been assessed at a range of concentrations and so it was unknown at what concentration they begin to take effect. This is of particular importance when considering medicines for children where only the absolute minimum concentration of excipient, necessary to carry out its functional role, should be used. This is important for all patient populations but especially for children who have a more limited metabolising capacity compared to adults (Kearns *et al.*, 2003).

3.1.2 Standardising assessment

The efficacy of novel taste-masking excipients for oral solutions must be evaluated, broadly, in two ways, using structural assessments and in vitro in vivo evaluations (figure 3.2).

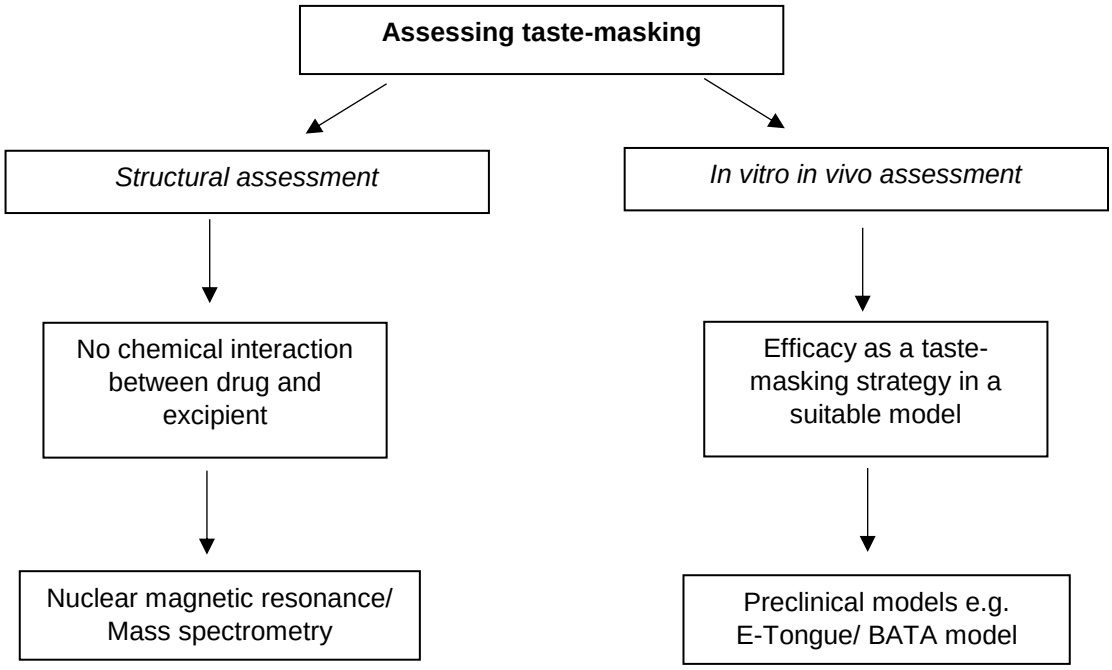


Figure 3.2 Assessing a compound for use as a taste-masking excipient. BATA; brief access taste aversion

The compound must demonstrate that it improves the perceived bitterness of API without covalent interaction, which might alter the action of the drug (Buggins, Dickinson and Taylor, 2007; Al-Kasmi *et al.*, 2018). There are a number of techniques to assess this including nuclear magnetic resonance (NMR) and mass spectrometry which produce spectra relating to the absorption peaks of the drug and of the excipient and of the two as a

complex. The spectra can be compared and highlight if chemical bonds have been formed, seen by unexplained changes in the two sets of peaks.

Palatability assessment can be carried out using various preclinical models ranging from in vitro cell lines to biomimetic systems (Malode and Gudsoorkar, 2014). There are a number of in vivo models which can be used to assess palatability. Less commonly used are fish and drosophila which can be used to taste-screen API. Fish can be fed with fluorescently dyed food/drug mixes and the intensity of the dye can be measured within the animals to give an indication of preference (Okada, 2015). Alternatively, Drosophila melanogaster have been shown to be able identify bitter tastes (Lvovskaya and Smith, 2013). Simple behavioural feeding assays can be used to understand if the flies find a particular drug solution bitter by measuring their proboscis extension responses (Sarah French *et al.*, 2015). These models have not been fully validated with against human data and do not offer sufficient information.

The most appropriate preclinical methods to assess the taste-masking ability of excipients on a bitter API within liquid formulations include the BATA model and electronic tongue as discussed in chapter 1 (Clapham *et al.*, 2021).

The Insent e-tongue has been shown to have good in vitro-in vivo correlation for a number of drugs (Woertz *et al.*, 2011a) this model uses electrodes with lipid membranes (seen in figure 1.7). The taste-sensor is immersed into the KCL reference solution, which is used as an alternative to human saliva and is used to obtain the membrane potential (Vr). Then the sensor is placed into the sample solution to obtain the potential (Vs), the difference between these two

(Vs-Vr) is the relative value and indicates the initial taste. This is followed by a light rinsing procedure and a measurement of aftertaste. Then the sensor is rinsed with reference solution and then alcohol solution before the next sample is measured (Woertz *et al.*, 2011b). However, it is important to note that the e-tongue should not be used as a standalone method of assessment as the model does not mimic the physiological conditions seen in vivo (Woertz *et al.*, 2011b). More validation of the model is needed to fully understand the limitations before it would be possible to make an absolute statement regarding the taste of novel samples from e-tongue data alone. However, the e-tongue can give concentration-response correlations and is useful to compare one solution directly with another for perceived bitter output.

A number of palatability models use rodents to investigate bitter-masking efficacy. The two bottle taste preference test exposes animals to two bottles; one with taste solution and one with, usually, water (Gaillard and Stratford, 2016). The volume consumed from each bottle is compared. This approach will give a preference ratio of tastant to water but does not allow for multiple concentrations of compound to be assessed. Other rodent based models require the animals to be trained. The high-throughput taste-assessment model uses trained rats to sample different solutions in a standard 96-well in an operant chamber. This first involves training the animal using a reference sample at a single concentration (for example 100mM sucrose) to perform a task such as pressing a level. Pressing the lever will generate a reward e.g. a food pellet. After training is complete, the animal then can be used for trials involving sampling from a 96-well plate. If the rats perform the trained task on sampling, this suggests the test sample has the same taste quality as the

reference sample. This model also counts the number of licks taken from each well to ascertain palatability (Palmer *et al.*, 2013). This particular model involves significant training which can take months to achieve and training involves just one concentration of standard and so there is a risk of establishing a discrimination based on stimulus intensity rather than taste quality (Palmer *et al.*, 2013; Mohamed-Ahmed *et al.*, 2016). Conversely, using negative reinforcement instead of rewarding with food, the conditioned taste aversion (CTA) model trains animals to associate reference solutions (e.g a bitter compound) with negative outcomes, such as intraperitoneal (IP) injections of lithium chloride. When the animal is then exposed to a novel compound with a similar taste profile to the reference compound, they will avoid it in order to prevent the negative outcome (Gore-Langton *et al.*, 2015). Not only is this model unpleasant for the animal, but it is also unable to assess the palatability of a drug or taste-masked API but only indicates its taste-quality.

The operant taste discrimination model involves training the animal to perform a specific task when given a certain control compound and a different task when presented with another control compound. New formulations can then be administered and the task the rat performs will indicate which control the new compound is most similar to. This model can only assess the taste quality (in reference to the controls) and cannot give a measure of the palatability (Spector and Kopka, 2002).

The brief access taste aversion (BATA) model uses mildly water deprived animals within a Davis Rig (as shown in figure 1.8). The animals are presented with various solutions of API, usually with and without taste-

masking excipients at various concentrations (Mohamed-Ahmed *et al.*, 2016). The DiLog equipment, as used in this work, uses an electrical lickometer; each time the rat's tongue makes contact with the drinking tube, the circuit of the sensor is complete (Smith, 2001) and the signal is sent to the computer which stores this lick data. The number of licks to each sample correlates to how palatable that solution is perceived to be. The lick count for each solution is compared to the control, water, which is set as the fully palatable reference. The rat BATA model has been shown to have very good correlation with human results for predicting the bitterness of API (Clapham *et al.*, 2012; Rudnitskaya *et al.*, 2013; Cocorocchio *et al.*, 2016; Soto *et al.*, 2018) and is beginning to be used routinely in industrial settings to assess the palatability of new drugs before they progress to the market. The BATA rats have been shown to predict bitterness in humans consistently with a half-log unit of molar concentration offset (Rudnitskaya *et al.*, 2013; Soto *et al.*, 2018), meaning the rats can tolerate slightly more bitter solutions likely due to the fact they are encouraged to drink whereas the corresponding human panel is not.

3.2 Aims and objectives

The first aim of this work was to investigate the bitter-blocking efficacy of sodium acetate, sodium gluconate and NaAMP, initially using the e-tongue to highlight at what concentrations these compounds can begin to be effective, and then in the BATA model for confirmation of findings. The second aim of this work was to investigate any covalent interaction that may occur between each bitter-blocker and the model drug Quinine HCL.

3.3 Materials and methods

3.3.1 E-tongue

3.3.1.1 E-tongue materials

Quinine HCL, sodium acetate, sodium gluconate, tartaric acid, absolute ethanol and hydrochloric acid (32%) were purchased from Sigma-Aldrich (Gillingham, UK). Adenosine 5'-monophosphate sodium salt was purchased from Insight biotechnology (Middlesex, UK). Potassium chloride was purchased from VWR International (Leicester, UK).

3.3.1.2 Reference solutions

Potassium chloride inner solution (for sensors and reference electrodes) was made to 3.33M with distilled water. The alcohol washing solution for the sensor in between samples was made up using 100mM hydrochloric acid in 30% ethanol in distilled water. The reference washing solution, used for taste-sensor preconditioning and light wash between initial and aftertaste readings for the same sample, was made up using 30mM potassium chloride and 0.3mM tartaric acid dissolved in distilled water. 10mM potassium chloride solution was made up in distilled water for reference solution, this is used as the solvent for test solutions and is the blank control.

3.3.1.3 Test solutions

Quinine HCL was made to 0.26mM (human EC50) in 10mM KCL as the bitter control.

Ten different concentrations of each bitter-blocker were tested for their bitter-blocking of 0.26mM Quinine HCL. The concentrations chosen were based around the reported efficacious concentration in human panels (derived from

the literature) of 100mM sodium acetate (Keast and Breslin, 2002), 300mM sodium gluconate (Mennella, Pepino and Beauchamp, 2003) and 20mM NaAMP (Keast and Breslin, 2002).

The concentrations prepared of the blockers in 10mM KCL are as follows (table 3.3);

Table 3.3 Bitter-blocker solutions prepared in 10mM KCL for e-tongue testing

Bitter blocker	mM	mg/mL
Sodium acetate	10, 20, 25, 30, 35, 50, 75, 100, 150 and 200	0.82, 1.64, 2.05, 2.46, 2.86, 4.1, 6.15, 8.2, 12.3 and 16.4
Sodium gluconate	75, 100, 150, 175, 200, 250, 275, 300, 400 and 600	16.4, 21.8, 32.8, 38.2, 43.6, 54.5, 60.0, 65.4, 87.3 and 130.9
NaAMP	0.5, 1, 2, 3, 4, 5, 10, 20, 30 and 40	0.19, 0.37, 0.74, 1.11, 1.48, 1.85, 3.7, 7.4, 11.1 and 14.8

3.3.1.4 E-tongue measurements

The Insent TS-5000Z electronic tongue (Insent Inc., Atsugi, Japan) was equipped with a lipid membrane sensor SB2AN0 (Insent Inc., Atsugi, Japan) representing bitterness. The sensor was filled with 0.2mL inner solution and the reference electrodes were filled with 0.4mL. All sensors were immersed in standard solution for 24 hours for preconditioning before measurement. Sensor checks were carried out prior to each measurement and each sample was measured four times. As recommended by the supplier, the first run each time was rejected to enable sensor conditioning. First, the reference solution was measured then the sample solution was measured. Then a light washing procedure occurred followed by measurement of the after taste then a full clean in the alcohol wash.

3.3.1.5 Data analysis

The data was analysed and graphical representations were produced using OriginPro (2019) using one-way analysis of variance (ANOVA) and tukey post hoc statistical tests.

3.3.2 BATA assessment

BATA assessment was carried out in collaboration with colleagues at GlaxoSmithKline (GSK) (PPL number P5CCAC30D) whilst the model was unavailable at UCL (owing to license renewal issues) and was being set up elsewhere as part of this PhD (discussed in chapter 4). Previous inter-laboratory comparison between UCL and GSK laboratories has been undertaken (Ives, Tuleu and Bachmanov, 2018) and found very good reproducibility of data. For example, Quinine HCL (used as a bitter control) has an historical IC50 value of approximately 0.1mM at GSK (Rudnitskaya *et al.*, 2013) and 0.08mM at UCL (Soto *et al.*, 2018). Furthermore, the majority of controlled conditions under which the BATA studies were run were found to be very similar between the laboratories. This work highlighted only four factors which were not fully aligned between the two laboratories being; age of rats, housing, technical staff and study design/ analysis. In terms of study design differences, 12 rats are used at the GSK facility owing to an increased number of lickometer instruments enabling more than one animal to be assessed at one time. Conversely at UCL facilities, and at the external laboratory set up (as discussed in chapter 4), 10 rats were used as only one animal can be assessed at a time with the one lickometer available. This is because each animal undergoes a 40 minute Davis Rig session, then he is weighed and put back in his home cage for a 1hr 20minute rehydration period.

There is then a short window of 5 minutes to fill up any tubes which may be low and wipe down the Davis Rig ready for the next animal who then is weighed before his session begins. Carrying out this process back to back for each animal involves 8-hours of experimentation. Time is also needed in the morning, to set up the Rig and to fill the tubes with fresh solutions, and at the end of the day, to derig and clean the equipment before removing the last animal's water following his rehydration period.

The other difference between the two laboratories study design is regarding the randomisation of test solutions to tubes. At GSK, the test solutions to be presented are randomly allocated to the sipper tubes and this order is kept the same between the two test days. However, at UCL, the randomisation of tube contents is different between test day 1 and test day 2. This is because every animal is using the same lickometer at UCL and so randomising the solution order between the two days protects against the impact of any technical issues. For example, there has been a rare occurrence of a particular tube at a specific position glitching and giving false read-outs which is recorded at the time by the operator and the data produced from that tube is subsequently deleted. Keeping the solutions in the same tubes between test days may mean an entire data set for a particular test solution is discarded. However, at GSK, where there are multiple lickometers used, if such a technical issue was experienced the impact would be minimal to the overall data set.

3.3.2.1 BATA materials

Quinine HCL, sodium acetate, sodium gluconate and NaAMP were supplied by Sigma-Aldrich (Gillingham, UK).

3.3.2.2 Test solutions

Each bitter-blocker was assessed at 5 concentrations based around e-tongue findings; 20mM sodium acetate, 75mM sodium gluconate and 4mM NaAMP. Each blocker was first assessed as a standalone solution to understand their own palatability and subsequently assessed alongside IC50 (0.14mM, 0.052mg/mL) and IC90 (1.8 mM, 0.714mg/mL) Quinine HCL to assess taste-masking action. The concentrations prepared of the blockers are as follows (table 3.4):

Table 3.4 Bitter-blocker concentrations for BATA assessment

Bitter blocker	mM	mg/mL
Sodium acetate	3, 10, 30, 100 and 300	0.25, 0.82, 2.46, 8.2 and 24.6
Sodium gluconate	10, 30, 100, 300 and 1000	2.18, 6.54, 21.8, 65.4 and 218.1
NaAMP	1, 3, 10, 30 and 100	0.37, 1.11, 3.7, 11.1 and 36.9

0.14 mM and 1.8 mM Quinine HCL control solutions were also prepared. All solutions were made using distilled water.

Each solution was made 1 day prior to use and stored in the fridge (2-8 °C) protected from light. Solutions were taken out of the fridge for a few hours on the morning of each test day to reach room temperature. Each study first attained a dose-response curve to the bitter-blocker alone and then assessed the various concentrations against Quinine HCL at IC50 and IC90 levels. An example of how test solutions containing blocker + Quinine HCL were prepared is given in table 3.5.

Table 3.5 Sodium gluconate solution table

Identifier	Quinine HCL (mM)	Sodium Gluconate (mg/mL)
A	0.14	2.18
B	0.14	6.54
C	0.14	21.8
D	0.14	65.4
E	0.14	218.1
F	1.8	2.18
G	1.8	6.54
H	1.8	21.8
I	1.8	65.4
J	1.8	218.1
1.8 mM Quinine HCL	1.8	0
0.14 mM Quinine HCL	0.14	0

For each study the 10 test solutions (A-J in table 3.1), 2 Quinine HCL controls and 2 water controls were randomly assigned to tube position 1-16 in the Davis Rig, except for tube 3 and tube 8 which were are always water (water in tube 3 is presented first so the rats are motivated to continue and tube 8 is a water rinse between samples). The random assignment of solutions to tubes was kept the same for the two test days.

3.3.2.3 Animals

12 male CD (Sprague Dawley) rats (approximately 12 weeks of age) were tail marked 1 to 12, weighed and housed in groups of four. Following discussion with a statistician, it was previously decided that these rats do not require randomisation on arrival as all are within the same treatment group and act as their own controls. Animals were housed in large tower caging and provided with 2 nest boxes, 2 shelves, 2 to 3 wood perches, 1 red tunnel, 2 or 3 cardboard tubes suspended from roof of cage, 4 wood chews and 1 handful of nesting material. The room was maintained at 19-21^{o}C with 45-55% humidity and with a 12:12 h light/dark cycle. The animals used in this BATA study had

previously been used in other BATA studies and so did not require any further training on the Davis Rig.

3.3.2.4 Trial overview

This work was conducted over three separate BATA studies, one assessing each bitter-blocker. The commercially available “Davis MS-160” lickometer from DiLog Instruments (Tallahassee, Florida, USA) was used for these experiments.

Water was removed from home cages 21 hours before the Davis rig run on the following day. The first day re-habituated the animals to the Davis Rig for up to 40 minutes and randomly presented 16 bottles twice each containing water only. Each rat was allowed free access to water in their home cage for at least 2.5 hours, prior to a further 21-hour water deprivation. Following this time period, each rat in turn was placed in the Davis Rig for up to 40 minutes and randomly presented bottles containing water and various concentrations of bitter-blocker to confirm rats’ acceptance at each concentration. On completion, the rats were again allowed free access to water prior to further Davis Rig sessions (each following a 21-hour water restriction) on the following two days which exposed the rats to the test solutions. Following completion of the study, water was freely available to rats in their home cage.

Each bottle was presented for eight seconds on two occasions during each session in the Davis Rig, with a two second water rinse (bottle 8) between each compound presentation. The first bottle presented each day was always water (bottle 3). The total number of licks from each presentation was counted by the computer.

All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and GSK Policy on the Care, Welfare and Treatment of Animals.

3.3.2.5 Animal welfare

Rats were health assessed and observed to ensure they all exhibited normal behaviours both during and after the study. Food consumption was expected to decrease during the water restriction phase so daily water and food consumption were recorded. Transient weight loss was also expected; this is not deemed detrimental to the welfare of the rats as weight is usually recovered during the rehydration period however weight gain was monitored daily. If at any point during the study, the recorded weight dropped by more than 15% of either the initial weight or previous day weight, advice would of been sought from the study director, Named Veterinary Surgeon (NVS)/ Named Animal Care Welfare Officer (NACWO) before removing the animal from the study. If any other unexpected variations to health or welfare were seen during the daily checks, the study director and NVS/NACWO would have been informed and the animal may have been euthanised by either an IP overdose of pentobarbitone or by exposure to a rising concentration of CO_2. Death would have been confirmed by cervical dislocation.

3.3.2.6 Data processing

All data were imported into Microsoft Excel 2016 (©Microsoft, Redmond, Washington) to process before analysis. Licks equal to 0 or 1 were excluded to avoid incorporating falsely registered licks, for example if the animal made contact with the spout with his nose whilst sniffing without actually tasting the

sample. Also ‘rinse’ data, which represented the licks taken in the 2 second rinse in between test samples was excluded.

Statistics and graphs were produced using R-studio software (version 3.5.0). The normality of the data distribution was identified using the Shapiro-Wilk test. If this test highlighted the data does not follow parametric distribution, then Kruskal-Wallis rank sum test with Gao post-hoc analysis was used to compare differences in data sets (Gao *et al.*, 2008). If the data distribution was normal, the one-way analysis of variance was performed with Tukey’s post-hoc analysis.

Results were displayed using notched box-plots, showing the median and interquartile ranges, with whiskers that represent 1.5 times the 25^{th} and 75^{th} percentile. The notches display the 95% confidence interval of the median. Outliers are shown as circles.

3.3.3 Structural assessment

Two methods of structural assessment were used to confirm no interaction was occurring between Quinine HCL and the bitter-blockers. Mass spectrometer studies used clinically relevant concentrations of compounds and NMR studies used a higher set concentration of 5mg/mL of each compound. This was to see if concentration had any impact on covalent interaction with Quinine HCL.

3.3.3.1 Materials

Quinine HCL, sodium acetate and sodium gluconate were purchased from Sigma-Aldrich (Gillingham, UK). Adenosine 5’-monophosphate sodium salt

was purchased from Insight biotechnology (Middlesex, UK). D_2O (99.9%) was purchased from Cambridge Isotope Laboratories (Andover, UK).

3.3.3.2 Mass spectrometer studies

Bitter-blockers, at concentrations found to be efficacious in human panels as shown in table 3.2 (100mM (8.2 mg/mL) sodium acetate, 300mM (65.4 mg/mL) sodium gluconate and 20mM (7.4 mg/mL) NaAMP), in formulation with EC50 0.26mM (0.1 mg/mL) Quinine HCL were run through TOF (time of flight) mass spectroscopy using a Q-TOF premier mass spectrometer. Spectra were compared to Quinine HCL alone to confirm no covalent interaction occurred and that the peak corresponding to Quinine HCL remained. Solutions for mass spectroscopy were made up in deionised water. Compound and fragment structures were drawn using ChemDraw 19.0.

3.3.3.3 NMR studies

^1H NMR spectra of each bitter blocker alone, of Quinine HCL alone and of each blocker in formulation with Quinine HCL in solution were recorded to investigate the potential interaction. These studies used a set concentration of 5mg/mL of each bitter-blocker (corresponding to 61.0 mM sodium acetate, 22.9 mM sodium gluconate and 13.5mM NaAMP) alongside 0.26mM (0.1 mg/mL) Quinine HCL to see if a higher concentration of bitter-blocker had any effect on drug interaction. Solutions of samples were prepared in 1mL D_20 (reference solvent). ^1H NMR spectra were recorded on a Bruker Avance 400 mHZ NMR spectrometer. The scan number was set to 200 and the spectra were processed using ‘topspin’ software (version 4.0.7). The solvent peak was used to calibrate the axis. Compound structures were drawn using ChemDraw 19.0.

3.4 Results and discussion

3.4.1 E-tongue

3.4.1.1 Sodium acetate

Ten concentrations of sodium acetate alone from 10-200mM were assessed. The larger the sensor output, the more bitter the formulation is being perceived to be by the e-tongue. There was a clear concentration response seen with sodium acetate with increasing concentration resulting in higher output (figure 3.3). 30, 25, 20 and 10mM sodium acetate did not produce a detectable response so are not seen on the figure.

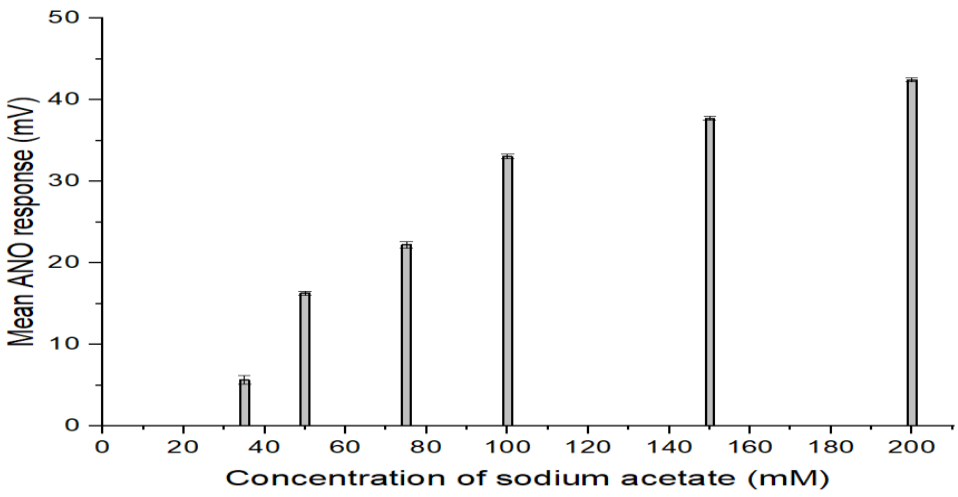


Figure 3.3 Dose response curve to sodium acetate alone. Concentrations ≤ 30mM did not produce a detectable mV response

Sodium acetate was also evaluated in solution with 0.26mM Quinine HCL (figure 3.4). 0mM sodium acetate represented the Quinine HCL control solution. Each concentration of sodium acetate significantly reduced the

sensor response compared with Quinine HCL alone ($p<0.05$) with 20mM producing the most significant reduction in output (figure 3.4).

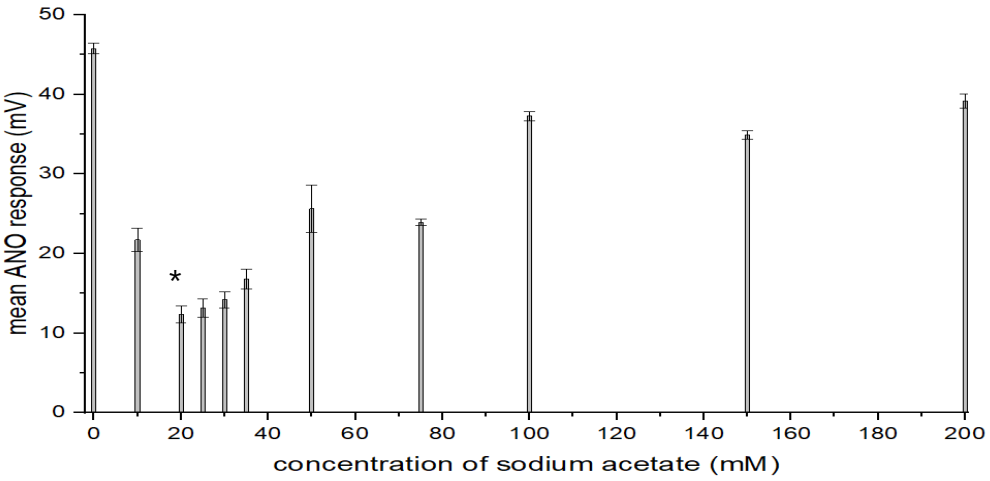


Figure 3.4 E-tongue response to Quinine HCL in solution with sodium acetate. All concentrations of sodium acetate significantly reduced sensor output compared to Quinine HCL alone with *20mM reducing the sensor response the most ($p<0.05$).

The measurement of after taste (denoted as CPA1) after a light washing of the sensor, determined the bitter output of Quinine HCL to be approximately 9mV; a 5-fold reduction in bitterness. The bitter output for the solutions containing sodium acetate remained around 14-17Mv, a reduction from the initial values but suggesting more intensity of bitterness remained compared to the Quinine HCL control (figure 3.5).

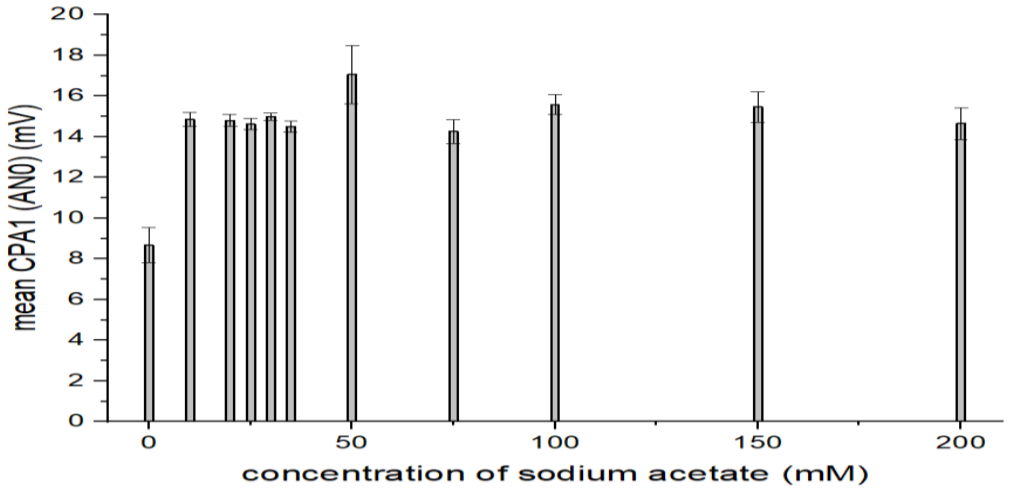


Figure 3.5 E-tongue aftertaste response to Quinine HCL in solution with sodium acetate.

3.4.1.2 Sodium gluconate

Ten concentrations of sodium gluconate alone from 75-600mM were assessed. Overall, there was a concentration-dependent response seen with increasing concentration resulting in increasing output, except for 275mM which gave an unexpectedly high output. It is not clear why this is the case but perhaps is due to lingering bitter-blocker which had not been sufficiently washed off during the previous cleaning process (figure 3.6). 75mM sodium gluconate did not produce a detectable response so is not seen on the figure.

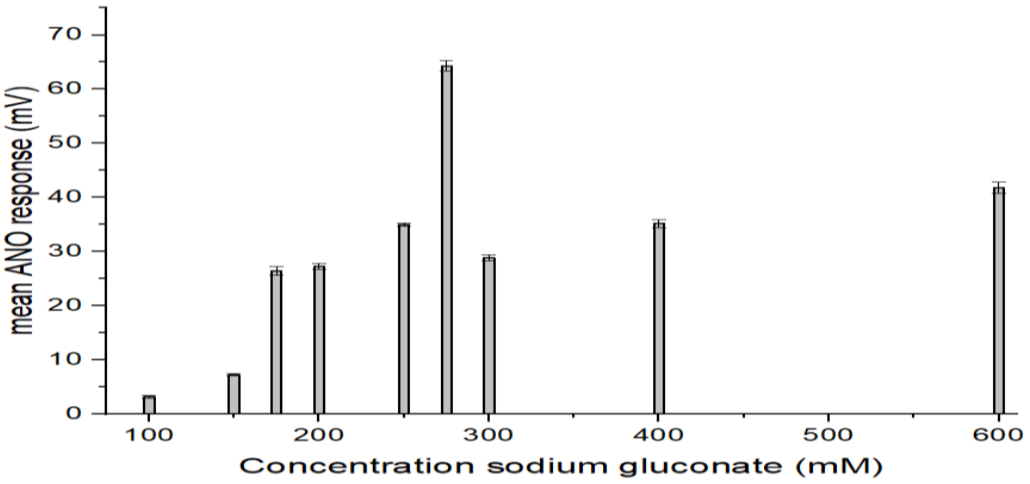


Figure 3.6 Dose response curve to sodium gluconate alone. 75mM did not produce a detectable mV response

Sodium gluconate was also evaluated in solution with 0.26mM Quinine HCL. 0mM sodium gluconate represented the Quinine HCL control solution. Each concentration of sodium gluconate significantly reduced the sensor response compared with Quinine HCL alone ($p<0.05$) with 75mM producing the most significant reduction in output (figure 3.7).

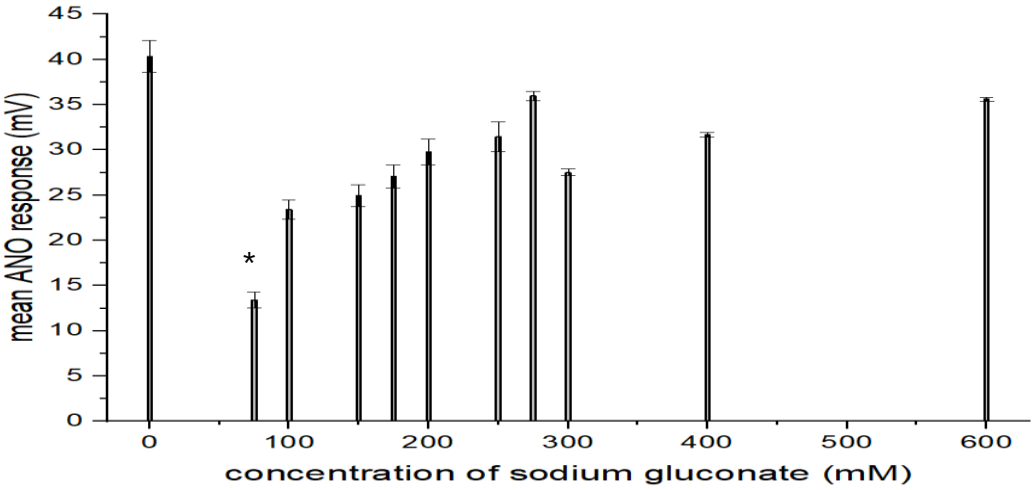


Figure 3.7 E-tongue response to Quinine HCL in solution with sodium gluconate. All concentrations of sodium gluconate significantly reduced sensor output compared to Quinine HCL alone with *75mM reducing the sensor response the most ($p<0.05$)

The measurement of after taste determined the bitter output of Quinine HCL to be significantly reduced compared to the initial mV response. The bitterness of all the solutions containing sodium gluconate also reduced substantially but remained significantly higher than Quinine HCL alone (figure 3.8).

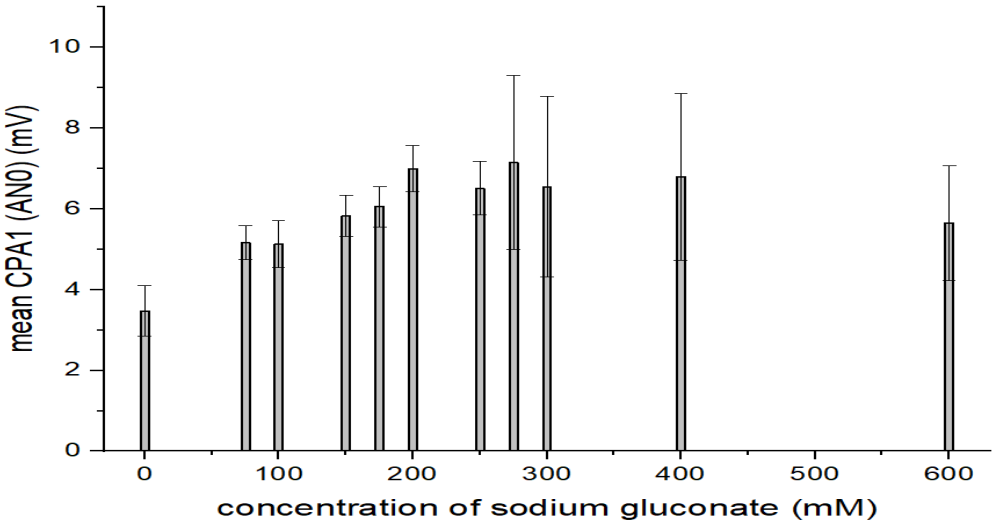


Figure 3.8 E-tongue aftertaste response to Quinine HCL in solution with sodium gluconate.

3.4.1.3 NaAMP

Ten concentrations of NaAMP alone from 0.5-40mM were assessed. There was a concentration-dependent response seen with increasing concentration resulting in increasing output (figure 3.9). 5, 4, 3, 2, 1, 0.5mM NaAMP did not produce a detectable response so are not seen on the figure.

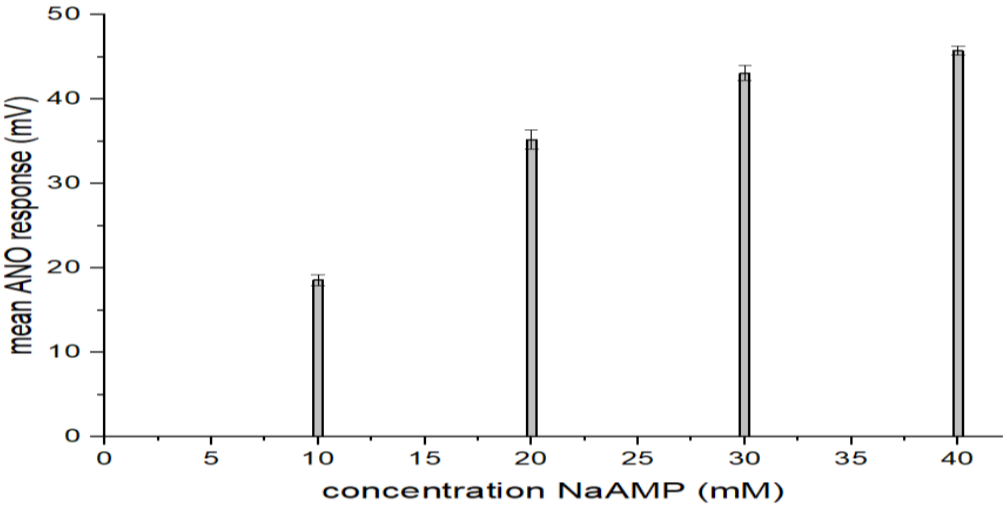


Figure 3.9 Dose response curve to NaAMP alone. Concentrations ≤ 5mM did not produce a detectable mV response

NaAMP was also evaluated in solution with 0.26mM Quinine HCL. 0mM NaAMP represented the Quinine HCL control solution. Each concentration of NaAMP (except 40mM) significantly reduced the sensor response compared with Quinine HCL alone ($p<0.05$) with 4mM producing the most significant reduction in output (figure 3.10).

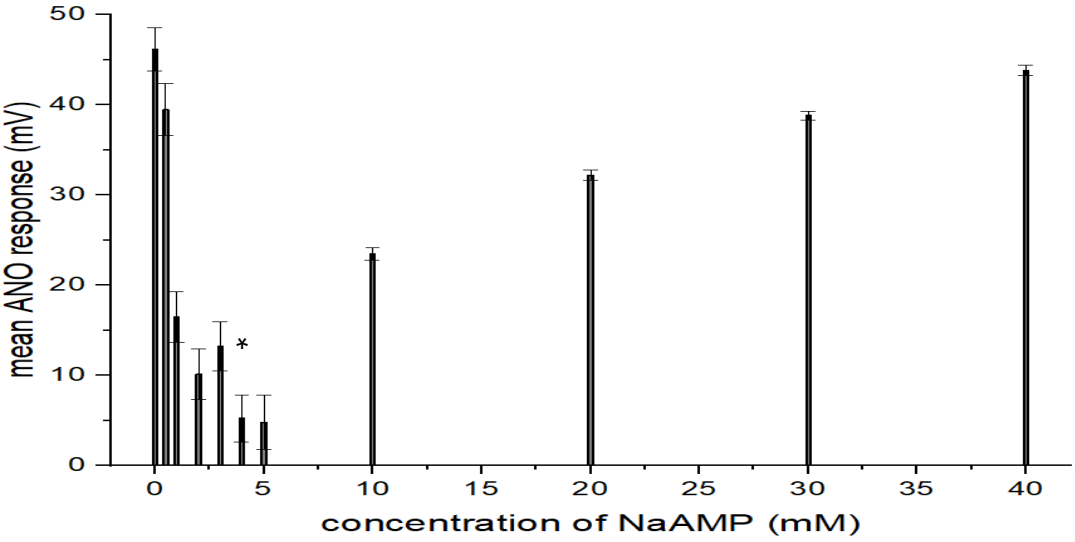


Figure 3.10 E-tongue response to Quinine HCL in solution with NaAMP. All concentrations of NaAMP (except 40mM) significantly reduced sensor output compared to Quinine HCL alone with *4mM reducing the sensor response the most ($p<0.05$).

The measurement of after taste determined the bitter output of Quinine HCL to be significantly reduced compared to the initial mV response. The aftertaste of all the solutions containing NaAMP remained significantly higher than Quinine HCL alone (figure 3.11).

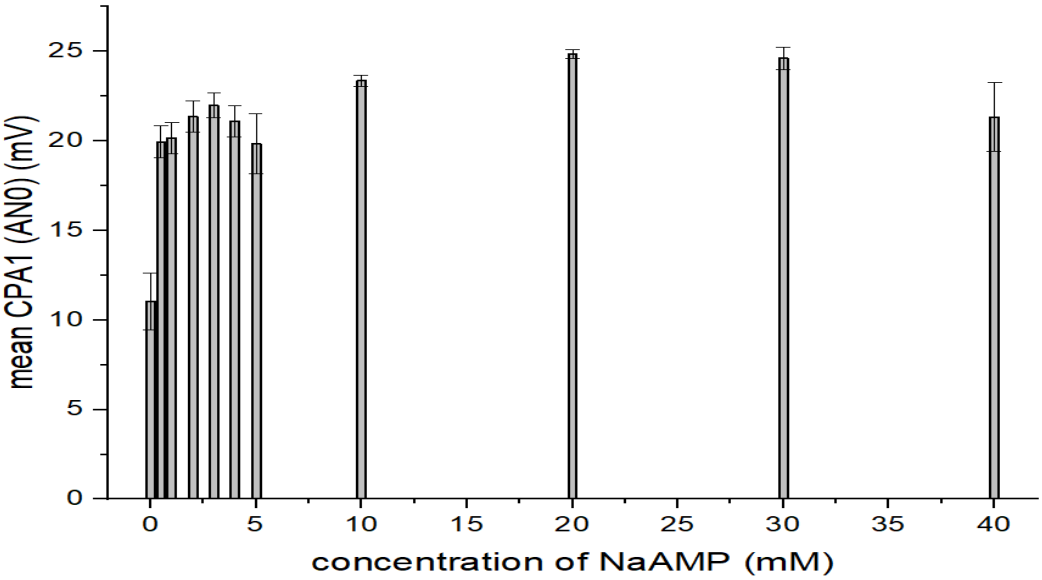


Figure 3.11 E-tongue aftertaste response to Quinine HCL in solution with NaAMP

3.4.2. Interpreting E-tongue results

3.4.2.1 Different sensor types

When using the e-tongue as a preclinical tool of taste-assessment there are a range of different sensors available to detect different taste qualities of a sample, for example sweetness, bitterness or saltiness (Podrażka *et al.*, 2017). It is possible to use a combination of these sensors in order to generate a principal component analysis which displays a test solution's (e.g. taste-masked API) taste attributes with respect to a control (e.g. bitter API alone). The experiments presented in this chapter exclusively used a bitter

sensor (ANO) because the aim was to evaluate the effect of sodium acetate, sodium gluconate and NaAMP on Quinine HCL (which is known to be bitter) in order to inform subsequent BATA studies. The aim was not to fully investigate the taste profile of the bitter-blockers themselves therefore, the use of multiple sensors was not deemed necessary. Furthermore, the tastes of these bitter-blockers is known in humans; sodium acetate has a strong salty flavour compared with sodium gluconate which has a slight saltiness (Breslin and Beauchamp, 1995) and NaAMP has a subtle savoury taste (Keast and Breslin, 2002). In the future, as an extension of developing the e-tongue as a preclinical tool, it would be interesting to use alternative sensors, for example one which responds to saltiness, to understand how the e-tongue perceives the taste-qualities of the bitter-blockers and if that corresponds to the human perception.

3.4.2.2 E-tongue summary

The results of this e-tongue work, which looked exclusively at the taste-attribute of bitterness, suggested that each tested concentration of the three bitter-blockers was able to significantly supress the bitterness to Quinine HCL (with the exception of the highest concentration of NaAMP, 40mM, where the Quinine HCL control had a significantly similar output to the taste-masked formulation). For all three bitter-blockers, the e-tongue suggested a far lower concentration could confer bitter blocking than was assessed in human panels in the literature. These human panels did not assess a range of bitter-blocker concentrations and so it is plausible that lower concentrations could convey taste-masking. However, as eluded to previously, the e-tongue should not be used as a standalone model to predict palatability and in vivo systems, such

as the BATA model, need to be used to confirm findings (Woertz *et al.*, 2011b).

3.4.2.3 Aftertaste vs adsorption

The E-tongue findings suggested that the aftertaste of bitter-blocker containing solutions were stronger than that of the Quinine HCL control. Given the mechanism of action of the bitter-blockers which directly target receptors rather than acting on Quinine HCL itself, it is likely that the salts are interacting with the e-tongue sensor and absorbing to its surface – washing off fully during the alcohol rinsing step. This manifests as a stronger output and assumed after-taste which may not be a true representation of what is happening. This theory is supported by the fact that when each bitter-blocker was run through the e-tongue alone (without Quinine HCL) to assess the bitter response to the salts themselves, the more bitter-blocker present the stronger the mV response. Given this mechanism of action, the e-tongue is not best placed to assess these compounds but offered a useful stepping stone to inform the BATA studies which are a more predictive tool in palatability assessment.

3.4.3 BATA

3.4.3.1 Sodium acetate

Sodium acetate was assessed at 5 concentrations (3, 10, 30, 100 and 300mM), based around e-tongue findings. Each concentration of sodium acetate was first assessed as a standalone solution and then alongside IC50 (0.14mM) and IC90 (1.8mM) Quinine HCL. The higher the number of licks, the more palatable the solution is deemed to be. Water represents the palatable control.

Sodium acetate alone was very well tolerated by the rats, only 300mM sodium acetate had a statistically different lick count to water ($p<0.05$) with the rest being as palatable as water (figure 3.12).

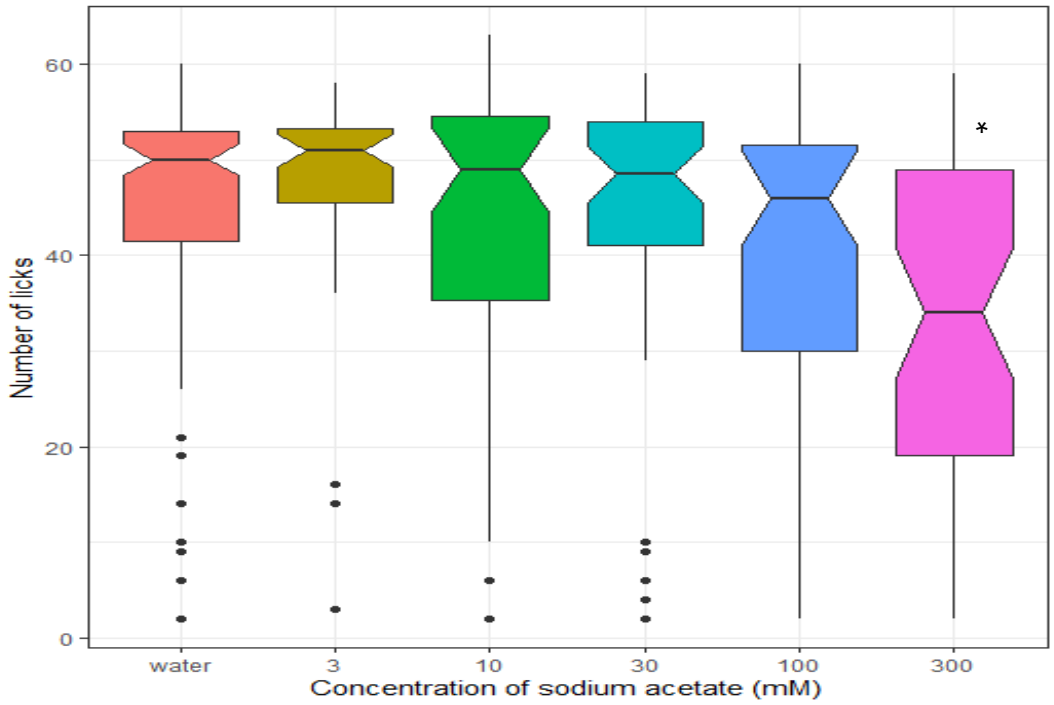


Figure 3.12 Sodium acetate dose response curve in the BATA. *Denotes significant difference in lick rate compared with water control.

No concentration of sodium acetate in solution with either IC50 (figure 3.13a) or IC90 (figure 3.13b) Quinine HCL, improved the perceived bitterness to the respective Quinine HCL control.

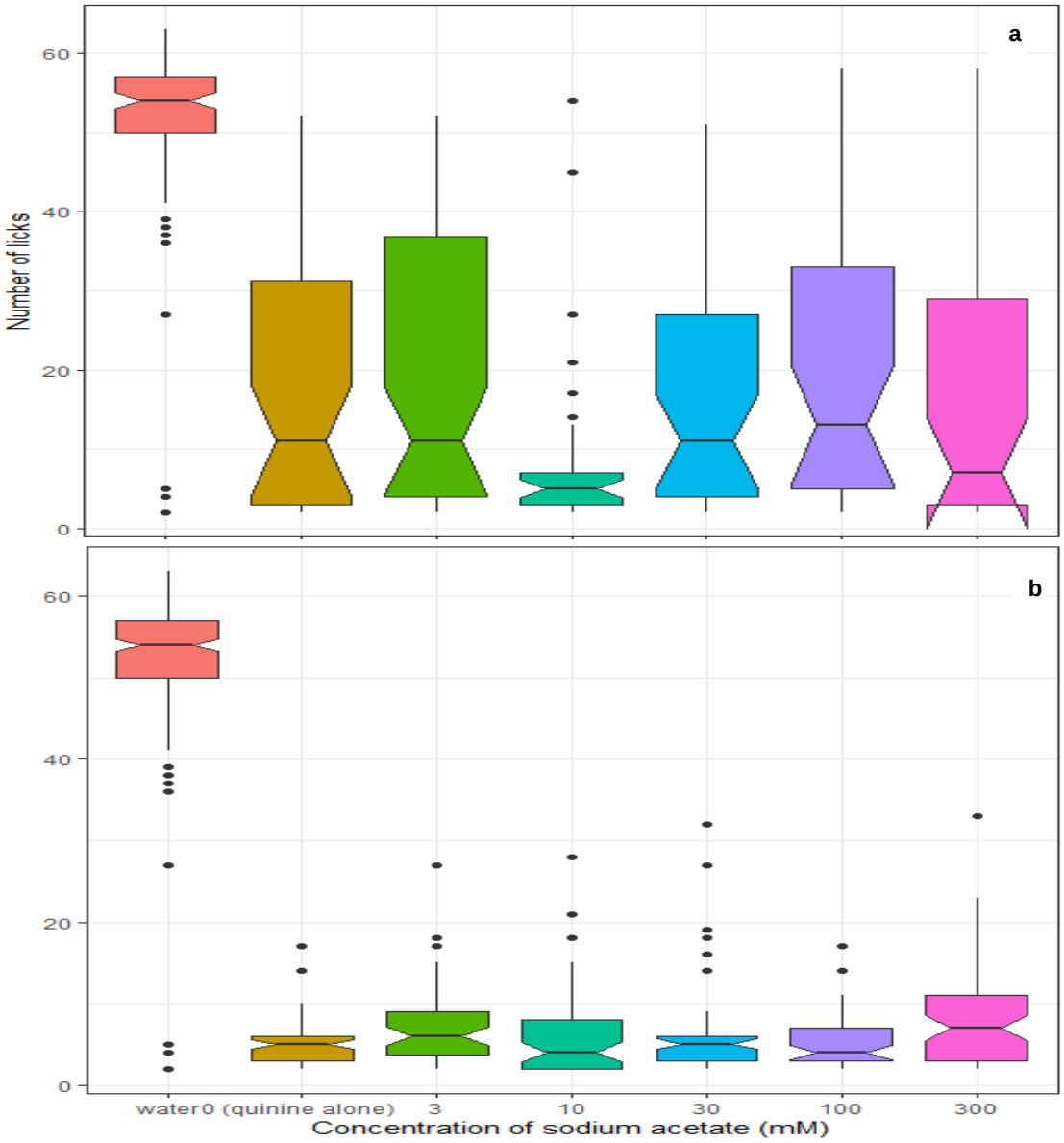


Figure 3.13 Sodium acetate + Quinine HCL dose response curve in the BATA a) IC50 Quinine HCL b) IC90 Quinine HCL

3.4.3.2 Sodium gluconate

Sodium gluconate was assessed at 5 concentrations (10, 30, 100, 300 and 1000mM), based around e-tongue findings. Each concentration of sodium gluconate was first assessed as a standalone solution and then alongside IC50 (0.14mM) and IC90 (1.8mM) Quinine HCL.

The rats gave a varied response to sodium gluconate solutions, resulting in 30 and 300mM being statistically comparable to water but not 10, 100 or 1000mM alone (figure 3.14). Overall the concentrations were well tolerated with the lowest mean lick count (in response to 1000mM) still around 70% of the average lick count for water.

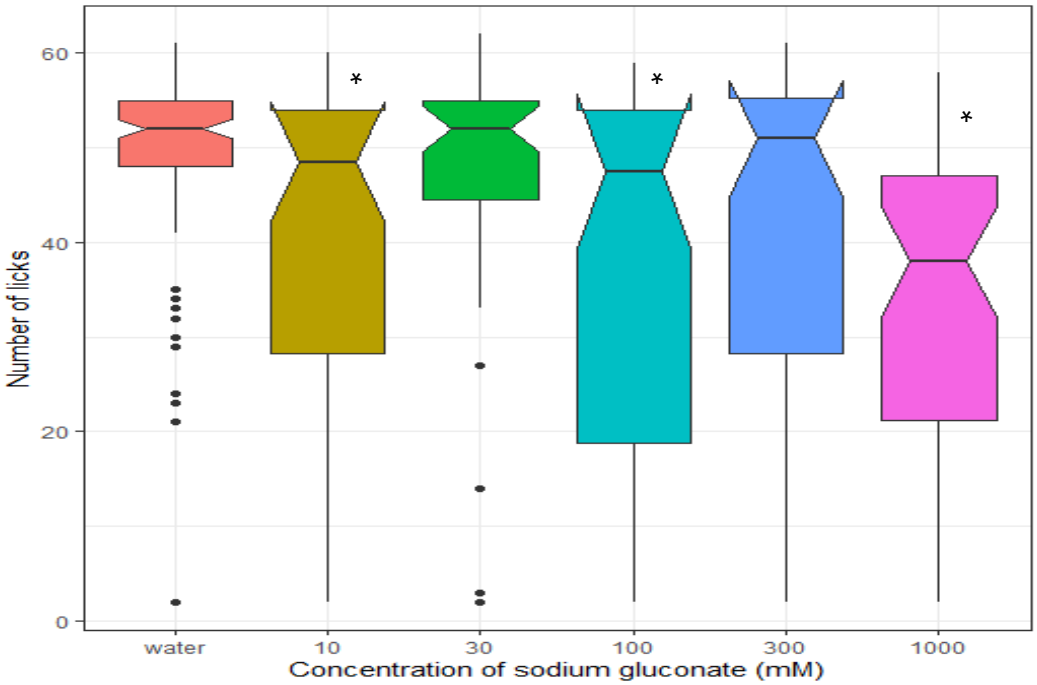


Figure 3.14 Sodium gluconate dose response curve in the BATA. *Denotes significant difference in lick rate compared with water control.

No concentration of sodium gluconate significantly taste-masked IC50 Quinine HCL (figure 3.15a). 1000mM sodium gluconate did improve the perceived bitterness of IC90 Quinine HCL and was significantly different to IC90 Quinine HCL control (figure 3.15b).

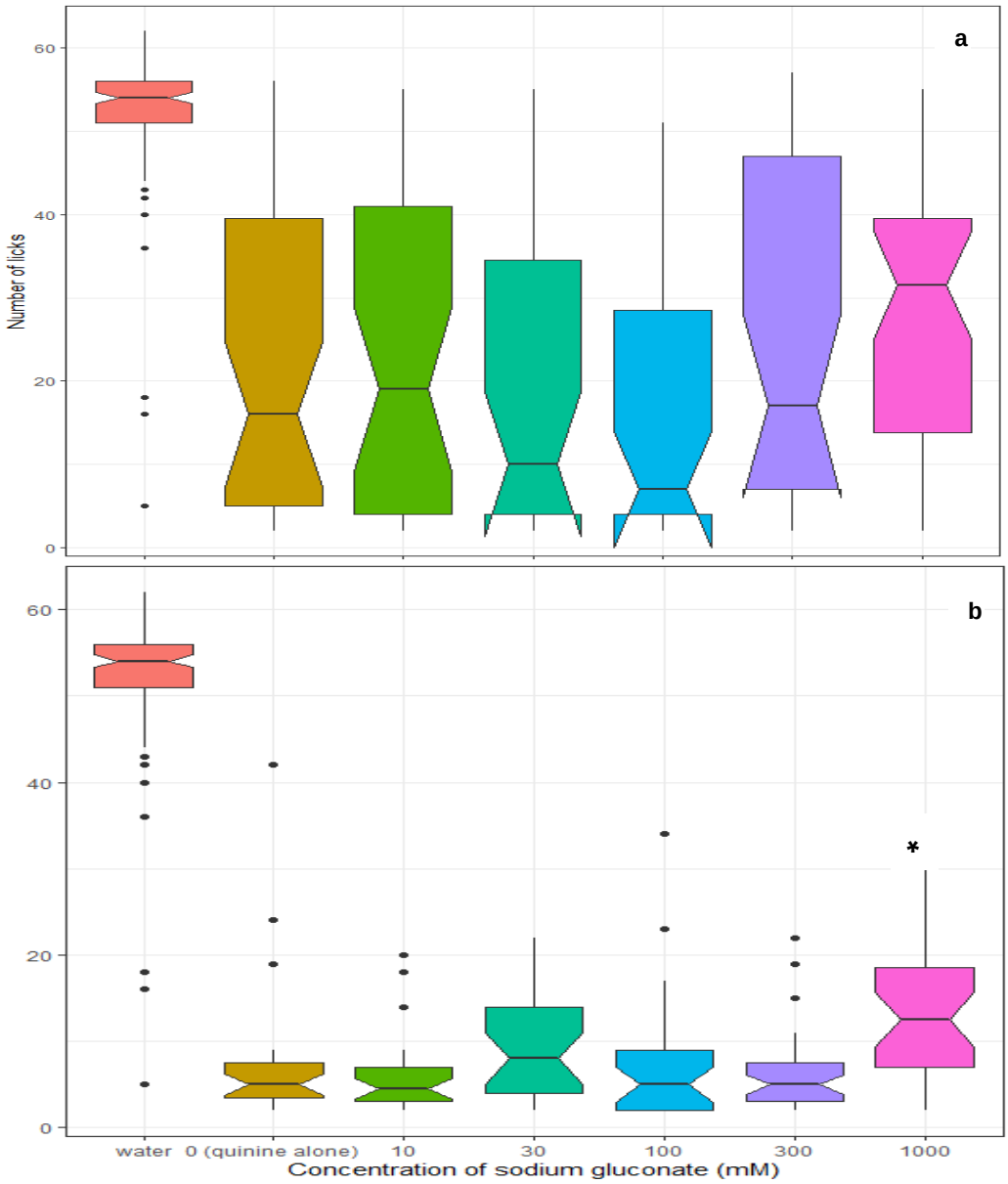


Figure 3.15 Sodium gluconate + Quinine HCL dose response curve in the BATA a) IC50 Quinine HCL b) IC90 Quinine HCL. *Denotes statistically significant improvement compared to Quinine HCL control

3.4.3.3 NaAMP

NaAMP was assessed at 5 concentrations (1, 3, 10, 30 and 100mM), based around e-tongue findings. Each concentration of NaAMP was first assessed as a standalone solution and then alongside IC50 (0.14mM) and IC90 (1.8mM) Quinine HCL.

None of the concentrations of NaAMP were perceived as statistically comparable to water (figure 3.16). The solutions were relatively well tolerated with the lowest mean lick count (in response to 100mM) around 50% of the average lick count for water.

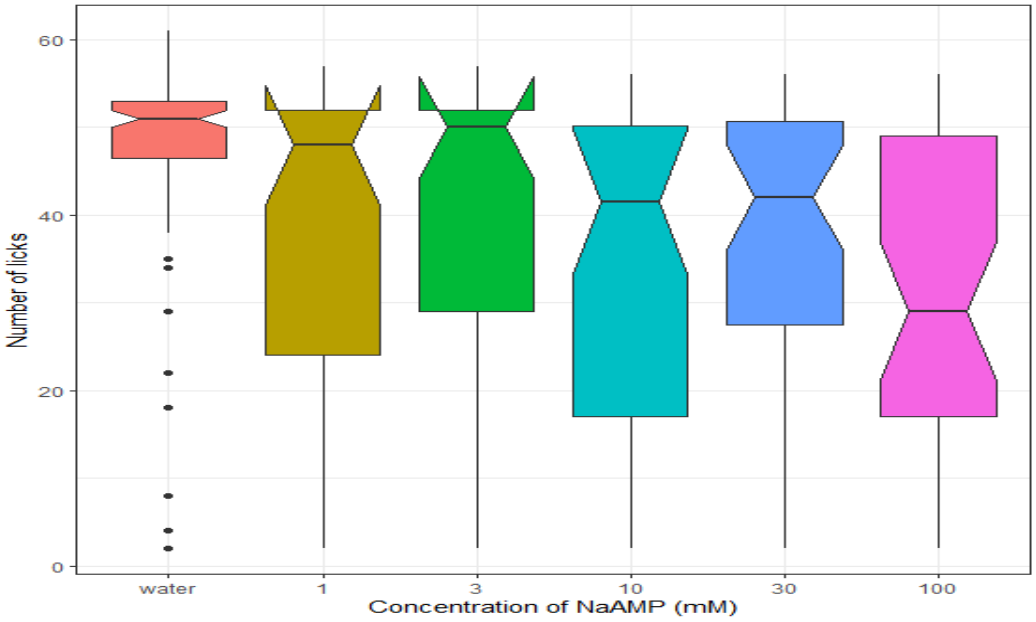


Figure 3.16 NaAMP dose response curve in the BATA.

30 and 100mM NaAMP significantly improved the palatability of both IC50 (figure 3.17a) and IC90 (figure 3.17b) Quinine HCL compared to respective Quinine HCL controls.

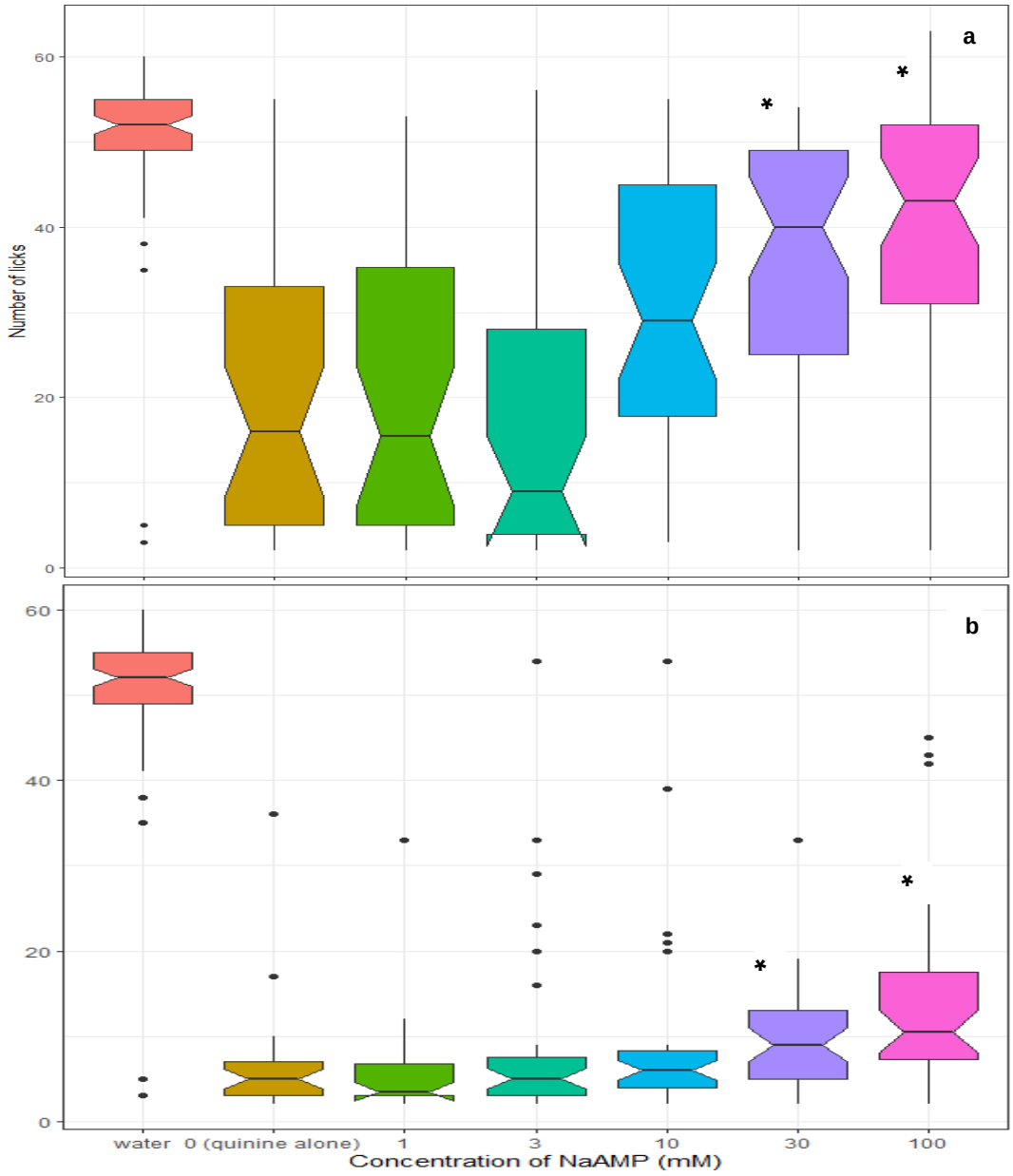


Figure 3.17 NaAMP + Quinine HCL dose response curve in the BATA a) IC50 Quinine HCL b) IC90 Quinine HCL. *Denotes statistically significant improvement compared to Quinine HCL control

3.4.4 Interpreting BATA results

The BATA results showed each of the bitter-blockers alone was well tolerated at low levels, with sodium acetate being the most well tolerated with only the highest concentration tested (300mM) giving a statistically different lick response than water. The salts do not need to be as palatable as water, they

just need to provide enough improvement in palatability to increase compliance to whatever drug they are formulated with.

In terms of bitter-blocking ability the BATA studies gave mixed results. Sodium acetate did not effectively bitter-block Quinine HCL and sodium gluconate only showed a small improvement at 1000mM. Both theses excipients are either not blocking the TAS2Rs that convey Quinine HCL's bitterness, or they are doing so at an insufficient level. At present it is unknown which TAS2Rs are inhibited by sodium acetate and sodium gluconate. Some work has investigated the bitter-blocking effect of sodium gluconate (and another sodium salt, monosodium glutamate) on a range of bitter compounds including 6-n-propylthiouracil in humans (Mennella *et al.*, 2014). 6-n-propylthiouracil is a compound which is perceived as highly bitter in some people or undetectable in others, largely due to TAS2R38 gene variation (Cabras *et al.*, 2012). Neither sodium gluconate nor monosodium glutamate was able to block the perceived bitterness of 6-n-propylthiouracil, suggesting they do not act on TAS2R38 (Mennella *et al.*, 2014). Aside from the knowledge that sodium containing compounds likely do not act at this receptor, very little is known about their exact mechanism of action (Mennella *et al.*, 2014). It would be highly useful to understand which members of the TAS2R family are inhibited by promising bitter-blockers, and also activated by bitter API, so they can be matched within formulations. This is because the use of any bitter-blocker will only be effective if the aversive API is hitting that receptor too. The exact role of each subset of bitter receptor, and how they influence taste perception for different medicinal compounds, is not yet fully understood. Databases such as the 'BitterDatabase' (BitterDB) (Dagan-

Wiener *et al.*, 2019) are beginning to fill these gaps by collating information on bitter molecules and their receptors, for example Quinine HCL (table 3.6). As more knowledge is generated, the use of bitter-blocking compounds can be better directed and make the art of taste-masking more precise.

Table 3.6 TAS2Rs activated in humans by Quinine HCL, information from BitterDB

Bitter drug	**Receptors targeted**
Quinine hydrochloride	TAS2R4 TAS2R7 TAS2R10 TAS2R14 TAS2R39 TAS2R40 TAS2R43 TAS2R44 TAS2R46

In this work, bitter blocking was achieved with NaAMP. It is likely that the umami taste of NaAMP will be acceptable to humans as it has previously been shown that monosodium glutamate (which has a similar savoury taste) is well accepted by both adults and children (Mennella *et al.*, 2014).

AMP has been demonstrated to inhibit the activation of α-Gustducin in bitter receptors by Quinine HCL and other bitter compounds in vitro (Ming, Ninomiya and Margolskee, 1999). α-Gusducin is a G-protein responsible for signal transmission in 25-30% of type II taste-bud cells (which express receptors for sweet, umami and bitter tastants) and therefore a big contributor to bitter taste transduction (Mclaughlin, Mckinnon and Margolskee, 1992; Ahmad and Dalziel, 2020). This mechanism of action means NaAMP is potentially a good candidate for taste-masking as its action is less specific compared to the direct targeting of a handful of bitter-receptor subtypes.

AMP's action is dependent on the bitter drug being a substrate for α-gustducin containing G-proteins.

Sodium, present in all three bitter-blockers, has been shown to be an effective bitter-blocker (Kroeze and Bartoshuk, 1985; Breslin and Beauchamp, 1995; Keast and Breslin, 2002) but the exact mechanism is not well defined and so suitable drug candidates are difficult to predict.

3.4.4.1 Bitter-blockers in children

There is known to be age-related differences in how children perceive certain tastes which could impact the efficacy of these sodium salts as bitter-blockers. Children are more sensitive to bitterness (Forestell, 2017) and to saltiness (Weiffenbach, Baum and Burghauser, 1982; Mojet, Christ-Hazelhof and Heidema, 2001) compared with adults. The taste of sodium gluconate has been evaluated in both adults and children and was found to be well received in children who liked the slight salty flavour more so than adults (Mennella *et al.*, 2014). Moreover, when in formulation with bitter tastants it has been shown that sodium gluconate reduced the bitterness in four out of five API tested (including 0.01mM Quinine) in adults. Whereas, for the same formulations, only two (including 0.01mM Quinine) had reduced bitterness to children (Mennella *et al.*, 2014). This is likely due to the increased sensitivity children show towards bitterness.

Previous work has demonstrated that the majority (70%) of male rats are known as 'super tasters' based on their response to 6-n-propylthiouracil (Keeley *et al.*, 2017) whilst the other 30% are 'medium-tasters'. The super tasters perceived 6-n-propylthiouracil as significantly more aversive than NaCl

(the control taste) whilst the medium tasters found the tastant to be as aversive as NaCl. None of the animals were ‘non-tasters’ which is when the animal perceives 6-n-propylthiouracil as significantly less aversive than NaCl. Children (between 6-12 years old) have also been studied for responses to 6-n-propylthiouracil and this work found 19% were non tasters, 49% were medium tasters and 32% were super tasters (Rupesh and Nayak, 2006) which means over 80% of the children evaluated are sensitive to the bitter tastant. Conversely, work in adults shows almost as many people are non-tasters (27%) as they are super tasters (29%) (Drewnowski *et al.*, 1997) which means fewer adults are sensitive to 6-n-propylthiouracil. It is known that super tasters are more sensitive to bitterness found in alcohol and vegetables (Robino *et al.*, 2014) which suggests they may also be more sensitive to bitter API. Given that children show increased sensitivity to bitter API perhaps the rats are well placed to predict taste-masking in this demographic.

3.4.4.2 Pre-clinical model predictability vs Quinine HCL concentration

NaAMP at 30mM improved the perceived bitterness of IC50 and IC90 Quinine HCL in the BATA model – this is a reflection of the literature reported human panel where 20mM NaAMP improved the bitterness of 0.1mM Quinine HCL (Keast and Breslin, 2002). Conversely, sodium acetate did not offer improvement to the perceived bitterness to either concentration of Quinine HCL. Sodium gluconate did not offer significant improvement to IC50 Quinine HCL however the highest concentration (1000mM) did improve the IC90 Quinine HCL lick response. These results may seem contrary to the literature findings which reported 100mM sodium acetate and 300mM sodium gluconate significantly improved the palatability of Quinine HCL. However, it is

important to look at the concentrations of Quinine HCL used in the literature reports compared to these experiments. In these experiments the IC50 concentration given to the rats was 0.14mM, this is the concentration that in house data has historically shown to produce approximately half of the water lick response. The human EC50 for Quinine HCL is 0.26mM, which has been shown from in house data to produce approximately half the palatability score of water on a sliding scale of bitterness (Soto *et al.*, 2018). This is the concentration which should be used as a benchmark in human panels. However, in the literature, 100mM Sodium acetate was shown to reduce the bitterness of 0.1mM Quinine HCL by 36% in a human panel (Keast and Breslin, 2002) and 300mM sodium gluconate was shown to significantly improve the palatability of 0.119mM Quinine HCL by around 20% in children in a forced-choice test (Mennella *et al.*, 2014).

The human panel (from the literature), e-tongue and BATA results are summarised in table 3.7.

Table 3.7 Comparison of effective bitter-blocking concentrations using the e-tongue, BATA and human panels reported in the literature – the latter used Quinine HCL at low concentrations.

Compound	**Human**	**E-tongue**	**BATA**
Sodium acetate	100mM	20mM	No improvement up to 300mM
Sodium gluconate	300mM	75mM	1000mM (vs IC90 Quinine HCL)
NaAMP	20mM	4mM	30 and 100mM (vs IC50 and IC90 Quinine HCL)

The e-tongue experiments used the appropriate concentration (EC50 0.26mM) of Quinine HCL as the bitter control yet predicted far lower concentrations of bitter-blocker to be effective than was seen in vivo. As previously discussed, the adsorption seen by the sodium salts may give misleading results – perhaps the salt which had adsorbed to, and built up on, the sensor's surface was physically preventing Quinine HCL from acting on the sensor and so the API was perceived as less bitter than it would in an in vivo system.

3.4.4.3 Rat response to saltiness

As explored in chapter 2, rats can display neophobic tendencies to novel tastes; sodium acetate has a stronger salty flavour compared with sodium gluconate (Breslin and Beauchamp, 1995) and NaAMP (which has a slight savoury taste) (Keast and Breslin, 2002). This saltiness is new to the animals. Separating the lick response to each concentration of sodium acetate by day, there is an indication of this effect with the day 1 licks consistently lower than day 2 (figure 3.18). However, this effect is not significant and using day 2 data alone did not impact the results.

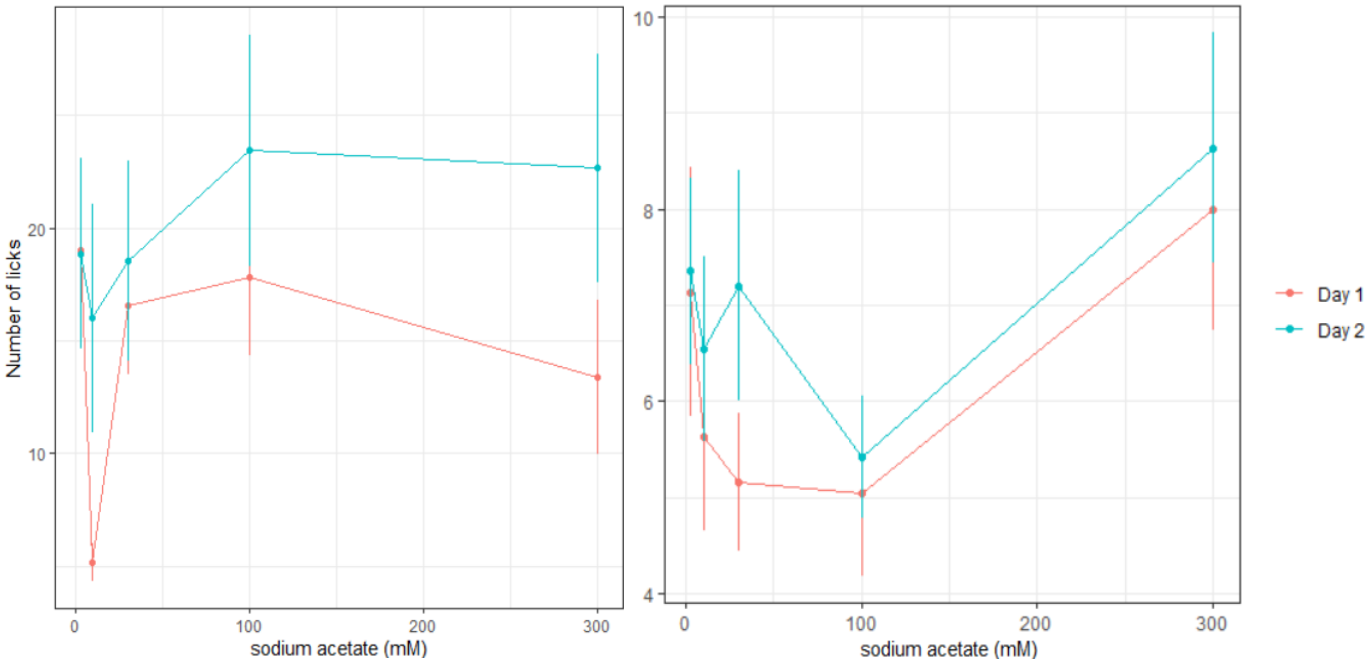


Figure 3.18 BATA results of sodium acetate in solution with IC50 Quinine HCL (left) and IC90 Quinine HCL (right) by day, day 1 licks are consistently lower than day 2 for comparable concentrations of sodium acetate.

The rat's response to sodium gluconate did not follow this pattern, with no clear difference between day 1 and day 2 responses, likely owing to the more subtle flavour of sodium gluconate (figure 3.19).

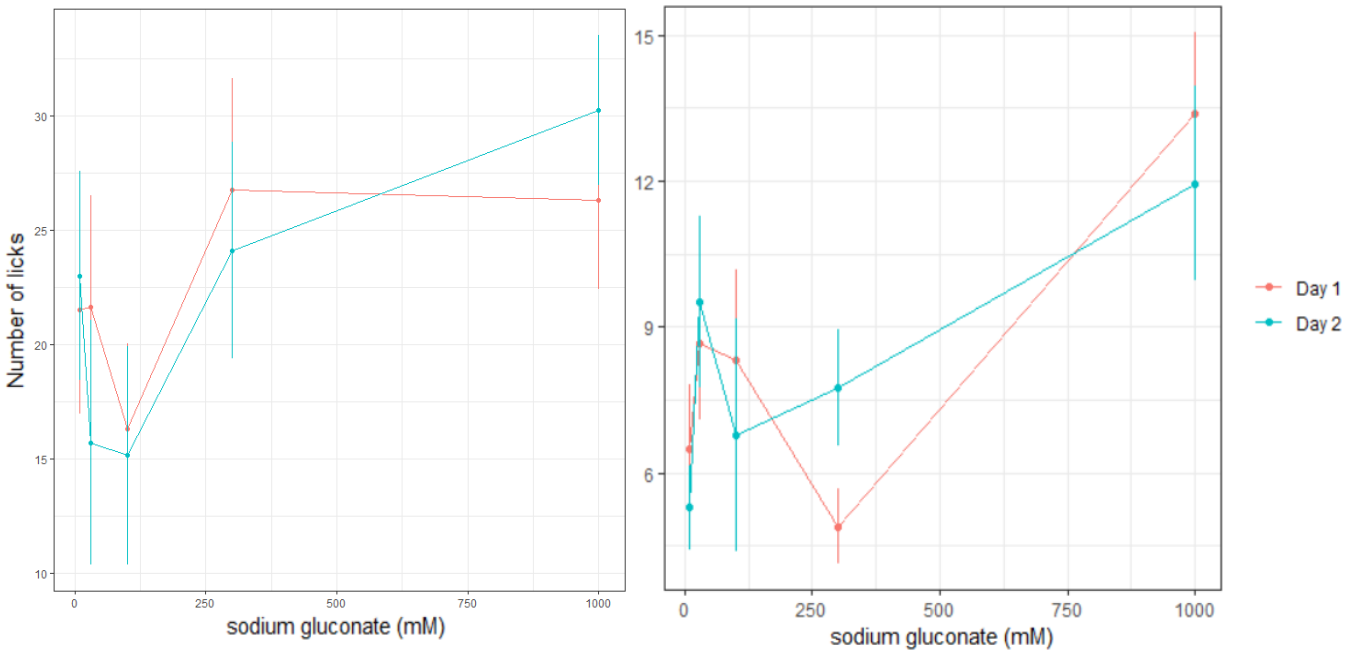


Figure 3.19 BATA results of sodium gluconate in solution with IC50 Quinine HCL (left) and IC90 Quinine HCL (right) by day

The rat's displayed signs of neophobia to the slight umami taste of NaAMP when in solution with 0.14mM Quinine HCL. However, there was no evidence of this when in solution with 1.8mM Quinine HCL (figure 3.20). This is likely to be because this IC90 concentration is highly aversive and bitter to the rats and so this bitterness is overriding any slight taste of the bitter-blocker itself. Using day 2 data alone did not impact the results.

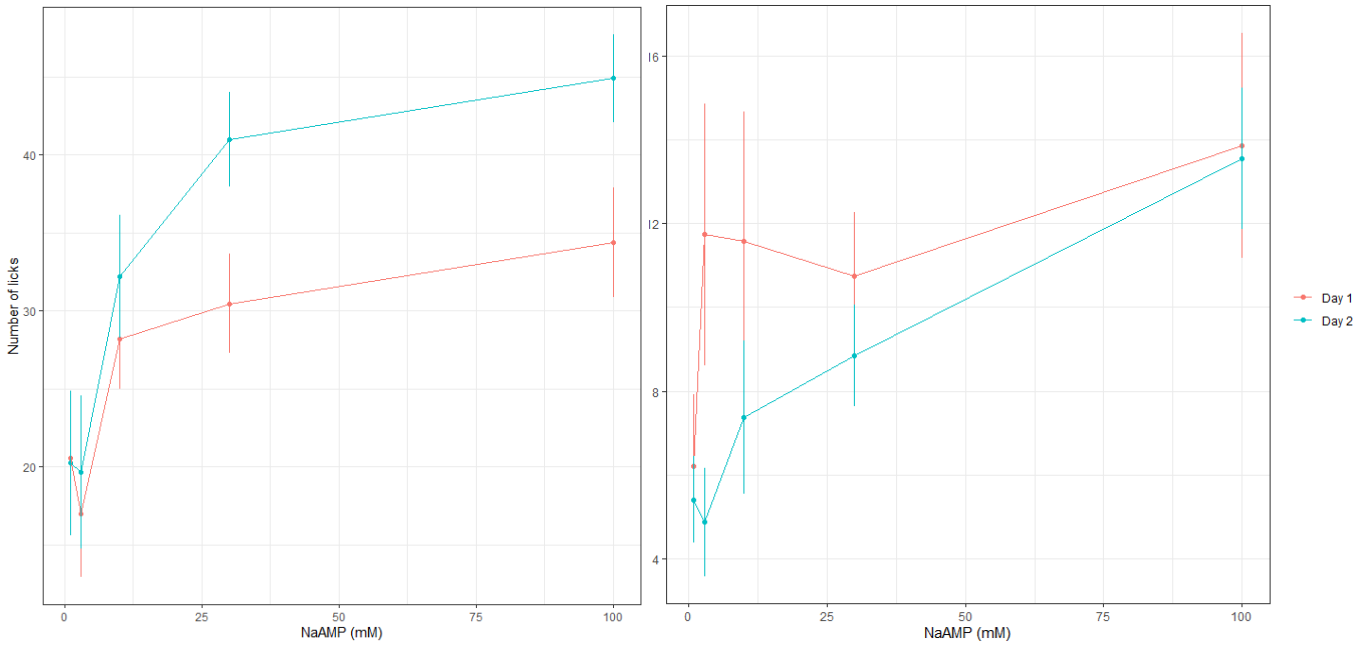


Figure 3.20 BATA results of NaAMP in solution with IC50 Quinine HCL (left) and IC90 Quinine HCL (right) by day.

3.4.5 Structural assessment

3.4.5.1 Mass Spectrometry

3.4.5.1.1 Quinine HCL

Quinine HCL (molecular weight 360.9g/mol) was run alone to determine its mass spectra without excipients (figure 3.21). The peak at 325 m/z corresponds to the molecular weight of Quinine (without HCL) of 324.42g/mol, with peaks below this corresponding to fragments; the peak at 127 corresponds to $C_9H_5N^2$. Peaks higher than 325 m/z are adducts e.g. 353 m/z is likely to be Quinine + C_2H_4 (molecular weight 28) fragment.

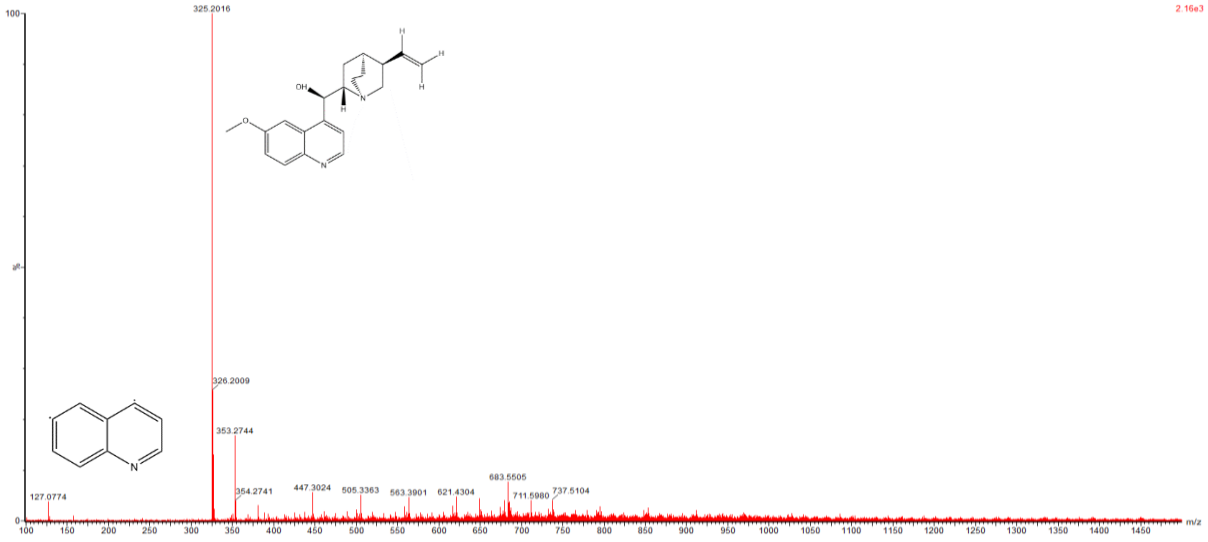


Figure 3.21 Mass spectra of Quinine HCL. 324 g/mol is the molecular weight of Quinine (without HCL), 325 m/z corresponds to Quinine + H^+ with the strucutre of Quinine shown, the structrure of $C_9H_5N^2$ is shown at 127 m/z

3.4.5.1.2 Sodium acetate

Sodium acetate was run alone to determine its mass spectra (figure 3.22). The peaks are all higher than the molecular weight of sodium acetate (82.03g/mol) therefore they are all likely to be adducts of various sodium (molecular weight 22.99g/mol) and acetate (molecular weight 59.04g/mol) for example the peak at 254 is perhaps corresponding to $11Na^+ + H^+$

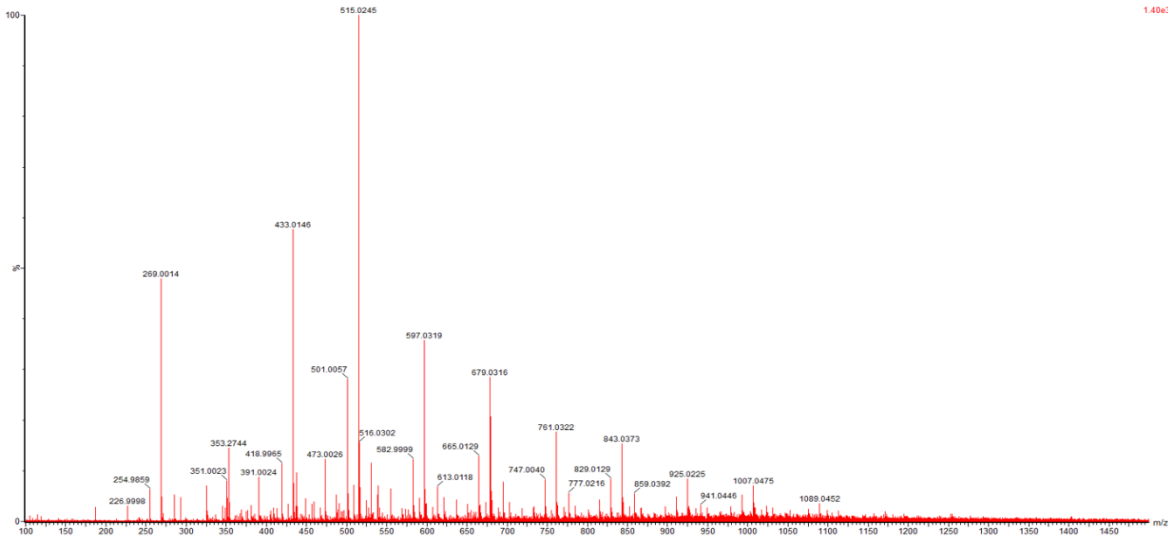


Figure 3.22 Mass spectra of Sodium acetate alone

Sodium acetate in solution with Quinine HCL showed no covalent interaction (figure 3.23). The peak at 325 m/z (corresponding to Quinine HCL) remains clear, the new peaks (269, 433, 501, 515, 597 and 679m/z) are indicative of sodium acetate and feature in the excipient alone spectra. Importantly, none of peaks showed evidence of covalent interaction of any ratio of drug and excipient e.g. no peak around 384m/z or 370m/z which would correspond to 1:1 Quinine:acetate or 1:2 Quinine:sodium respectively.

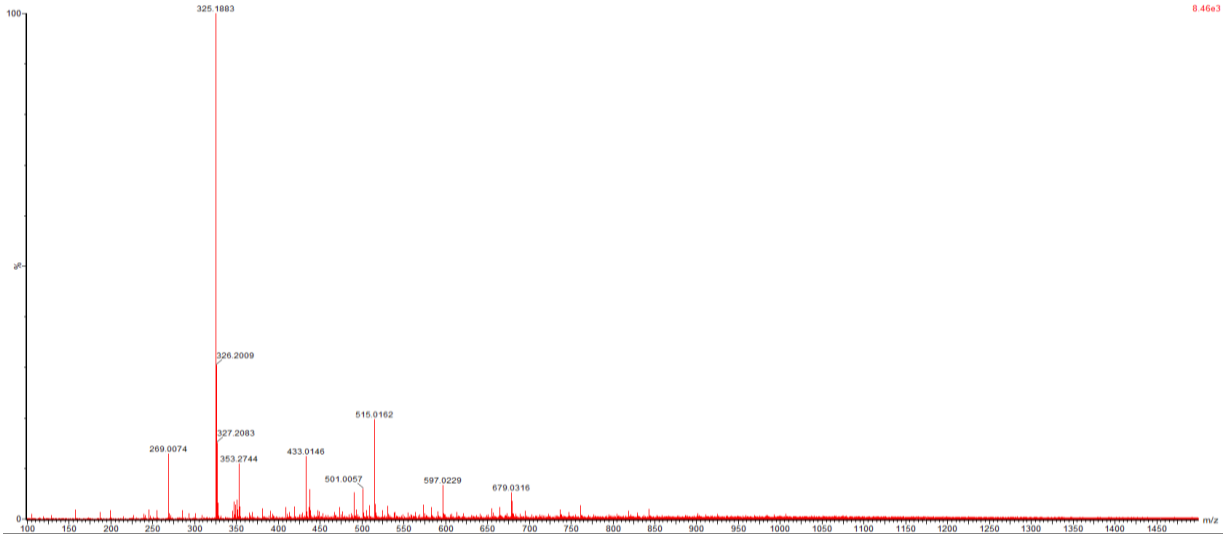


Figure 3.23 Mass spectra of sodium acetate + Quinine HCL 325 m/z corresponds to Quinine HCL + H^+

3.4.5.1.3 Sodium gluconate

Sodium gluconate was run alone to determine its mass spectra (figure 3.24). The peaks are all higher than the molecular weight of sodium gluconate (218.14g/mol) therefore they are all likely to be adducts of various sodium (molecular weight 22.99g/mol) and gluconate (molecular weight 196.16g/mol) for example the peak at 786 is perhaps corresponding to gluconate + gluconate + gluconate + gluconate + H^+ ; 4 $C_6H_{12}O_7$+ H^+

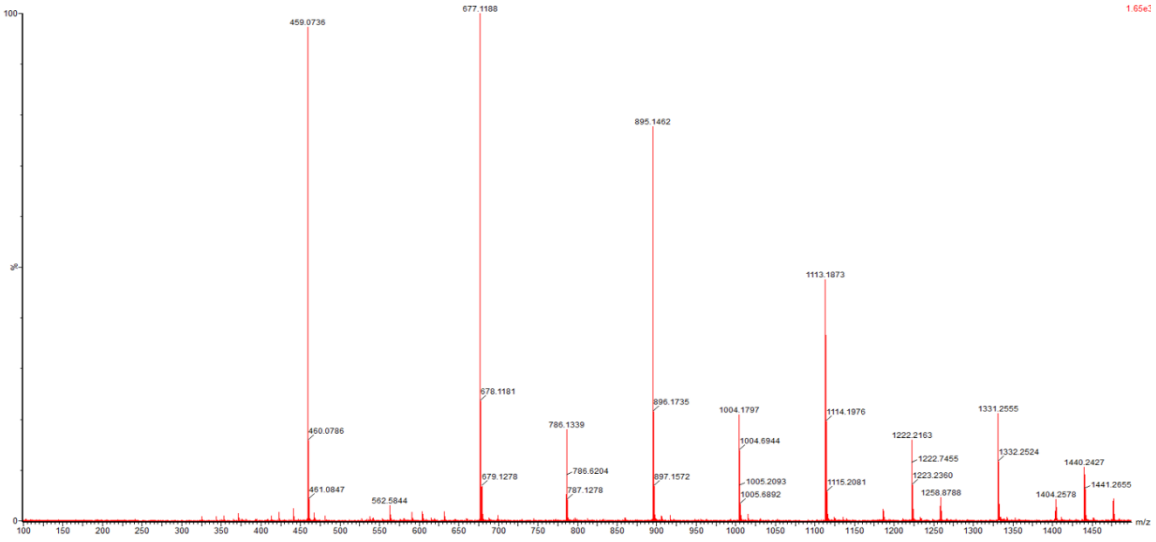


Figure 3.24 Mass spectra of sodium gluconate alone

Sodium gluconate in solution with Quinine HCL showed no covalent interaction (figure 3.25). The peak at 325 m/z (corresponding to Quinine HCL) remains clear, all the new peaks (except 409m/z and 537m/z) feature in the sodium gluconate alone spectra.

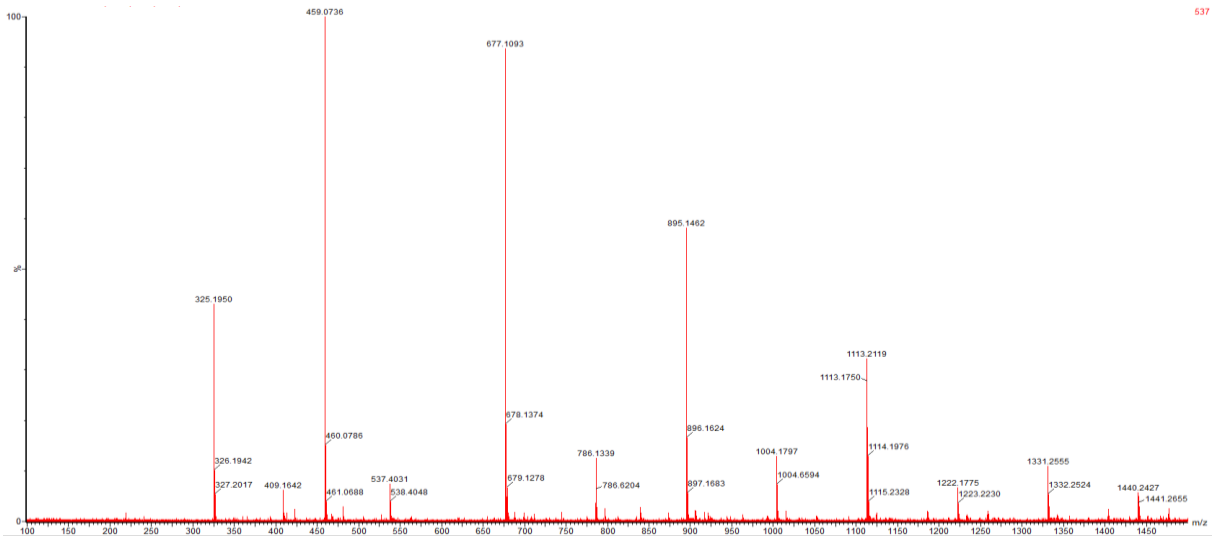


Figure 3.25 Mass spectra of sodium gluconate + Quinine HCL 325 m/z corresponds to Quinine HCL + H^+

It is likely the two previously unseen peaks are still indicative of sodium gluconate, just different adducts are created (figure 3.26). 409m/z is likely to be gluconate + gluconate + oxygen; $C_6H_{12}O_7 + C_6H_{12}O_7 + O^2$ and 537m/z is likely to be gluconate + gluconate + $C_6H_8O_4^{3-}$.

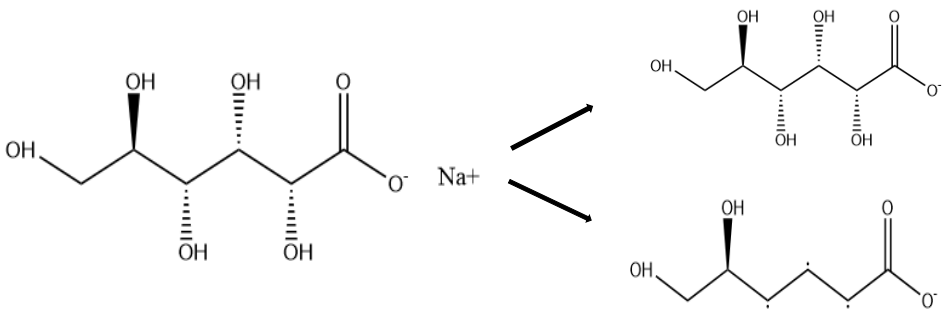


Figure 3.26 Structure of sodium gluconate and fragments Sodium gluconate on the left ($C_6H_{11}NaO_7$) and potential fragments; $C_6H_{12}O_7$ (top right), $C_6H_8O_4^{3-}$ (bottom right)

Importantly, none of peaks showed evidence of covalent interaction of any ratio of drug and excipient e.g. no peak around 520m/z or 763m/z which would correspond to 1:1 Quinine:gluconate or 1:2 Quinine:sodium gluconate respectively.

3.4.5.1.4 NaAMP

NaAMP was run alone to determine its mass spectra (figure 3.27). There are peaks above and below the molecular weight of NaAMP (368.22g/mol) suggesting both adducts and fragments are seen of sodium (molecular weight 22.99g/mol) and AMP (molecular weight 347.22g/mol). There is a peak at 370 m/z which likely corresponds to NaAMP ($C_{10}H_{13}N_5NaO_7P$) + H^+. There is also a peak at 393 m/z which is likely corresponding to NaAMP + Na^+ + H^+.

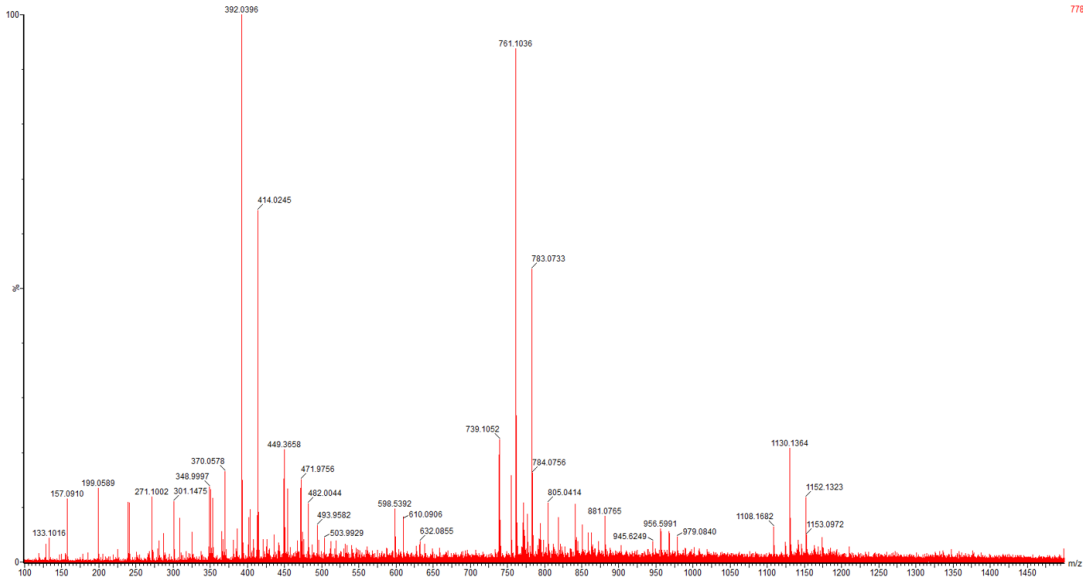


Figure 3.27 Mass spectra of NaAMP alone

NaAMP in solution with Quinine HCL showed no covalent interaction (figure 3.28). The peak at 325 m/z (corresponding to Quinine HCL) remains clear, all the new peaks (except 694m/z) feature in the NaAMP alone spectra. The 694m/z peak is still indicative of NaAMP and is likely to be two AMP ($C_{10}H_{14}N_5O_7P$) adducts.

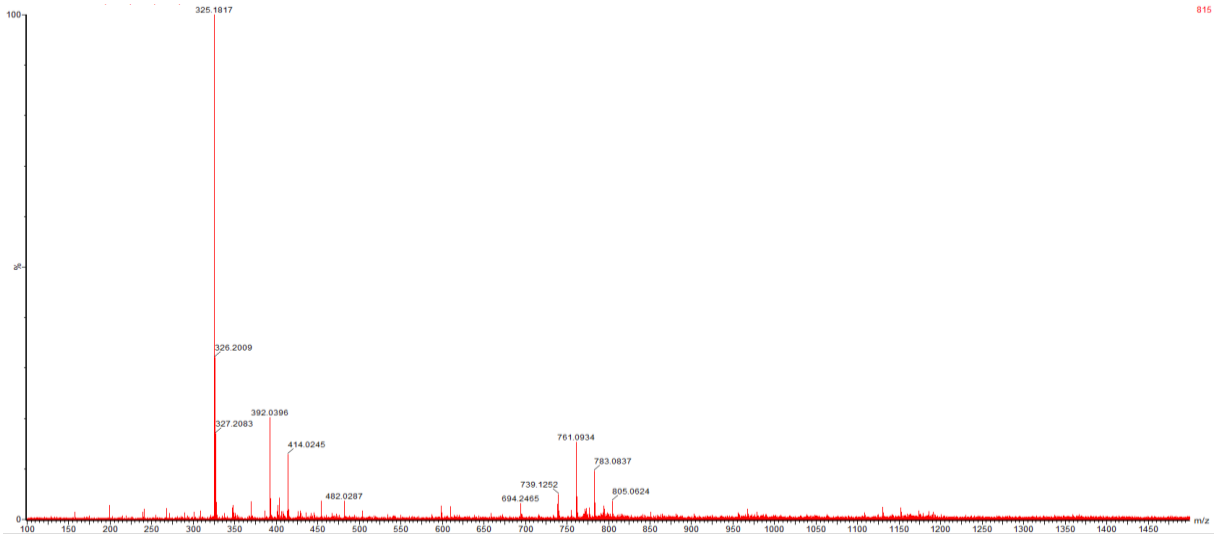


Figure 3.28 Mass spectra of NaAMP + Quinine HCL 325 m/z corresponds to Quinine HCL + H^+

Importantly, none of peaks showed evidence of covalent interaction of any ratio of drug and excipient e.g. no peak around 1019m/z or 1065m/z which would correspond to 1:2 Quinine:AMP or 1:2 Quinine:NaAMP respectively

3.4.5.2 NMR

3.4.5.2.1 Quinine HCL

Quinine HCL was run alone to determine its spectra without excipients (figure 3.29). The peak at 4.65ppm corresponded to D_2O – the solvent used. Quinine HCL has a number of identifying peaks, the largest (in terms of ppm) is 8.65 and the largest (in terms of strength of signal) being at 3.81 so these were chosen as peaks to look for in the excipient/ Quinine HCL mixtures.

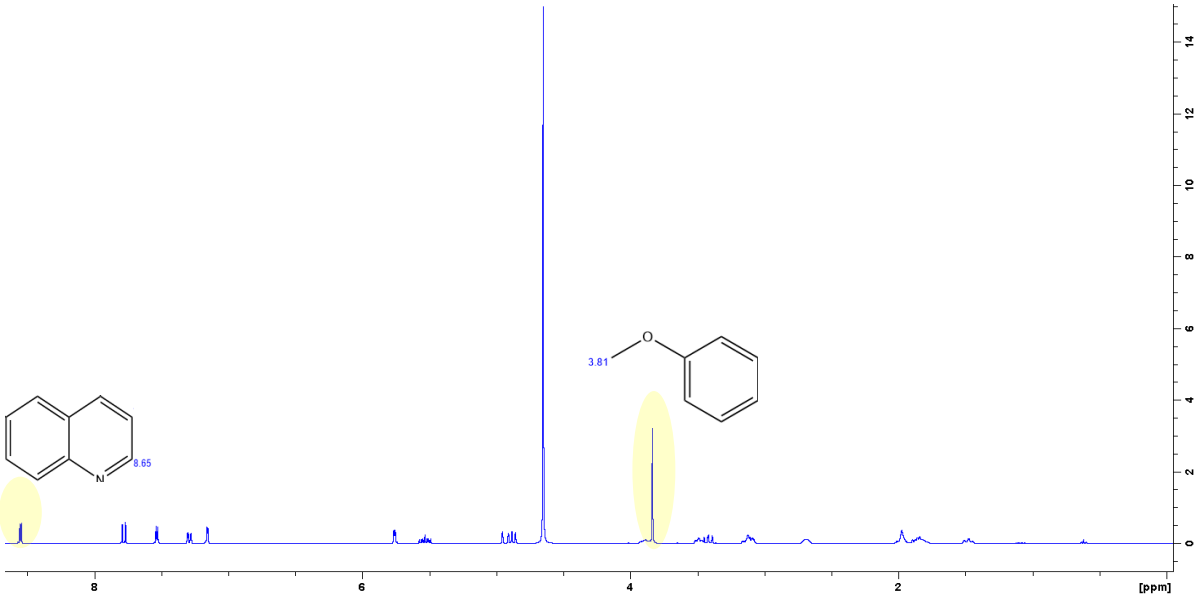


Figure 3.29 ^{1}H NMR spectra of Quinine HCL alone, peak at 4.65ppm corresponds to D_2O, two identifying peaks are highlighted, 8.65 and 3.81ppm, with their respective structures shown

3.4.5.2.2 Sodium acetate

The NMR spectra for sodium acetate showed one peak at 1.80ppm, this corresponded to its structure shown in figure 3.30.

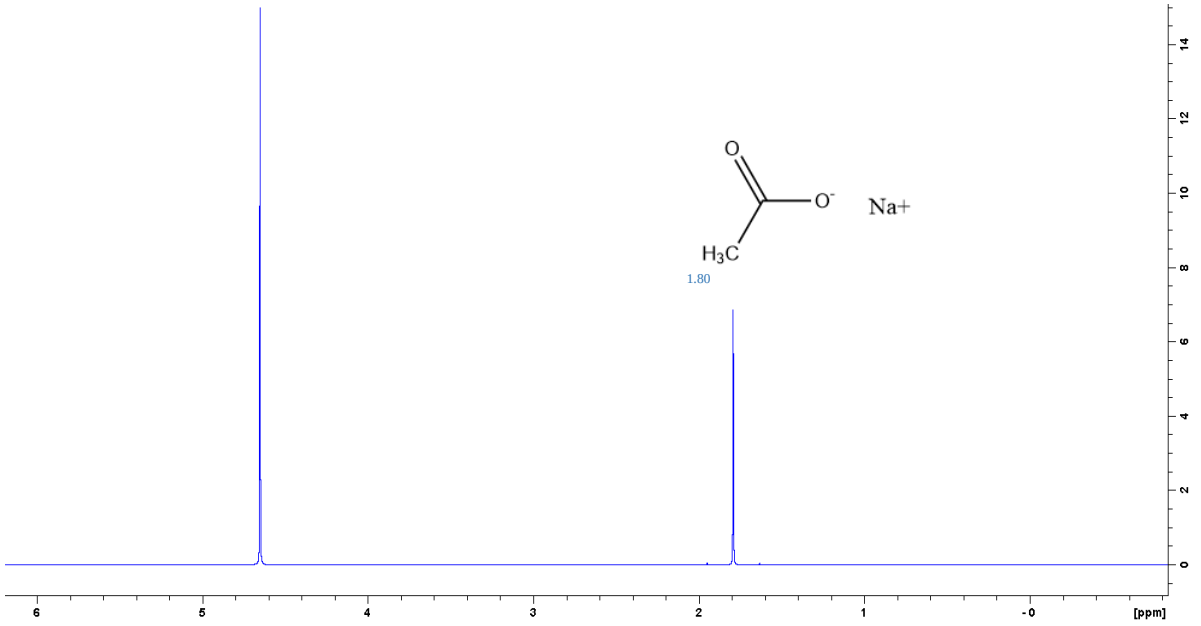


Figure 3.30 ^{1}H NMR spectra of sodium acetate alone, peak at 4.65ppm corresponds to D_2O

The NMR spectra for sodium acetate in solution with Quinine HCL confirmed no covalent interaction had occurred (figure 3.31) with the only new peak at 1.80ppm corresponding to sodium acetate.

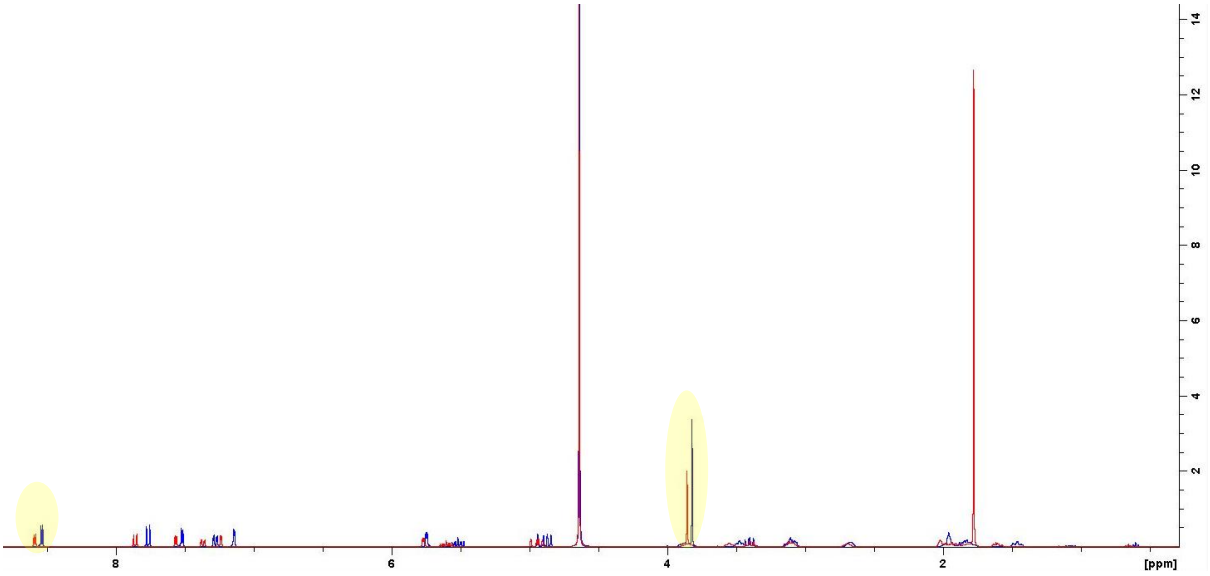


Figure 3.31 ^{1}H NMR spectra of sodium acetate & Quinine HCL in solution (red) and Quinine HCL alone (blue), peak at 4.65ppm corresponds to D_2O, peak at 1.80ppm corresponds to sodium acetate, the two peaks highlighted at 8.65 and 3.81ppm are Quinine HCL's identifying peaks shown in figure 3.17

3.4.5.2.3 Sodium gluconate

The NMR spectra for sodium gluconate showed peaks around 3.5-4.0ppm, this corresponded to its structure (figure 3.32)

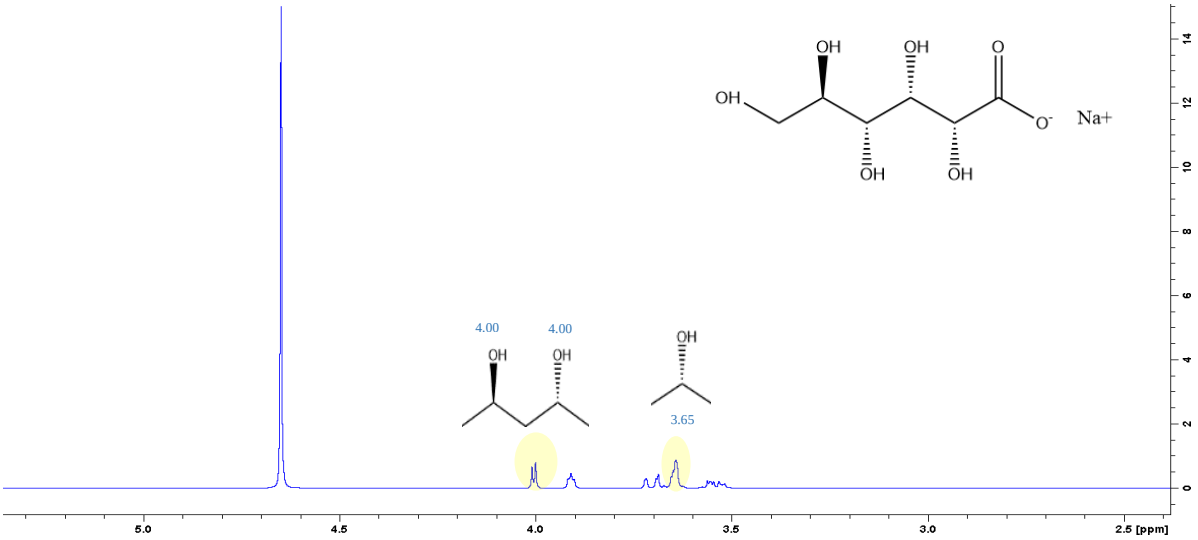


Figure 3.32 ^{1}H NMR spectra of sodium gluconate alone, peak at 4.65ppm corresponds to D_2O. Sodium gluconate's structure is shown on the right and some identifying peaks are highlighted at 4.00 and 3.65ppm

The NMR spectra for sodium gluconate in solution with Quinine HCL confirmed no covalent interaction had occurred (figure 3.33) with the only new peaks present at 3.5-4.0ppm corresponding to sodium gluconate.

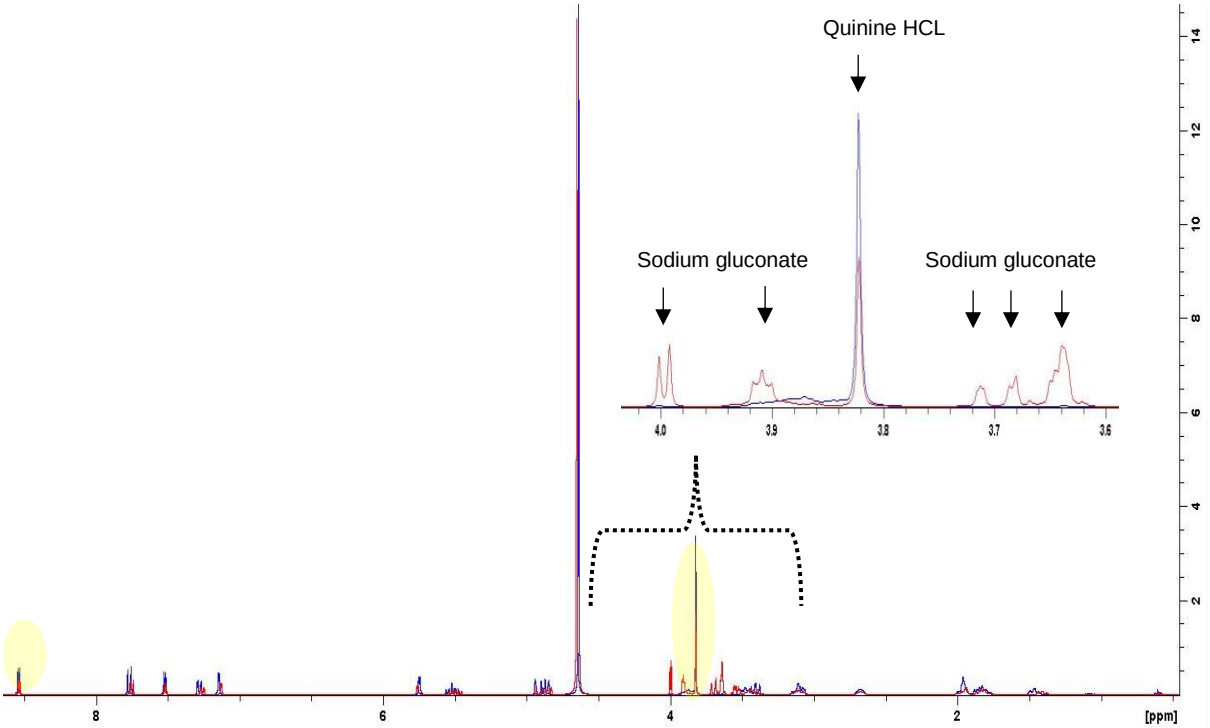


Figure 3.33 ^{1}H NMR spectra of sodium gluconate & Quinine HCL in solution (red) and Quinine HCL alone (blue) peak at 4.65ppm corresponds to D_2O, the two peaks highlighted at 8.65 and 3.81ppm are Quinine HCL's identifying peaks shown in figure 3.17. New peaks at 3.5-4.00ppm corresponds to sodium gluconate

3.4.5.2.4 NaAMP

The NMR spectra for NaAMP showed peaks grouped around 3.8-4.6ppm and 5.8, 7.8 and 8.2ppm this corresponded to its structure (figure 3.34)

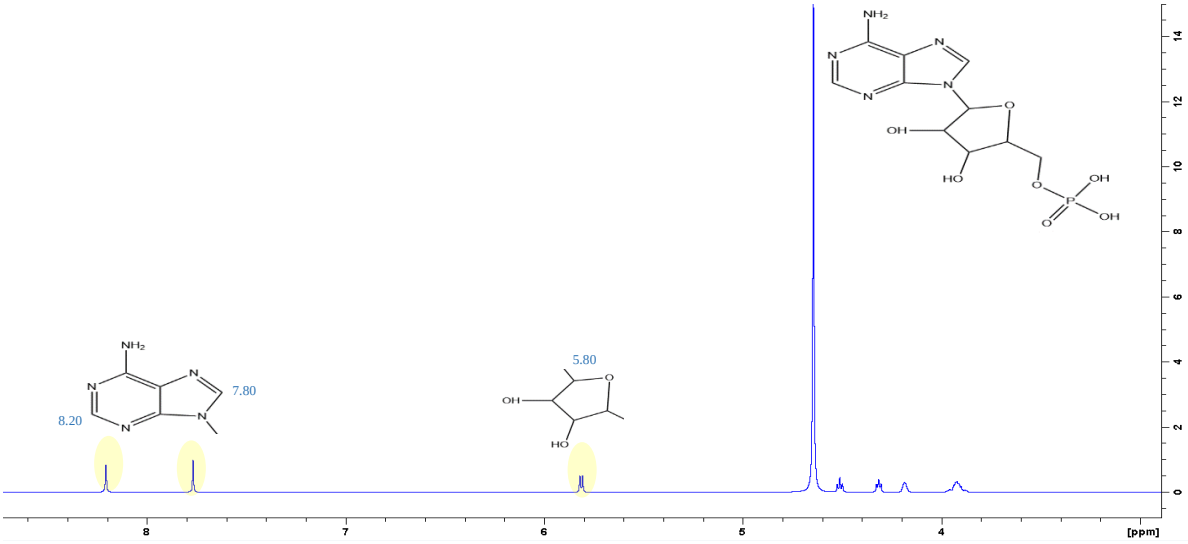


Figure 3.34 ^{1}H NMR spectra of NaAMP alone, peak at 4.65ppm corresponds to D_2O. NaAMP's structure is shown on the right and some identifying peaks are highlighted at 8.2, 7.80 and 5.80ppm

The NMR spectra for NaAMP in solution with Quinine HCL confirmed no covalent interaction had occurred (figure 3.35) with the only new peaks present around 3.8-4.6ppm and 5.8, 7.8 and 8.2ppm corresponding to NaAMP.

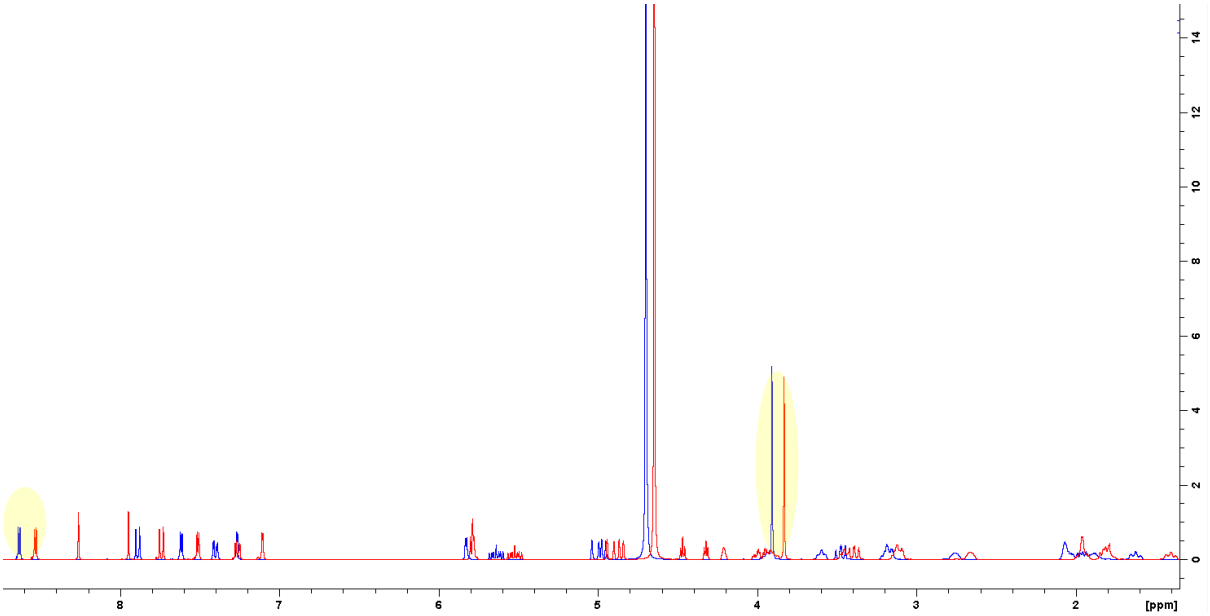


Figure 3.35 ^{1}H NMR spectra of NaAMP & Quinine HCL in solution (red) and Quinine HCL alone (blue), peak at 4.65ppm corresponds to D_2O, the two peaks highlighted at 8.65 and 3.81ppm are Quinine HCL's identifying peaks shown in figure 3.17. New peaks around 3.8-4.6ppm and 5.8, 7.8 and 8.2ppm correspond to NaAMP

3.4.6 Interaction study findings

The interaction studies showed no covalent interactions occurred between any bitter-blocker (at both efficacious and higher concentrations) and Quinine HCL. This suggests the use of these compounds as excipients would not impact upon the pharmacokinetics of the API.

3.5 Conclusions

Palatability plays an important role in a child's adherence to a medicinal regime. Bitter-blockers are a category of bitter-masking compounds which directly target the taste-pathway at a molecular level. These could turn a bitter medicinal liquid into a taste-neutral product which a child is more likely to ingest as prescribed.

This chapter describes a set of experiments exploring the utility of sodium acetate, sodium gluconate and NaAMP as bitter-blocking excipients in formulation with Quinine HCL; the model bitter API. Structural assessment showed none of the compounds covalently interact with Quinine HCL and so they are deemed low risk for pharmacokinetic interference. Palatability assessment using the e-tongue suggested each compound could be effective at much lower concentrations than quoted in the literature. However, the mechanism of action of the bitter-blockers means the e-tongue is not suitable to assess them going forwards as they adsorb to the sensor surface and results could be misleading. The BATA results showed sodium gluconate and, in particular, NaAMP improved the taste of Quinine HCL but it is necessary to examine the taste-masking efficacy of these compounds with other bitter-drugs to see where they may offer benefit.

Chapter 4

Establishing the BATA model at a new location to assess bitter-blockers with model paediatric drugs

The BATA model is a useful tool to assess the aversiveness of formulations. This chapter describes the setup of the BATA model at a new location using well established bitter drugs (Caffeine citrate and Quinine sulphate) to validate the model with historical UCL data. Once established, the three bitter-blockers discussed in chapter 3 were assessed alongside two model paediatric drugs; Ranitidine hydrochloride and Primaquine phosphate. Both drugs require better strategies to improve their palatability and compliance. These experiments highlighted how rats can become tolerant to bitter-controls over time so an end-of-study life BATA experiment was carried out to compare rat responses to when they were young.

4.1 Introduction

The use of predictive animal models in preclinical research is paramount in developing new medicinal formulations. The BATA model is a recognised tool for assessing the aversiveness of pharmaceuticals (Soto *et al.*, 2018; Clapham *et al.*, 2021). In order for this PhD to continue to utilise the BATA model transferred to a new site, to assess the palatability of novel formulations containing bitter-blockers, it was necessary to demonstrate the data produced were reliable and reproducible.

4.1.1 Method transfer

The transfer of an analytical method from one location to another needs to be validated. The United States Pharmacopeia (USP) defines the transfer of an analytical procedure as ‘the documented process that qualifies a laboratory (a receiving unit) to use an analytical test procedure that originates in another laboratory (the transferring unit also named the sending unit)’ (The United States Pharmacopeial Convention, 2016a). There are different types of method transfers including comparative testing, covalidation and revalidation (Eurofins, 2021). Comparative testing involves samples from the same lot of product being analysed by both laboratories which must meet acceptance criteria laid out in a transfer protocol. Alternatively, a co-validation of laboratories can be used where both the transferring laboratory and the receiving laboratory work to validate the method. An assessment is conducted to evaluate the reproducibility of the process.

In this work, there was only access to the receiving laboratory so the appropriate method was a revalidation in order to generate comparison data to ensure the new laboratory was providing comparable outcomes to UCL historical data sets (The United States Pharmacopeial Convention, 2016b). Setting up at a new location meant ensuring the equipment was set up and running correctly and that the necessary training had been given to on site technicians.

For this model, it was important to verify suitability under actual conditions of use. As per USP (The United States Pharmacopeial Convention, 2016a) guidelines, the protocols for the pilot studies were written in collaboration with the receiving laboratory. The validation studies were designed to generate

comparison data and as such used Caffeine citrate and Quinine sulphate dihydrate (QSD), two well established bitter compounds, to ascertain full concentration response curves.

4.1.2 Comparing data sets

As with any in-vivo model, the BATA rats display inherent variability. This makes validating data from two laboratories less straight forward than with a non-animal analytical method. This variability is minimised by using a sufficient n number so overall data is representative. Previous work has established that an appropriate number of rats for each BATA study is 10 (Soto, 2016). This work found that using 8 rats led to significantly different median IC50 values compared to when 10 animals were used; using 10 rats reduced the variability. Moreover, 10 represents the maximum number of animals which can reasonably be tested per day with one lickometer as discussed in chapter 3.

One way of comparing the two data sets is to identify and compare the IC50 values of Caffeine citrate and QSD generated by each laboratory. A compound's IC50 is the concentration that inhibits 50% of the maximum lick number (Soto *et al.*, 2015) and can be calculated using a mathematical E_{max} model which can fit data where the maximum effect is attributable to the drug (Soto, 2016). This represents a way of comparing 1 value, produced in each laboratory, which encompasses all the data points. Using a published standard the IC50 values can be used to identify how aversive the rats were perceiving each drug (Soto *et al.*, 2018) in each setting (table 4.1).

Table 4.1 IC50 and corresponding level of aversiveness adapted from Soto et al 2018.

IC_{50} category	**Level of aversiveness**
0-0.1mM	Extremely aversive
0.1-1mM	Moderately aversive
1–10 mM	Mildly aversive
10–100 mM	Weakly aversive

If the IC50 values generated in both labs reflect the same level of aversiveness then this is a good indication the new rats are producing reliable results in line with historical outcomes.

Secondly, each concentration of test solution can be categorised on a scale from fully tolerated to highly aversive (Soto *et al.*, 2015; Ruiz *et al.*, 2019). This can be achieved by comparing the lick response of test solution to that of the water control (table 4.2).

Table 4.2 Classifying aversiveness of test compounds, adapted from Ruiz et al 2019.

Classification	**% lick inhibition vs water**
Fully tolerated	No significant difference
Well tolerated	Less than 30%
Tolerated	Between 31-50%
Aversive/ untolerated	Between 51-75%
Highly aversive/ untolerated	More than 75%

This can be used to further understand how each concentration of QSD and Caffeine citrate was perceived and whether the new rats reflected the outcomes seen historically.

4.1.3 Cross lab differences and standardisation

The conditions under which BATA studies were conducted between the two laboratories were very similar (figure 4.1). This is largely due to the legislation in place detailing the use and housing of animals for in vivo research. The codes of practice laid out by ASPA 1986 (Home Office, 2014) specifies all aspects of a laboratory animal's life from cage size to room humidity. The majority of factors which were known to differ between the two laboratories at the time of the BATA experiments are negligible (shown in red on figure 4.1), these include slightly different equipment/ treats used for play time when the animals are on an 'off week' and different members of staff tending to the animals. Age (and number of re-uses of animals) is also in red because it is has been demonstrated that age can influence IC50 values (Soto, 2016) as discussed later in this chapter. Other factors which may have differed between the animal houses are shown in orange and include diet because, although the same standard chow was used, it came from a different supplier. Also background noise levels, which may distract the animals when they are on study or cause them stress which could affect performance. Noise levels were always kept to a minimum when carrying out BATA experiments and the majority of the noise the BATA animals experienced whilst on study was from the lickometer itself (which the animals were acclimatised to by using training days) and was the same at both locations.

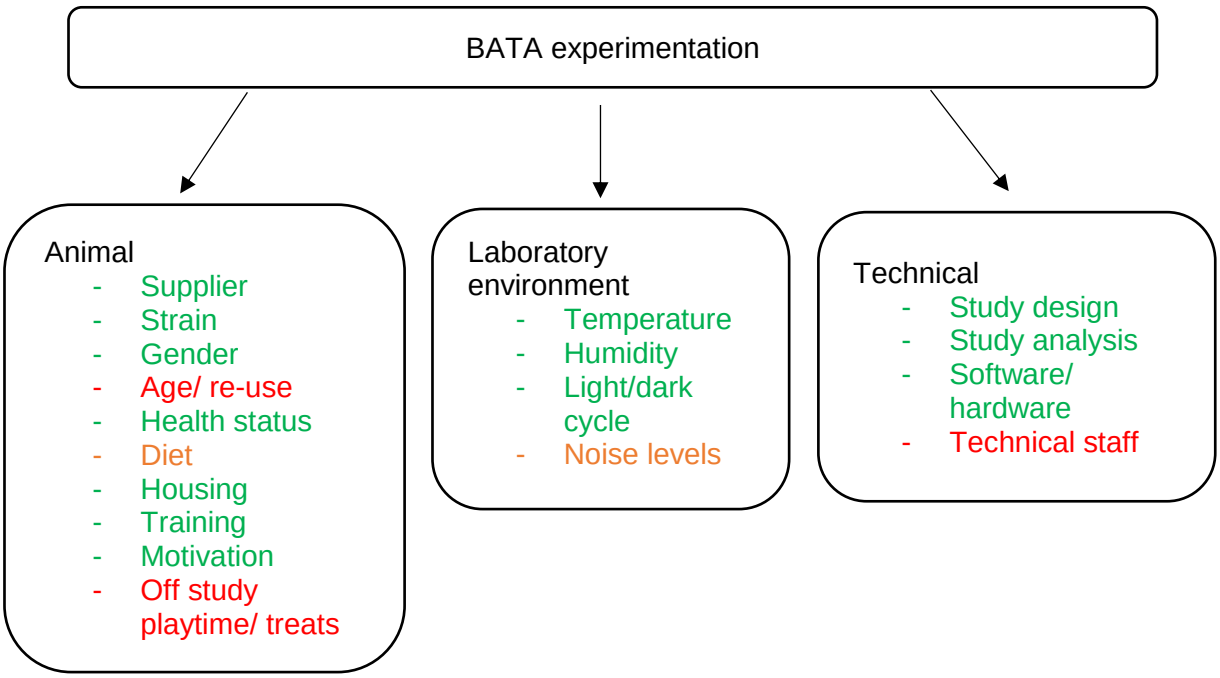


Figure 4.1 Factors affecting BATA experiments and their standardisation across the original and new laboratories. Factors in red were not identical between the two sites, in orange may have differed between the sites and green did not differ between the sites.

As discussed in chapter 3, previous work had been carried out within the research group to compare BATA data generated in different laboratories (Ives, Tuleu and Bachmanov, 2018). Here BATA outcomes were retrospectively compared from data produced at UCL (used as a benchmark in this chapter) and GSK (where the work detailed in chapter 3 was conducted). Human panel results from the two facilities were also compared. 22 APIs and 3 bitter controls (including Caffeine and Quinine) were assessed and the relationship between the data sets was found to be very well correlated across the two facilities, despite minor protocol deviations. This highlights the feasibility of method transfers for the BATA model across two sites.

Once satisfied with the set-up of the BATA model, the bitter-blockers of interest (as detailed in chapter 3) could be further assessed in formulation with bitter drugs.

4.1.4 Drugs of interest

Ranitidine HCL is a model drug that has been used widely in the BATA model with a well-established IC50 level. Ranitidine HCL was also commonly prescribed as an oral liquid dosage form which could be given, off-label, to young children. As such, a suitably taste-masked formulation was sorely needed which was not achieved with traditional excipients. However, there has been a recent update with Ranitidine products being voluntarily withdrawn by manufacturers in October 2019 due to the presence of low levels of an impurity called N-nitrosodimethylamine (NDMA) with a subsequent recommendation from the EMA to suspend all Ranitidine medicines in the European Union (Royal College of Paediatrics and Child Health, 2021). Following on from this in 2020, the United States FDA announced a request for manufactures to withdraw all prescription and over-the-counter Ranitidine drugs from the market (FDA, 2020). However, it was decided to continue to work on it for the purpose of this doctoral project as it was involved in various aspects of the work (see chapter 5).

Primaquine phosphate (PQP) is an anti-malarial medication and, at the time of this PhD, a novel paediatric formulation was under development with the aim of improving patient compliance through taste-masking (DPP project, 2021).

Therefore, these two drugs (one structurally related to Quinine, as shown in figure 4.2, but not the other with a milder bitterness) were chosen to be next assessed alongside the bitter-blockers.

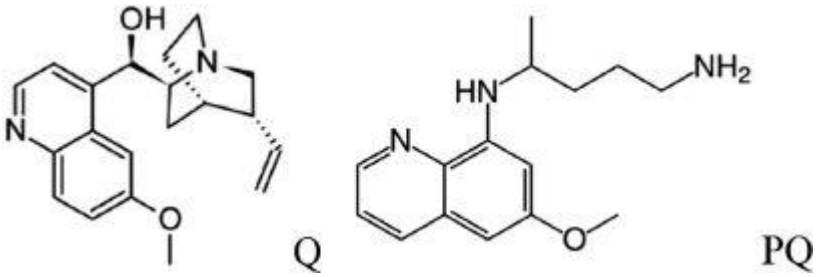


Figure 4.2 Molecular formulae of Quinine (Q) and Primaquinine (PQ)

These formulations were evaluated alongside bitter-controls. Work by Soto has demonstrated there to be an age-related decline in sensitivity to certain bitter compounds (Soto, 2016). This was reflected in the work presented here leading to a final experiment when the animals were end of study age. This was in order to further integrate in the revalidation the fact that the rats' perception of bitter compounds can also change over their study lifetime.

4.2 Aims and objectives

The aim of this work was first to validate the setup of the BATA model at a new location using two well-established bitter tastants over the time of re-use of the rats as per the license. Secondly, to assess the efficacy of sodium acetate, sodium gluconate and NaAMP in formulation with Ranitidine hydrochloride and Primaquine phosphate.

4.3 Materials and methods

4.3.1 Materials

Ranitidine hydrochloride, Caffeine citrate, Quinine sulphate dihydrate, Quinine hydrochloride dihydrate, sodium acetate, sodium gluconate, NaAMP and Chlorphenamine maleate (CPM) were purchased from Sigma-Aldrich (Gillingham, UK). Primaquine phosphate was supplied by IPCA laboratories (Mumbai, India).

4.3.2 Validation BATA experiments

The initial validation work was conducted over two separate BATA studies, one assessing Caffeine citrate and one assessing Quinine sulphate dihydrate with a rest/washout week in between.

4.3.2.1 Test solutions

Caffeine citrate solutions were prepared at 0.3, 1, 3, 10, 30 and 100 mM (0.1, 0.4, 1.2, 3.9, 11.6, 38.6mg/mL). QSD solutions were prepared at 0.003, 0.01, 0.03, 0.1, 0.3 and 1mM (0.0023, 0.0078, 0.0235, 0.0783, 0.2349 and 0.783mg/mL). Each study had Quinine HCL at IC50 concentration (0.08mM, 0.032mg/ml) as a control. All solutions were made up using distilled water. Each solution was made 1 day prior to first use and stored at ambient temperatures protected from light.

For each test day, the 6 concentrations of Caffeine citrate/ QSD and the QHCL control were each randomly assigned to two tubes in position 1-16 in the Davis Rig, except for tube 3 and tube 8 which were are always water (water in tube 3 was presented first so the rats were motivated to continue

and tube 8 was a water rinse between samples). Each tube was presented twice per session.

4.3.2.2 Animals

10 male CD (Sprague Dawley) rats (approximately 4.5-5.5 weeks of age during the first experimental week) were housed in pairs in standard cages in a room maintained at 21±2 °C with 55±15% humidity and with a 12:12 h light/dark cycle. Animals were provided with nesting materials, tunnels, tubes and wood chews. Animals had free access to chow (Special Diet Services, Essex) and tap water, except for training/testing periods where a water-restriction schedule occurred. Daily food and water consumption were monitored throughout the experiment and, as a welfare measure, it was checked that their weight did not drop below 85% of their free feeding weight. All procedures were carried out in accordance with ASPA 1986 (PPL number 8909500).

4.3.2.3 Overview

For the first validation study, assessing Caffeine citrate, the animals underwent a four-day experimental week. This included two training days using only water, first with only the shutter open and then with the equipment moving, to acclimatise them to the associated noises. This was followed by two test days. For the rest of the BATA studies, the animals underwent a three-day test week with the first training day removed. These animals were used for all the experiments detailed in this chapter. The Davis Rig equipment and the protocol for water-deprivation is as detailed in previous chapters.

4.3.2.4 Comparing the data sets

The IC50 values for the Caffeine citrate and QSD dose-response curves were generated from the data sets using a mathematical E_{max} model. This model was written by Soto 2016 (Soto, 2016).

Each concentration of Caffeine citrate and QSD could be categorised according to perceived aversion (table 4.2). This was achieved for each laboratory by comparing the mean lick response of test solution to that of the respective water control. The raw data set from the original laboratory was not available so mean licks for each concentration of Caffeine citrate and QSD were estimated from graphs published in Soto 2016 (Soto, 2016).

4.3.3 Bitter-blocker BATA experiments

Work evaluating the three bitter-blockers in formulation with Primaquine phosphate and Ranitidine hydrochloride was conducted over three separate BATA studies with a week rest in between each. The first study assessed sodium acetate in combination with the drugs, the second NaAMP and the third sodium gluconate.

The animals, equipment and protocol were as detailed previously

4.3.3.1 Test solutions

Ranitidine hydrochloride was assessed at 4mM (1.4mg/mL) and 48mM (16.84mg/mL) which represent the historical IC50 (Soto *et al.*, 2018) and the therapeutic dose respectively.

Primaquine phosphate was assessed at 5.8mM (2.63mg/mL) which contains 1.5mg/mL Primaquine base. This concentration was chosen as it was the

proposed therapeutic dose for a paediatric formulation of the drug which is known for its extreme bitterness.

The bitter-blockers were assessed at three concentrations reflecting the lowest efficacious concentration found in the e-tongue and BATA (vs Quinine as detailed in chapter 3) and human panel (reported in the literature (Keast and Breslin, 2002; Mennella, Pepino and Beauchamp, 2003)). Sodium acetate was assessed at 20, 100 and 300mM, NaAMP at 4, 20 and 30mM, sodium gluconate at 75, 300, 1000mM. The rationale for bitter-blocker concentration choice is summarised in table 4.3.

Table 4.3 Rationale for bitter-blocker concentrations for use with Primaquine phosphate and Ranitidine hydrochloride in the BATA model

Compound	**Human panels (literature)** Effective concentration vs low Quinine (0.1mM)	**E-tongue (chapter 3)** Effective concentration vs 0.26mM Quinine (human EC50)	**BATA (chapter 3)** Effective concentration vs 0.14 and 1.8mM Quinine (rat IC50 and IC90)
Sodium acetate	100mM	20mM	No improvement up to 300mM
Sodium gluconate	300mM	75mM	1000mM increased lick response to IC90 Quinine by 67.3%
NaAMP	20mM	4mM	30 and 100mM increased lick response to IC50 by 75.3 & 91.8% respectively and to IC90 by 54.8 & 116.1% respectively

Each solution was made 1 day prior to use and stored in the fridge (2-8 °C) protected from light. Solutions were taken out of the fridge for a few hours on

the morning of each test day to reach room temperature. These three studies also used QHCL as a control. All solutions were made up using distilled water.

For each test day, the controls (water, QHCL, 4mM Ranitidine, 48mM Ranitidine and 5.8mM PQP) and the 3 concentrations of bitter-blocker with; 4mM Ranitidine, 48mM Ranitidine and 5.8mM PQP were randomly assigned to a tube in position 1-16 in the Davis Rig except for tube 3 and tube 8 which were always water. Each tube was presented twice per session.

4.3.3.2 QHCL control

The initial QHCL concentration of 0.08mM became tolerated by the rats as the experiments went on and the lick rate increased. This is in line with previous findings evaluating rat performance overtime which found the concentration of QHCL needed to be gradually increased as they became more tolerant to it (Soto, 2016). For the final experiment in this series, using NaAMP as the bitter blocker, the QHCL concentration was increased to 0.16mM however this was still tolerated too well. Therefore, when these animals were at the end of their study life, a final study was undertaken to evaluate how the IC50 of a range of compounds evolved over time.

4.3.4 End-of –study life IC50 BATA experiment

To understand the deviation in IC50 levels from age 1 month (start of BATA experimentation) to age 7 months (end of study life for animals, 6-months from first use), a series of compounds were assessed in the BATA in one final experiment. These were given at concentrations reflecting the historical IC50 and, for Caffeine citrate and QSD, at a higher predicted IC50 level reflecting their age.

The animals, equipment and protocol were as detailed previously

4.3.4.1 Test solutions

The IC50 concentrations established in the two initial validation studies were used for Caffeine citrate (9.86mM, 3.81mg/mL) and for QSD (0.0428mM, 0.034mg/mL). Previous work has shown that IC50 concentration of Caffeine citrate can increase approximately 4-fold as the rats age (Soto, 2016) so the original IC50 value was quadrupled to represent a potential higher IC50 (39.44mM, 15.24mg/mL). A similar age-related change in QHCL's IC50 has also been shown so 0.32mM (0.128mg/mL) was the concentration chosen to represent a potential higher IC50. QSD has not been evaluated for tolerance over time but since it is also a Quinine derivative, a 4-fold increase was chosen for the potential IC50 in these aged animals of 0.1712mM (0.134mg/mL).

Ranitidine HCL had not been assessed for tolerance over time so the original 4mM IC50 value was used to see if this was still accurate. To maximise data from the animals, CPM was also assessed at a predicted IC50 of 2.02mM (0.788mg/mL) (Keeley *et al.*, 2019).

All solutions were made up using distilled water. Each solution was made 1 day prior to first use and stored at ambient temperatures protected from light. Each solution (water, QHCL, QSD at two concentrations, Caffeine at two concentrations, Ranitidine, CPM) was randomly assigned to two tubes in position 1-16 in the Davis Rig except for tube 3 and tube 8 which were are always water. The randomised order was different for each test day. Each tube was presented twice per session.

4.3.5 Data analysis

All data were imported into Microsoft Excel 2016 (©Microsoft, Redmond, Washington) to process before analysis. Licks equal to 0 or 1 were excluded to avoid incorporating falsely registered licks, for example if the animal made contact with the spout with his nose whilst sniffing without actually tasting the sample. Also 'rinse' data, which represented the licks taken in the 2 second rinse in between test samples was excluded.

Statistics and graphs were produced using R-studio software version 3.5.0. The normality of the data distribution was identified using the Shapiro-Wilk test. If this test highlighted the data did not follow parametric distribution, then Kruskal-Wallis rank sum test with Gao post-hoc analysis was used to compare differences in data sets (Gao *et al.*, 2008). If the data distribution was normal, the one-way analysis of variance was performed with Tukey's post-hoc analysis. Outliers were calculated by R using the interquartile range criterion, the observations fulfilling the following statements were considered outliers 1) data points above third quartile + (1.5 x interquartile range) or 2) data points below first quartile – (1.5 x interquartile range) (Soetewey Antoine, 2020).

Results were displayed using notched box-plots, showing the median and interquartile ranges, with whiskers that represent 1.5 times the 25^{th} and 75^{th} percentile. The notches display the 95% confidence interval of the median. Outliers are shown as circles.

4.4 Results and discussion

4.4.1 Validation studies

Caffeine citrate and Quinine sulphate were assessed at a range of concentrations to ascertain dose response curves (figure 4.3).

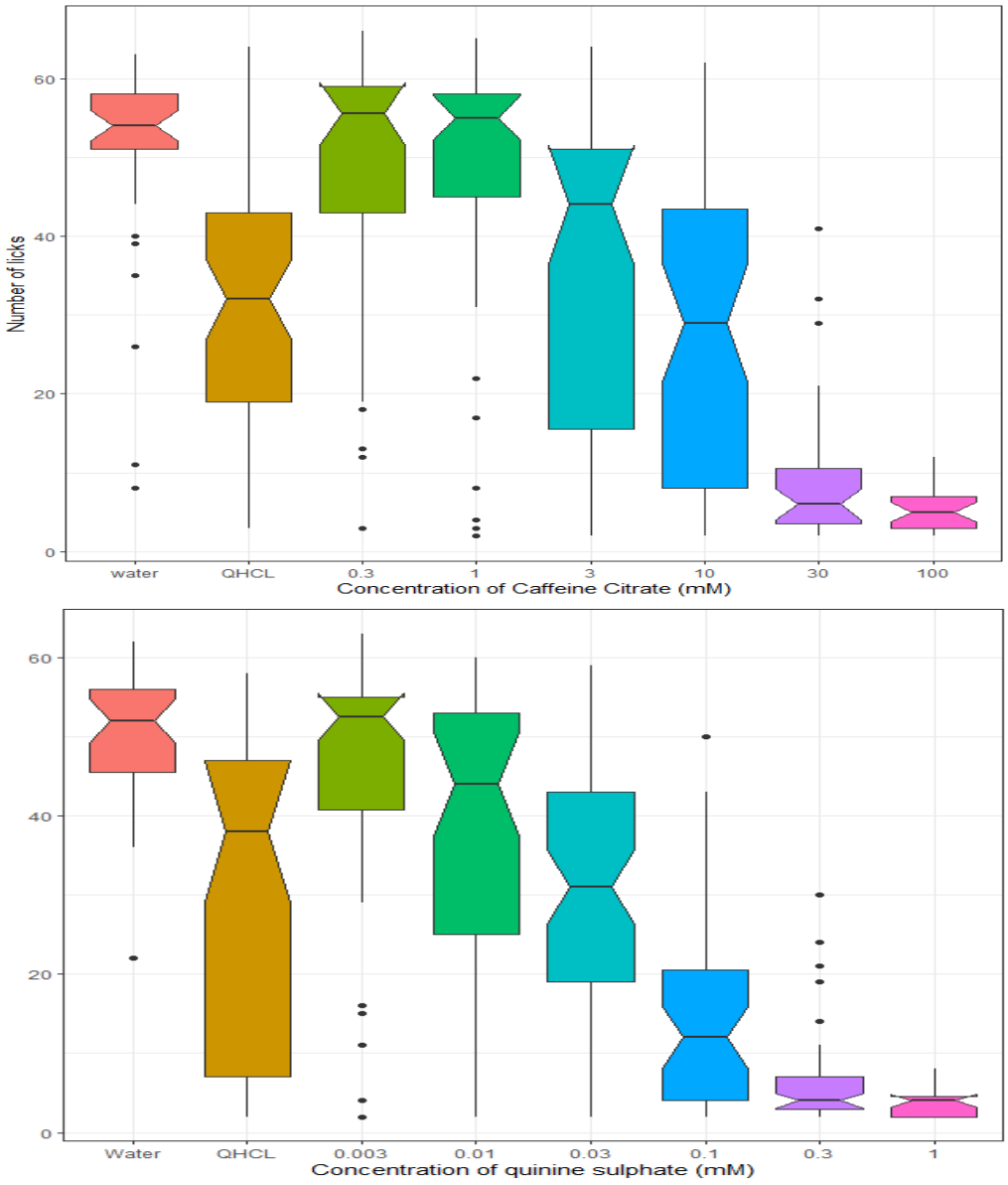


Figure 4.3 Dose response curves of Caffeine citrate (top) and Quinine sulphate (bottom). QHCL is presented at 0.08mM, the IC50 concentration, as a bitter control.

From these concentration-response curves the IC50 was generated. The IC50 is presented alongside the mean QHCL lick rate (bitter control) and the water lick rate (fully palatable control) in table 4.4.

Table 4.4 Caffeine citrate and QSD IC50 values, QHCL and water mean licks. QHCL is presented at the IC50 level and so should be approximately half of the water response.

	Week 1 Caffeine citrate	**Week 2 QSD**
Drug IC50	9.86mM (3.81mg/mL)	0.0428mM (0.034mg/mL)
QHCL mean lick/ 8 seconds	31.5	29.9
Water mean lick/ 8 seconds	50.6	50

4.4.2 Caffeine citrate

4.4.2.1 Inter-rat and day to day variation

As is typical of an in vivo model there was some variation between the rats' responses to the formulations (figure 4.4).

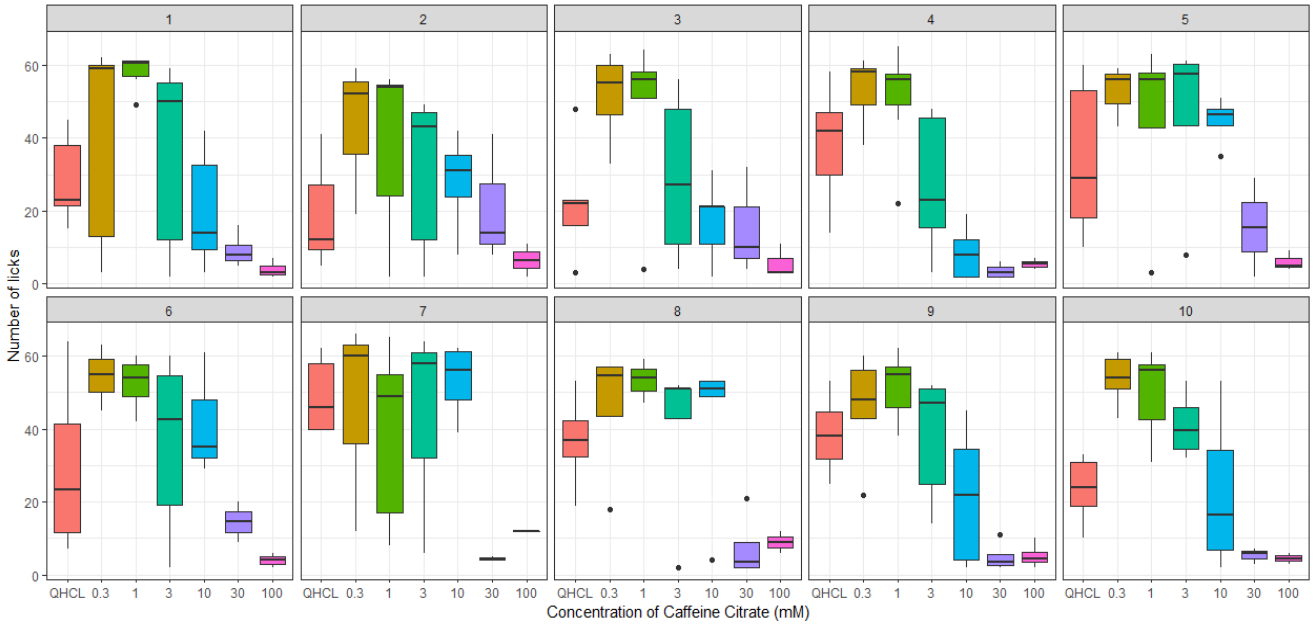


Figure 4.4 Individual rat BATA findings for Caffeine citrate validation study at new laboratory site

There was also some variation over the two test days – this is why the IC50 was generated from the overall data set. The variation seen for Caffeine citrate between day 1 and day 2 is shown in figure 4.5. Only the response to 10mM Caffeine citrate differed significantly by day.

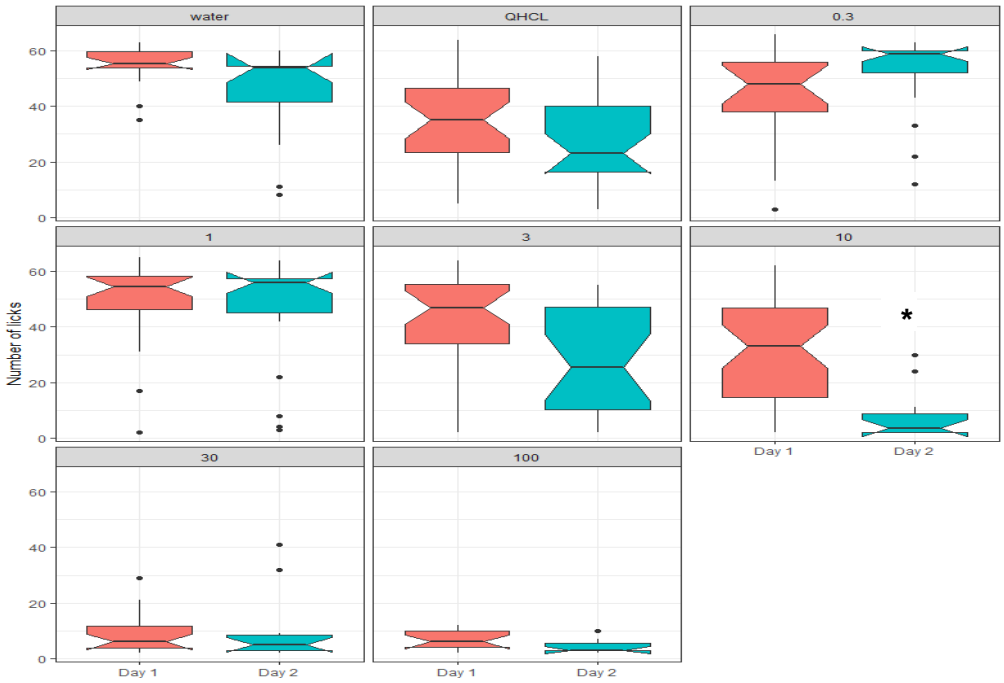


Figure 4.5 Caffeine citrate rat response by day during validation study at new laboratory site. *Denotes statistical difference by day using Gao post-hoc analysis ($p<0.05$)

Variation between days was also seen in the original laboratory at UCL. Figure 4.6 is taken from Soto 2016 (Soto, 2016) and shows an example of the variation seen for Caffeine citrate over two test days.

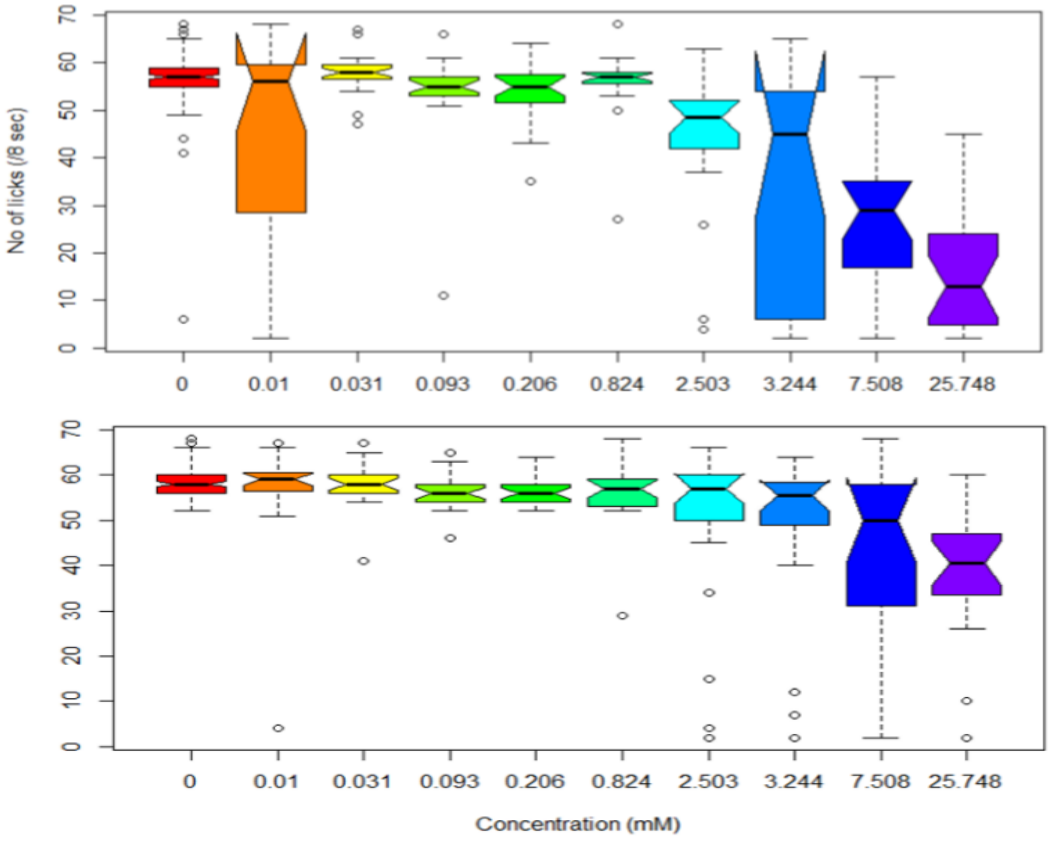


Figure 4.6 Lick response variation to Caffeine citrate on test day 1 (top) and test day 2 (bottom) taken from Soto 2016 carried out at the original laboratory.

4.4.2.2 IC50 comparison

The overall IC50 found for Caffeine citrate in this work was 9.86mM (3.81mg/mL). The historical IC50 generated for animals in the original laboratory was 7.76mM (3.00mg/mL) (Soto *et al.*, 2018). Both laboratories placed this drug in the mildly aversive category (table 4.1).

4.4.2.3 Tolerability comparison

The lick response to each concentration of Caffeine citrate vs water control from both laboratories is shown in table 4.5. The two laboratories gave the same results for each concentration except 10mM Caffeine citrate where the validation study placed this as just within the tolerated category but the

original laboratory placed it as just within the untolerated category. Importantly both studies found the same two concentrations (0.3 and 1mM) to be statistically comparable to water.

Table 4.5 Comparison of mean licks, percentage inhibition of water and aversiveness of Caffeine citrate at each concentration produced from the two laboratories. *Estimated from graphs published in Soto 2016

Caffeine citrate (mM)	**Validation study mean licks**	**Original laboratory mean licks***	**Validation study % lick inhibition vs water**	**Original laboratory % lick inhibition vs water**	**Validation study category**	**Original laboratory category**
0 (water)	50.6	48	n/a	n/a	n/a	n/a
0.3	48.5	46	4.2	4.2	Fully tolerated	Fully tolerated
1	48.3	47	4.5	2.1	Fully tolerated	Fully tolerated
3	35.8	38	29.2	20.8	Well tolerated	Well tolerated
10	26.8	21	47	55.2	*Tolerated*	*Untolerated*
30	9.5	9	81.2	81.3	Highly untolerated	Highly untolerated
100	5.7	6	88.7	87.5	Highly untolerated	Highly untolerated

4.4.3 Quinine sulphate

4.4.3.1 Inter-rat and day to day variation

There was some variation between the rats' responses to the formulations (figure 4.7).

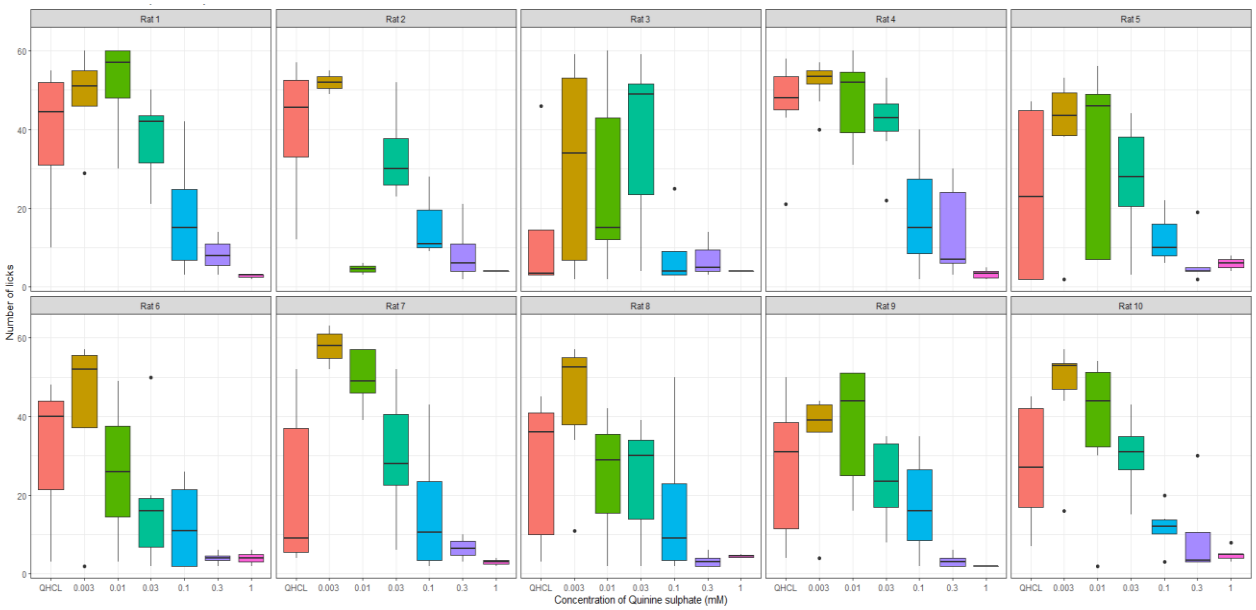


Figure 4.7 Individual rat BATA findings for Quinine sulphate during validation experiment at new laboratory site

The overall variation for Quinine sulphate seen between day 1 and day 2 is shown in figure 4.8. Only the response to QHCL differed by day and it ended up to be well accepted on day 2.

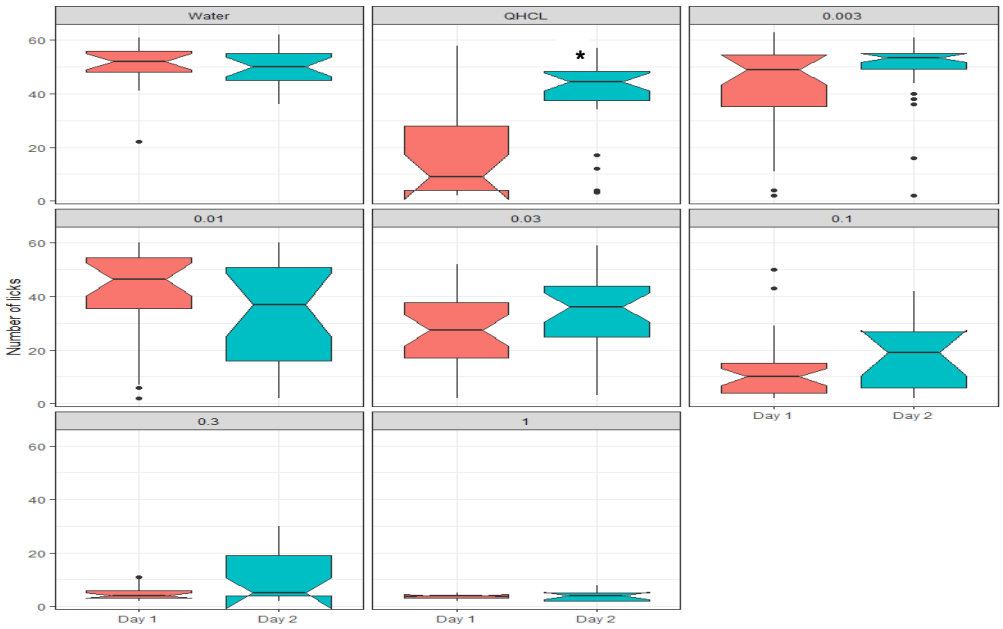


Figure 4.8 Quinine sulphate rat response by day during the validation study at the new laboratory site.

*Denotes statistical difference between days using Gao post-hoc analysis ($p<0.05$)

Some variation between days was also seen in the original laboratory at UCL. Figure 4.9 is taken from Soto 2016 (Soto, 2016) and shows an example of the variation seen for Quinine sulphate over two test days.

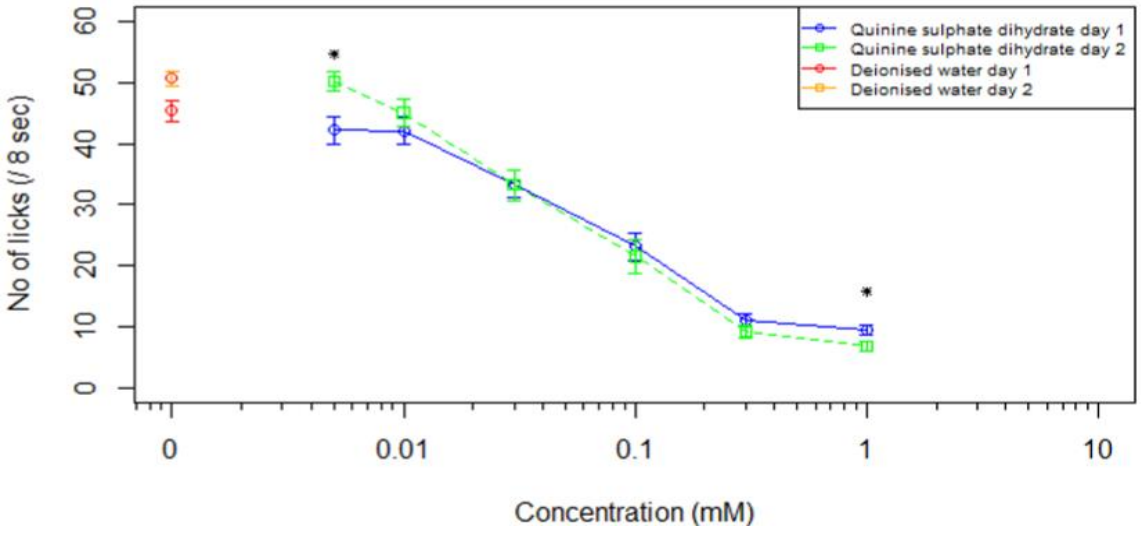


Figure 4.9 Lick response to Quinine sulphate on test day 1 (blue) and test day 2 (green) produced at the original laboratory. *Denotes statistical difference between days as reported by Soto 2016.

4.4.3.2 IC50 comparison

In this work, the overall IC50 for Quinine sulphate was 0.0428mM (0.03mg/mL). The historical IC50 generated in the original laboratory was 0.061mM (0.05mg/mL) (Soto, 2016). Both laboratories placed this drug in the extremely aversive category (table 4.1).

4.4.3.3 Tolerability comparison

The lick response to each concentration of Quinine sulphate vs water control from both laboratories is shown in table 4.6. The two laboratories gave the same results for each concentration.

Table 4.6 Comparison of mean licks, percentage inhibition of water and aversiveness of Quinine sulphate at each concentration produced from the two laboratories. *Estimated from graphs published in Soto 2016.

Quinine sulphate (mM)	Validation study mean licks	Original laboratory mean licks*	Validation study % lick inhibition vs water	Original laboratory % lick inhibition vs water	Validation study category	Original laboratory category
0 (water)	50.0	48.5	n/a	n/a	n/a	n/a
0.003	44.9	47.5	10.2	2.1	Fully tolerated	Fully tolerated
0.01	37.3	43	25.5	11.3	Well tolerated	Well tolerated
0.03	30.7	33	38.6	32.0	Tolerated	Tolerated
0.1	14.9	21	70.2	56.7	Untolerated	Untolerated
0.3	7.3	9	85.5	81.4	Highly untolerated	Highly untolerated
1	3.8	8	92.4	83.5	Highly untolerated	Highly untolerated

4.4.3.4 Limitations of validation experiments

The historical raw data from the original laboratory was unavailable so mean lick numbers were estimated from graphs published in Soto 2016 (Soto, 2016). Therefore, it is probable there would have been some small differences in mean lick number than was reported in tables 4.5 and 4.6. This could result in different percentage lick inhibition and thus affect tolerability category. In reality, it is likely that any effect on tolerability categories would have been minimal because only one of the estimated percentage lick inhibition for the original laboratory was on the cusp of a category: 0.03mM QSD had an

estimated 32% lick inhibition so an increase to the mean lick could have put it in the well-tolerated category opposed to the tolerated category.

If the full data set had been available, statistical analysis would have been carried out to compare the means at each concentration at each location using a Kruskal-Wallis rank sum test with Gao post-hoc analysis to compare differences in data sets (Gao *et al.*, 2008). This would have been more accurate as it would take into account the variation from all the data points.

4.4.3.5 Outcome of validation studies

The outcome of the validation studies suggested that the model was producing results in line with expected outcomes and 11 out of 12 concentrations tested gave the same tolerability category as historical data. The new laboratory rated the bitter-model drugs similarly as in the original work and differences seen between the two data sets were minimal. This meant the animals were reliably assessing the palatability of formulations new to them and that further experimentation could go ahead.

4.4.4 Bitter-blocker BATA experiments

Once the BATA model was established and revalidated at the new location, the three bitter-blockers of interest (sodium acetate, sodium gluconate and NaAMP) were assessed in formulation with Primaquine phosphate and Ranitidine hydrochloride

4.4.4.1 Sodium acetate

Sodium acetate offered no improvement to either concentration of Ranitidine hydrochloride or PQP (figure 4.10).

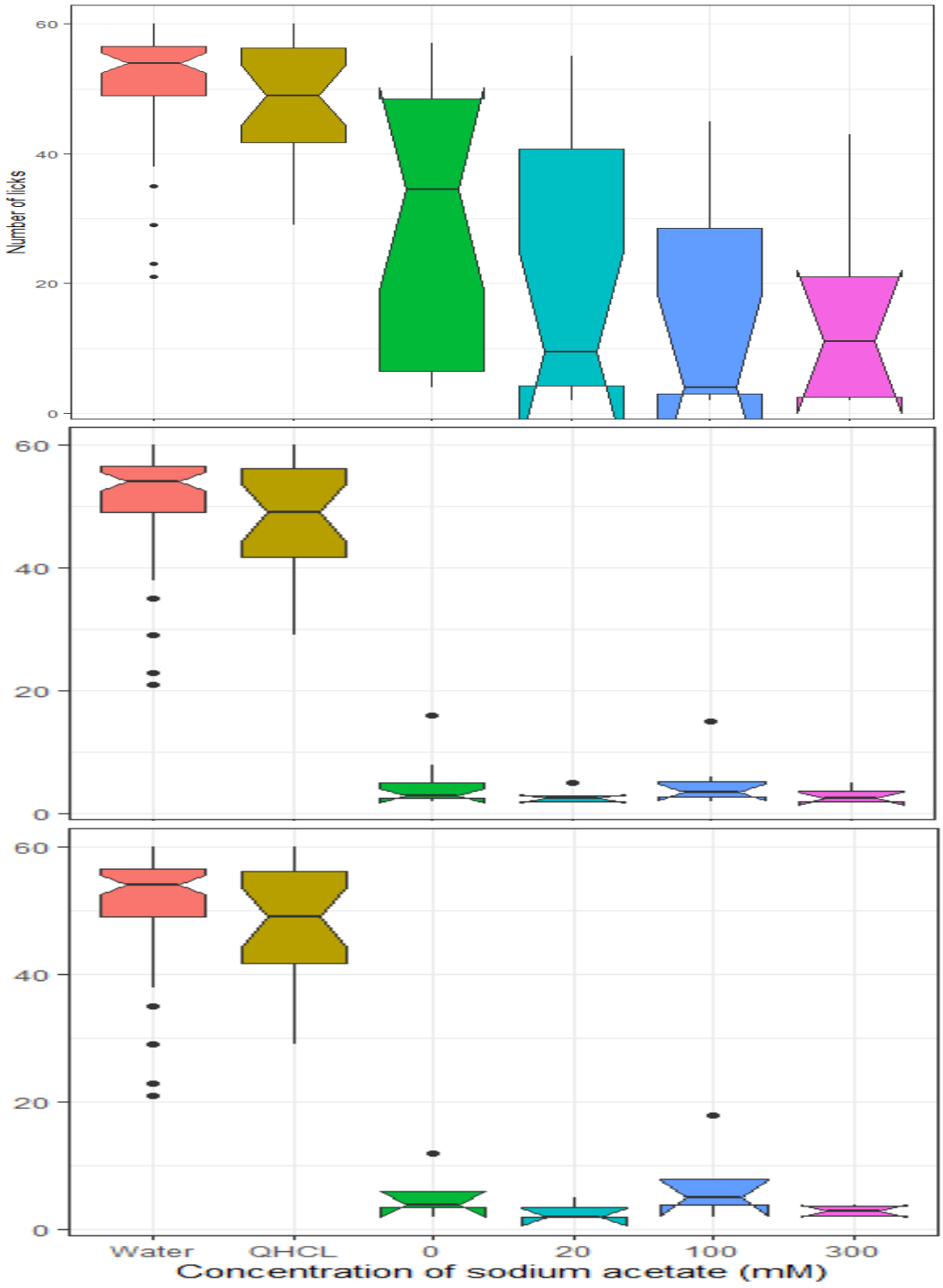


Figure 4.10 BATA findings for 4mM (top) and 48mM (middle) Ranitidine hydrochloride and PQP (bottom) in formulation with 0, 20, 100, 300mM sodium acetate. No concentration of sodium acetate improved the lick response to respective control (0mM excipient).

4.4.4.2 NaAMP

NaAMP offered no improvement to either concentration of Ranitidine hydrochloride. NaAMP at 4mM significantly increased the mean lick response to PQP alone by 76% (figure 4.11). Although this did indicate NaAMP improved the palatability of PQP, in reality the lick rates to this formulation were still extremely low. Therefore, NaAMP was not successful in creating a palatable PQP formulation.

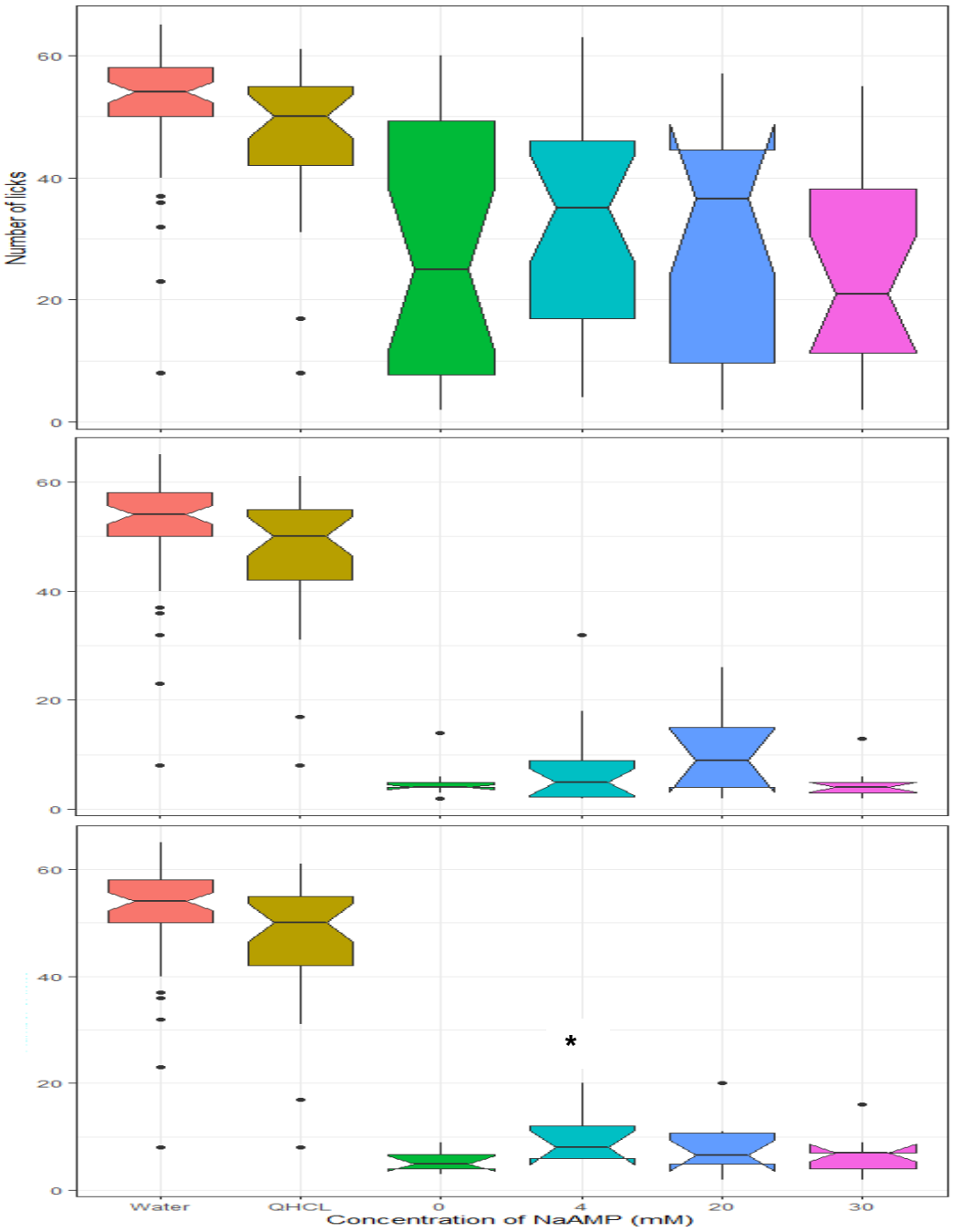


Figure 4.11 BATA findings for 4mM (top) and 48mM (middle) Ranitidine hydrochloride and PQP (bottom) in formulation with 0, 4, 20, 30mM NaAMP. *Denotes significant improvement compared to respective control using Gao post-hoc analysis ($p<0.05$).

4.4.4.3 Sodium gluconate

Sodium gluconate significantly improved the palatability of 4mM Ranitidine. Sodium gluconate at 75 and 300mM increased the lick response to control by 184 and 186% respectively, producing acceptably palatable formulations. 300mM sodium gluconate also significantly increased the lick response to PQP by 116% (figure 4.12). Although this did indicate sodium gluconate improved the palatability of PQP, in reality the lick rates to this formulation were still low. Therefore, sodium gluconate was not successful in creating a palatable PQP formulation.

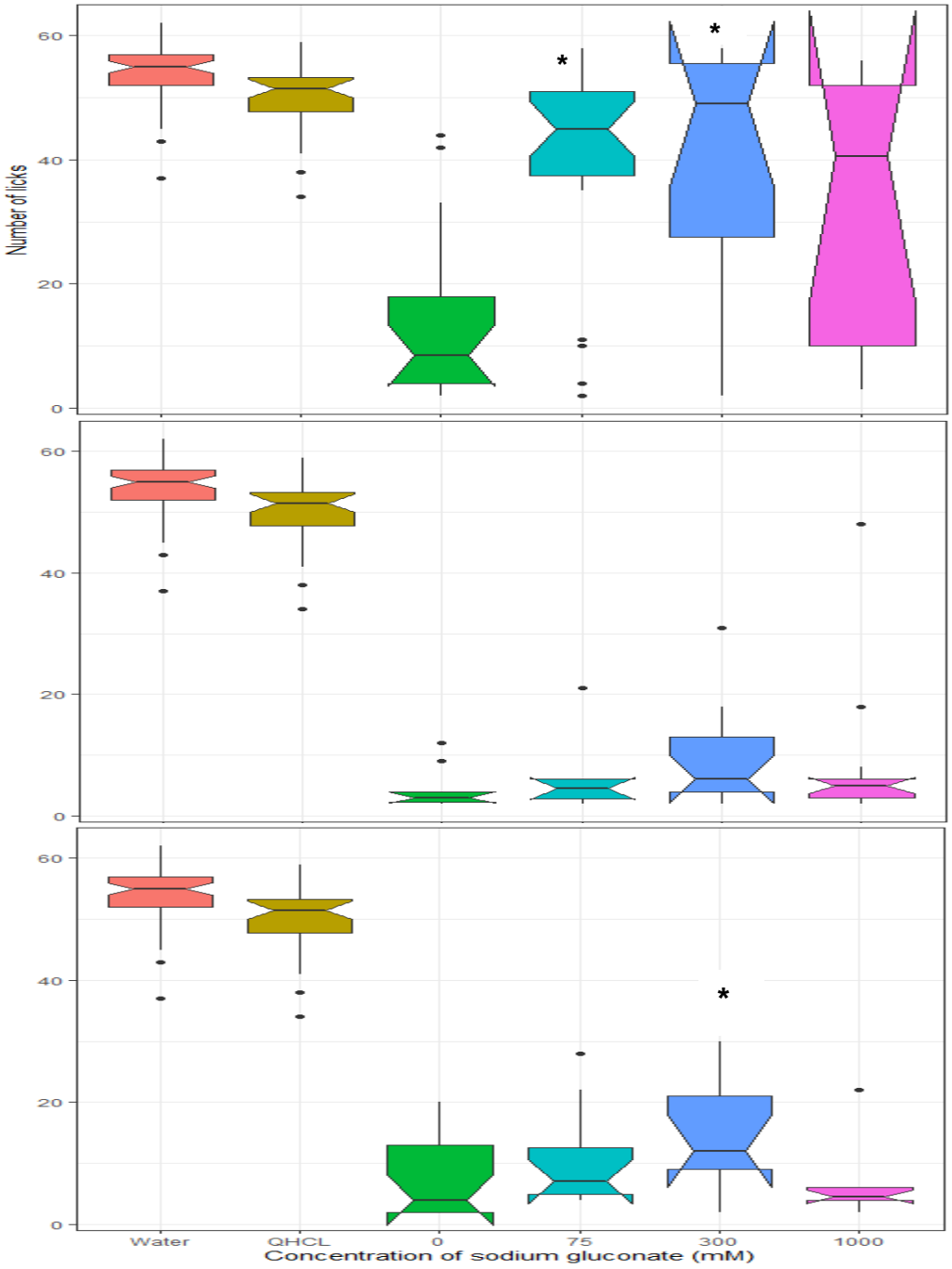


Figure 4.12 BATA findings for 4mM (top) and 48mM (middle) Ranitidine hydrochloride and PQP (bottom) in formulation with 0, 75, 300, 1000mM sodium gluconate. *Denotes significant improvement compared to respective control using Gao post-hoc analysis ($p<0.05$)

The lick response to 4mM Ranitidine alone was unexpectedly low over both test days (figure 4.13) as it should lie around the IC50 mark.

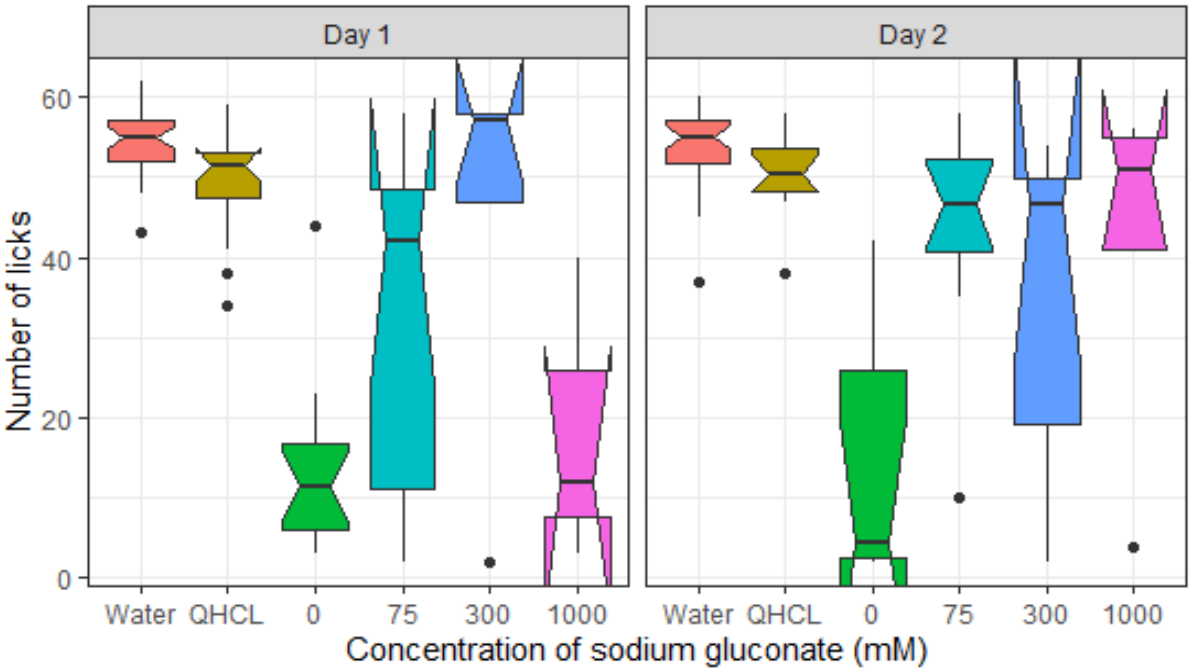


Figure 4.13 BATA findings for 4mM Ranitidine in formulation with sodium gluconate over the two test days.

To ensure this low Ranitidine data point was not skewing the data by making sodium gluconate appear more effective than it was, the data were reviewed another way. Table 4.7 lays out the mean lick response to each solution compared with water and the resulting tolerability category (Soto *et al.*, 2015; Ruiz *et al.*, 2019).

Table 4.7 Comparison of mean licks, percentage inhibition of water and aversiveness of 4mM Ranitidine hydrochloride in formulation with sodium gluconate.

Compound	Mean lick response overall	% inhibition vs water	Category
Water	54.1	n/a	n/a
QHCL	50.1	7.28 %	Well tolerated
4mM Ranitidine control	13.8	74.6 %	Aversive
Ranitidine + 75mM SG	39.1	27.7 %	Well tolerated
Ranitidine + 300mM SG	39.3	27.4 %	Well tolerated
Ranitidine + 1000mM SG	32.8	39.4 %	Tolerated

The Ranitidine formulations containing 75 and 300mM sodium gluconate were both well tolerated in comparison to water and so these solutions were deemed palatable irrespective of the Ranitidine control.

4.4.4.4 Summary of bitter-blocker experiments

The results of these BATA studies, assessing sodium acetate, sodium gluconate and NaAMP for their taste-masking of two paediatric drugs using the newly established model are summarised in table 4.8.

Table 4.8 Summary of bitter-blocker's impact on PQP and Ranitidine using the BATA model.

	4mM Ranitidine (rat IC50)	**48mM Ranitidine (therapeutic dose)**	**5.8mM PQP (therapeutic dose)**
Sodium acetate	No improvement	No improvement	No improvement
Sodium gluconate	75 & 300mM increased lick response to control *by 184 & 186 % respectively*	No improvement	300mM improved lick response to control *by 116%*
NaAMP	No improvement	No improvement	4mM increased lick response to control *by 76%*

None of the three bitter-blockers were able to provide an acceptably palatable formulation of PQP or Ranitidine at the therapeutic dose. Sodium gluconate and NaAMP (at well accepted concentrations as shown in chapter 3) did begin to improve the perceived palatability of PQP but this was not sufficient due to the aversiveness of the compound. Perhaps bitter-blockers may prove useful in combination with other taste-masking strategies. The risks associated with sweetener use (as discussed in chapter 1) stem from high exposure which can occur because they are found in many paediatric liquid formulations, with some used in high quantities, in order to achieve the necessary palatability for patient compliance (Al Humaid, 2018). Therefore, children can receive levels that far exceed even adult daily limits (Arthur and Burgess, 2017). This is even more of a concern for children with complex medical needs who are consuming multiple medications. Not only can this lead to side effects such as GI discomfort for the patient (Arthur and Burgess, 2017), there is evidence to show high levels of some taste-masking excipients can alter the bioavailability of the API and be detrimental to drug pharmacokinetics (Chen *et al.*, 2007; Abou-Donia *et al.*, 2008) as explored in chapter 5. With this in mind, if a bitter-blocker is unsuccessful at replacing a sweetener entirely, perhaps the two could be explored in combination (Gaudette and Pickering, 2012). This may enable lower levels of sweeteners to be used within paediatric formulations.

4.4.5 QHCL control

As seen in table 4.7 (and graphs 4.10-4.12) the QHCL bitter control was perceived as palatable – producing lick rates higher than, approximately, 50% of water. During the first two validation studies, the mean lick response to

0.08mM Quinine was 31.3 (week 1) and 28.4 (week 2). Week 3, which assessed a compound not part of this PhD project, had a mean lick response of 31.5, these were all in line with expected IC50 lick responses yet still a little higher than historical data. However, as the weeks progressed the mean Quinine HCL licks increased to 47.5 (week 4; sodium acetate assessment) and 46.4 (week 5; NaAMP assessment). For week 6 (sodium gluconate assessment) the QHCL concentration was increased to 0.16mM but this still was perceived as palatable with a mean lick rate of 50.2. Week 4,5 and 6 were all statistically different from week 1's lick response (figure 4.14).

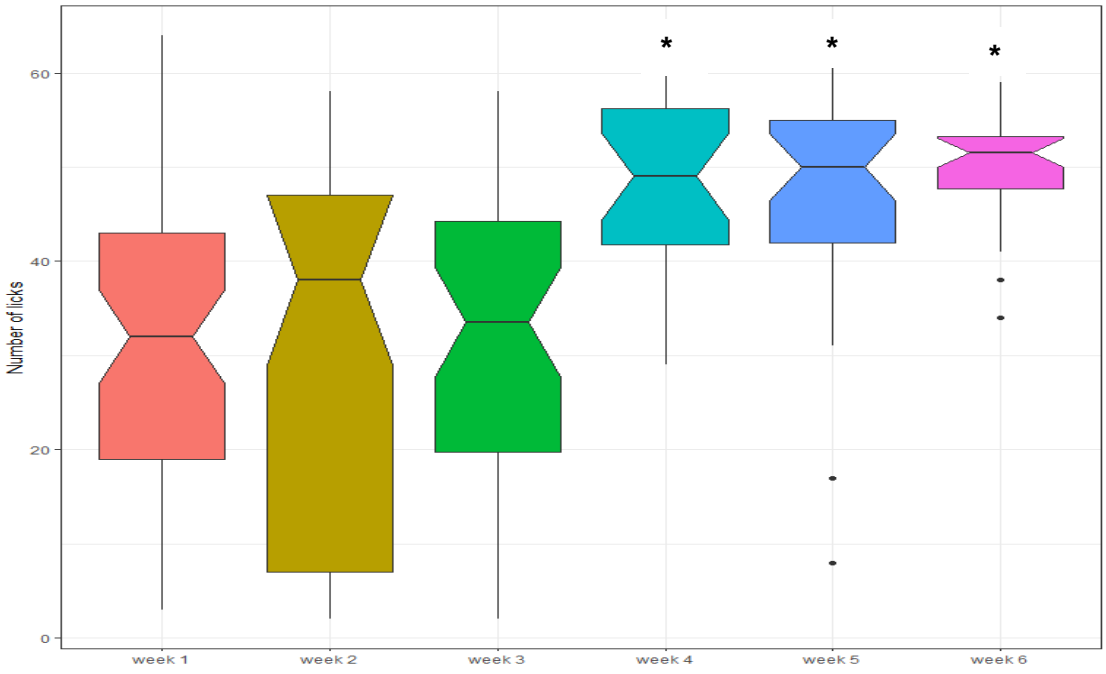


Figure 4.14 Rat lick response to QHCL control over experimental weeks at new laboratory site. *Denotes statistical difference from week 1. QHCL was used at 0.08mM to represent the IC50 value for weeks 1-5 inclusive but 0.16mM was assessed during week 6 to address the increase in tolerance.

The responses to QHCL each week were consistent between the two test days with only week 2 showing day to day difference, as discussed above, during QSD validation study week (figure 4.15)

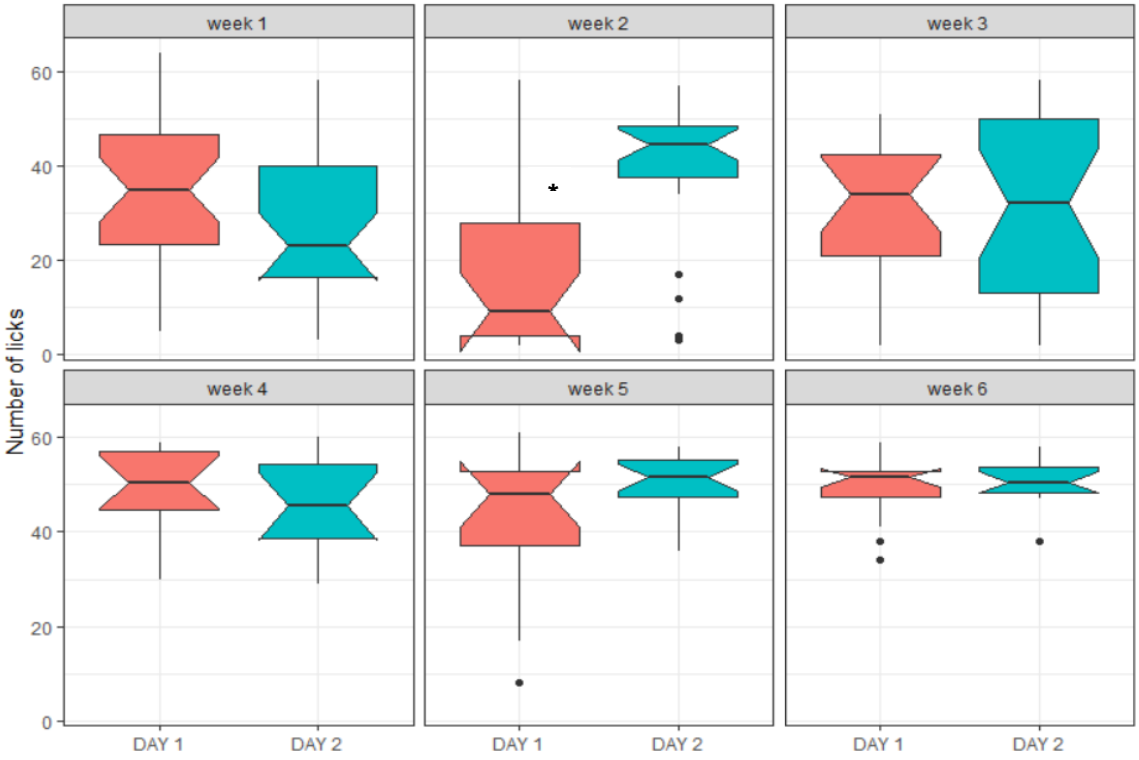


Figure 4.15 QHCL rat response by test day during experimental weeks 1-6 at the new laboratory site. *Denotes statistical difference to alternate day using Gao post-hoc analysis ($p<0.05$).

This phenomenon is known within the BATA model. As the animals age, they can become less sensitive to bitter-controls which are regularly presented (Soto, 2016). Soto documented how the IC50 value can change over the lifespan of a rat for three compounds (the highly bitter QHCL, Caffeine citrate which has low bitterness and Amlodipine besylate which has intermediate

bitterness) and the concentration needed to achieve IC50 can be much higher than when the animals were young (table 4.9).

Compound	IC50 (mM) at 4 weeks of age	IC50 (mM) at 7 weeks of age	IC50 (mM) at 10 weeks of age	IC50 (mM) at 13 weeks of age	IC50 (mM) at 21 months of age
Quinine hydrochloride dihydrate	0.113	0.115	0.145	0.084	0.377
Caffeine citrate	3.880	15.80	10.90	10.10	17.00
Amlodipine besylate	0.218	0.472	0.829	1.360	1.140

Table 4.9 Age-related changes to compound IC50 for QHD, Caffeine citrate and Amlodipine besylate as reported in Soto 2016.

During the validation studies, the animals were first exposed to QHCL, during week 1 assessment of Caffeine citrate, they were 4.5-5.5 weeks old and 0.08mM reflected the IC50 value. This is in line with previous work at UCL which showed rats at 1-month old to have an IC50 of 0.113mM for the drug (Soto, 2016) seen in table 4.9. As the animals got older this was insufficient to produce a 50% reduction in licks seen at week 4 of experimentation, when the animals were 10.5-11.5 weeks old, and tolerance is starting to show (figure 4.14).

4.4.6 End-of-study life IC50 experiment

Caffeine citrate was tested at two concentrations; 9.86mM and 39.44mM. This was to reflect the initial IC50 and a fourfold increase. The initial IC50 of 9.86mM was generated when the animals were 4.5-5.5 weeks old. This is in

line with data from the original laboratory; as seen in table 4.9 the IC50 increased fourfold from 4 weeks to 7 weeks of age, taking the midway point to represent a 5.5-week old animal the IC50 would be approximately 9.84mM. Similarly, Quinine sulphate dihydrate was tested at two concentrations; 0.0428mM and 0.1712mM to reflect the initial IC50 and a fourfold increase – no age-related work had been done previously on this compound.

As seen in table 4.9, the IC50 for QHCL in 21-month old adult animals can reach 0.377mM. The animals used in the end-of-study experiment were only 7-months old so a slightly lower IC50 of 0.32mM (representing a four-fold increase on the initial IC50) was chosen to reflect the IC50 in this age group.

Ranitidine hydrochloride was assessed at the original IC50 of 4mM and, to maximise data output, CPM was assessed at 2.02mM, the predicted IC50. Results are shown in figure 4.16.

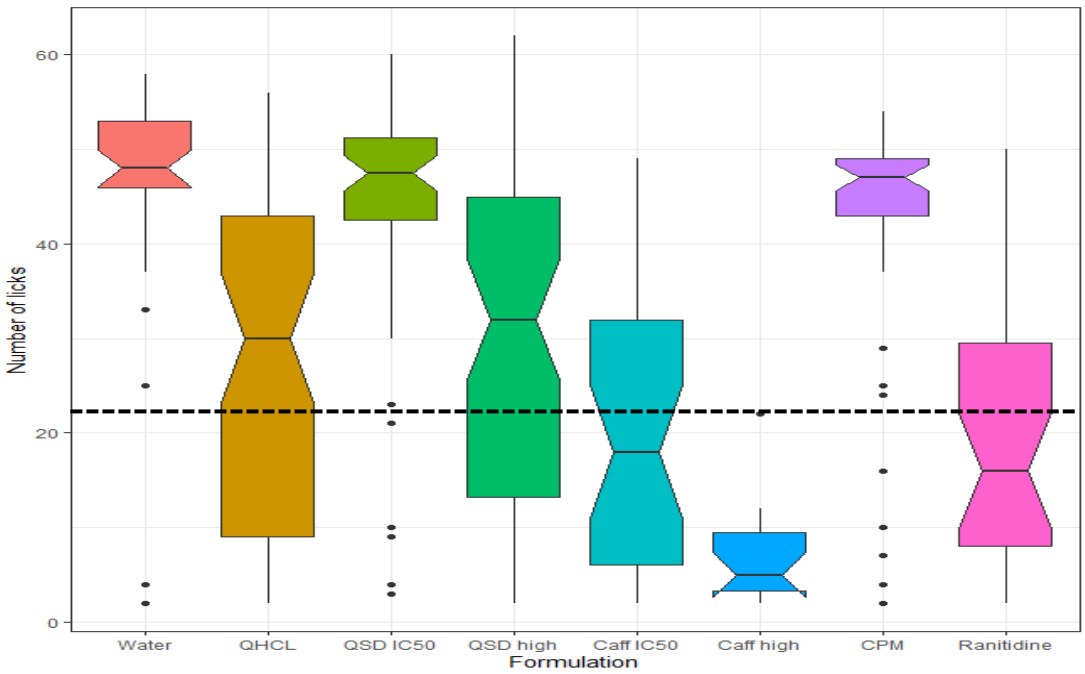


Figure 4.16 BATA findings for original vs predicted IC50 values. Caffeine citrate and QSD were presented at the original IC50 levels (labelled QSD IC50 and Caff IC50) and at an age-related predicted IC50 (labelled QSD high and Caff high). Dotted line approximately marks 22.23 which represents half the mean lick number of water (the IC50 level).

The number of outliers on figure 4.16 may seem high, however each of the ten animals was exposed to each solution on four occasions per day (each solution was in two tubes, with each tube presented twice per day), therefore there were 80 individual data points (over the two test days) which make up the boxplots and so the number of outliers is acceptable.

4.4.6.1 Inter-rat and day to day variation

Some variation between the rats' responses to the formulations were observed (figure 4.17)

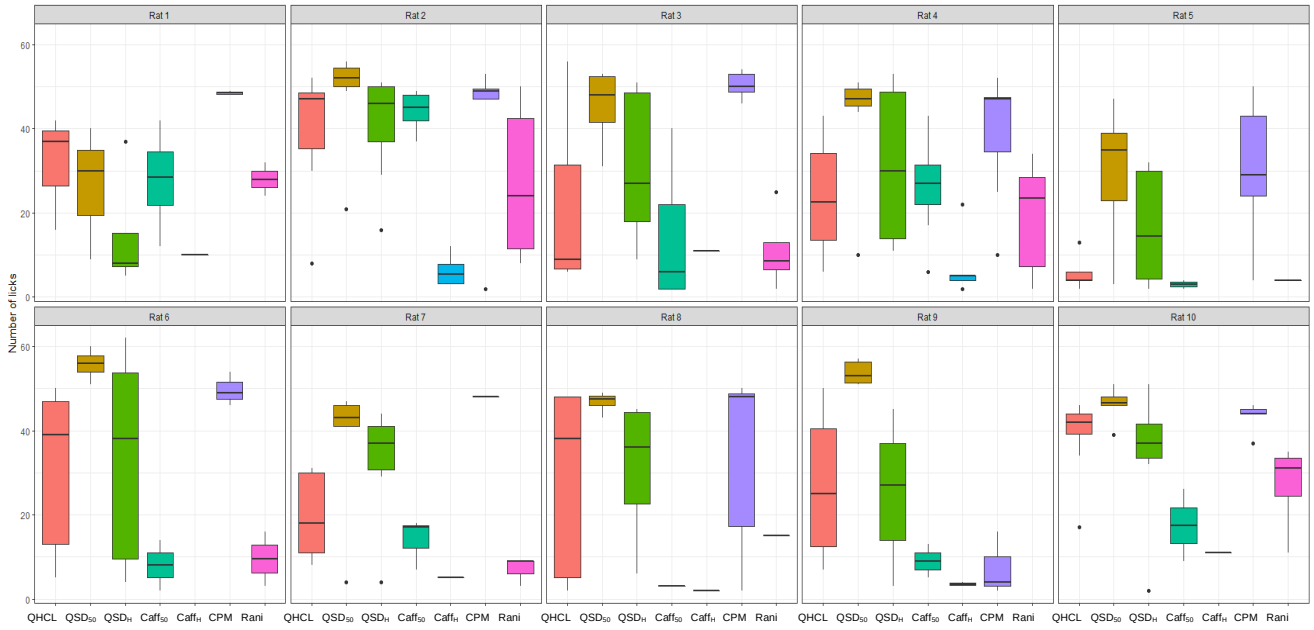


Figure 4.17 Individual rat BATA findings for original vs predicted IC50 values. QSD_{50}; QSD at original IC50. QSD_H; QSD at predicted IC50 (fourfold increase on original). $Caff_{50}$; Caffeine citrate at original IC50. $Caff_H$; Caffeine citrate at predicted IC50 (fourfold increase on original) Rani; Ranitidine

Yet there was no significant difference between day 1 and day 2 for any of the compounds, shown in figure 4.18.

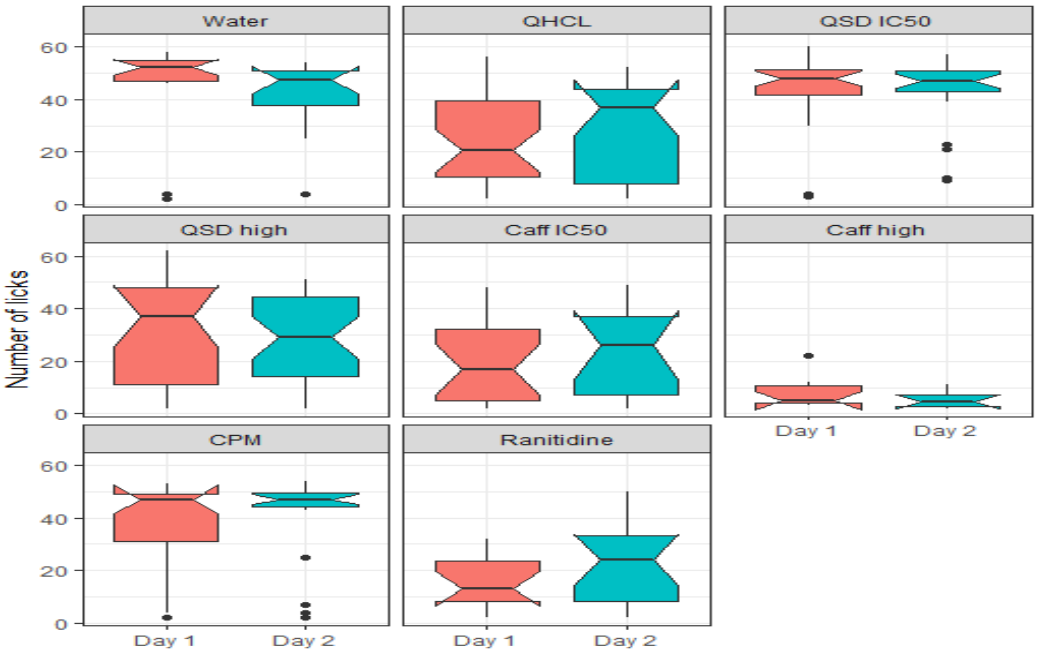


Figure 4.18 BATA findings for original vs predicted IC50 values over the two test days. Caffeine citrate and QSD were presented at the original IC50 levels (labelled QSD IC50 and Caff IC50) and at an age-related predicted IC50 (labelled QSD high and Caff high).

4.4.6.2 Interpreting Lick response compared to water control

The overall percentage inhibition of each compound compared to water is shown in table 4.10.

Table 4.10 Percentage inhibition of each compounds vs water

Compound	**Mean lick response**	**% inhibition vs water**
Water	44.5	n/a
QHCL (0.32mM)	27.1	39.0 %
QSD original IC50 (0.0428mM)	44.0	0.94 %
QSD high (0.1712mM)	30.0	32.6 %
CPM (2.02mM)	39.8	10.6 %
Caffeine original IC50 (9.86mM)	21.4	51.8 %
Caffeine high (39.44mM)	6.7	84.9 %
Ranitidine (4mM)	18.6	58.2 %

The predicted IC50 for this age of rat for QHCL (0.32mM) and QSD (0.1712mM) produced lick rate inhibitions of 39 and 32.6% respectively. This reflects the aging rats' tolerance to the bitter compounds. This is in line with historical UCL data (table 4.9) and also the literature which reports there to be changes in how rats respond to QHCL bitter stimulus over their lifetime (Inui-Yamamoto *et al.*, 2017). For animals in the first 6 months of life, increasing tolerance with age was seen for bitterness from juvenile (3-6 weeks), young adult (8-11 weeks) to adult animals (17-20 weeks). These changes were especially apparent for high bitterness compounds and less so for low bitterness compounds. Juvenile, young adult and adult animals showed similar responses to a low concentration of Quinine HCL (0.03mM) but showed marked increase in tolerance with age with a high concentration of Quinine HCL (0.3mM) (Inui-Yamamoto *et al.*, 2017) which was reflected in this work.

In this work, the animals were repeatedly exposed to Quinine, at least in its hydrochloride salt form, each experimental week (followed by a washout period of one week). This repeated exposure may account for the tolerance and it is a possibility that there was a decreased sensitivity to Quinine compounds. Previous work has shown that a one-week washout period was sufficient to make the rats naïve again to bitter-compounds including QHCL (Soto, 2016). Soto found that lick rate profile to QHCL was similar after a 1-week, 2-week or 4-week washout period with only a small increase in IC50 value seen when 1-week was used (0.231, 0.151, 0.159mM respectively). However, it may be that repeated exposure over many weeks, as carried out in the studies documented in this chapter, also drives decreased sensitivity.

Conversely, the original Caffeine citrate (9.86mM) IC50 concentration was still appropriate for these older animals, producing a 51.8 % lick inhibition. Previous work has shown there to be an age-related tolerance to Caffeine citrate (table 4.9) with the first major change seen in the first few months of life and another at 21-months of age after five exposures. Perhaps the shift in sensitivity reported by Soto is in part due to repeated exposure leading to tolerance rather than as a by-product of aging alone. However, in this work the animals were exposed to Caffeine citrate only on two occasions, at 4.5-5.5 weeks old and at 7-months old, likely explaining why a decline in sensitivity was not seen.

Tolerance on repeat exposure has also been demonstrated in mice for a number of bitter compounds including Caffeine and QHCL (Mura *et al.*, 2018). Exposure in drinking water over three weeks increased the animals' rejection threshold indicating the effect was not due to ageing but learned tolerance. However not all of the bitter drugs tested by Mura's group became tolerated – Salicin remained as aversive to the animals at the end of the experiment as it was in the beginning with authors suggesting the concentration was insufficient to induce tolerance. This was also seen in the work presented here with Ranitidine. Ranitidine was given to the animals during the three BATA studies, assessing the effect of the bitter-blockers, prior to this end of life experiment. However, the original IC50 concentration for Ranitidine (4mM) was also appropriate for these older animals, producing a 58.2% lick inhibition.

At first glance (over figures 4.10, 4.11, 4.12 to 4.16), it may seem as if the rats found the 4mM Ranitidine control more aversive overtime. However, the data

suggested there was no statistical difference in how the animals perceived it during their first exposure on week 1 (sodium acetate experimental week) compared to their fourth and final exposure during week 7 (end-of-study life experimental week) (figure 4.19). The only difference from week 1 was seen during week 3 when the 4mM Ranitidine control was unexpectedly low as discussed previously.

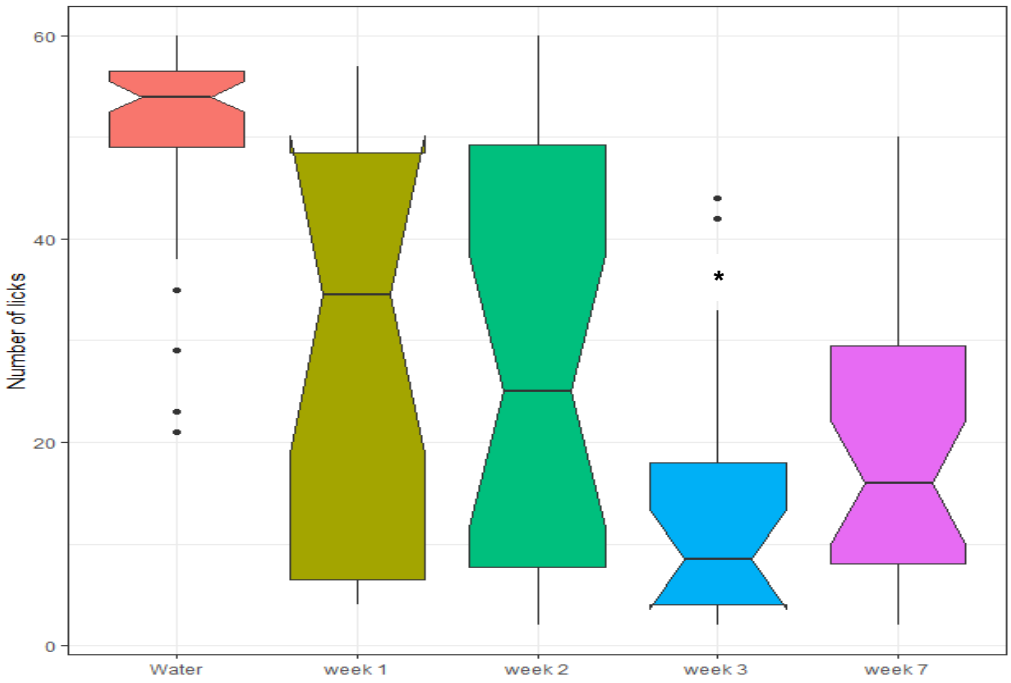


Figure 4.19 4mM Ranitidine control from weeks 1-3 and 7. Water is taken from week 1's results for comparison. *Denotes difference from week 1 using Gao post-hoc analysis ($p<0.05$)

No previous work has looked at animal response to Ranitidine over time. This data suggested there was no acquired tolerance to the compound from repeat exposure.

The IC50 of CPM was better tolerated than predicted. In the work presented here, the animals showed reduced sensitivity to some of the tested bitter-compounds overtime, as well as with repeated exposure. It is likely this concentration of CPM was not perceived as aversive due to the rats reduced bitter sensitivity at 7-months of age. The age of the rats the original IC50 value was obtained from is not reported (Keeley *et al.*, 2019).

4.4.7 Age-related sensitivity to bitter compounds

It has been reported in the literature that age can impact the gustatory system. Children can respond to certain tastes differently compared to adults. Children prefer more concentrated levels of sweetness which declines as they enter their teenage years (Mennella and Bobowski, 2015) and they are also more sensitive to bitterness (Forestell, 2017). The effect of age on taste-perception in humans seems to be taste-quality specific but these effects are inconsistently reported in the literature. An age-related decline in sensitivity to sour and salty tastes only has been reported in some studies (Mojet, Christ-Hazelhof and Heidema, 2001) whereas others found bitter and salty to be the taste-qualities affected (Weiffenbach, Baum and Burghauser, 1982). The mechanisms underlying age-related changes in human taste sensitivities are unclear. Initial work cited loss of taste buds (Arey, Tremaine and Monzingo, 1935) to be one explanation however other studies found no correlation between age and taste bud number (Arvidson, 1979).

Rodents also show similar age-related changes although the mechanisms driving this are unclear. Evidence from mice suggests there are age-related differences in levels of some blood serum components (Narukawa *et al.*, 2017), such as Angiotensin II, which are thought to modulate salty and sweet

taste sensitivity (Shigemura *et al.*, 2013). The age-related decline in levels is thought to impact taste sensitivities and correlate to a reduced preference for sweet and salty tastants. Furthermore, it has been shown, there are no changes to the number of taste-buds in old versus young mice but there was a significant reduction in taste bud size in 18-month animals compared with 2/10 month old animals (Shin *et al.*, 2012). Furthermore, a reduction in the number of taste-cells per taste bud in the older animals was found. Taste cells express receptors for taste, with type I cells involved in salty taste detection (Finger, 2005), type II cells transducing sweet, umami and bitter tastes (Miyoshi, Abe and Emori, 2001) and type III cells facilitating the perception of sourness (Finger, 2005). Specifically, a significant reduction in markers for type II and III cells were noted (Shin *et al.*, 2012). Such changes at the genotype level suggest sensitivity to umami, sweet, sour and bitter would all be impacted by age.

Inconsistencies have been reported in the literature with regards to how aging affects various taste sensations in rodents. Some studies have shown older mice have a higher preference for salty stimulus than younger mice but showed no difference for sweet, sour and bitter stimuli (Tordoff, 2007). Conversely, in another study older mice have been shown to have a lower sweet response than younger animals (Shin *et al.*, 2012). Other age-related changes in taste-preferences have been demonstrated with older mice having a decreased preference for MSG (an umami tastant) whilst having an increased preference to QHCL (likely due to decreased ability to detect bitterness) (Inui-Yamamoto *et al.*, 2017). The work by Inui-Yamamoto et al showed there was no linear age-related change in mice for preference to a

sour tastant (Hydrochloride) but responses to a salty tastant (sodium chloride) and to a sweet tastant (sucrose) were age-dependent, but the extent of this depended on the concentration of the stimulus. The lower concentration of NaCl (0.1 M) and sucrose (0.3 M) tested followed a linear age-dependent decrease in preference but the higher concentrations (0.3 and 0.5 M respectively) followed no clear pattern.

Moreover, as discussed, Soto 2016 (Soto, 2016) demonstrated that aged rats were less sensitive than their younger counterparts to the bitterness produced by Quinine hydrochloride (QHCL), Caffeine citrate and Amlodipine besylate. The aged rats were very old (21-22 months of age) compared with the older rats in this work of 7-months of age. Soto found 21-month old rats showed higher variability in all responses to the three bitter-tastants compared to the younger animals and concluded that rats should not be used after approximately 6-months in the BATA model.

As eluded to in this work, perhaps changes in perceived taste in rodents could be driven by the number of previous exposures to that, or to a similar, compound. This memory effect has been previously described (Soto, 2016) and the animals may still remember the aversiveness of a compound under assessment following the one-week washout period when a compound is repeatedly administered. This could lead to one of two possible outcomes; learned aversion to a compound or reduced sensitivity. The latter has been reported with QHCL when the washout period was reduced from one week to two days, suggesting this memory effect does not lead to learned aversion in the animals but to a higher tolerance (Soto, 2016). This is likely due to

attenuated neophobia as the animals learn that the taste is associated with a 'safe' outcome (Monk *et al.*, 2014).

However, as demonstrated with Ranitidine this may not be the case for all bitter drugs even on repeat exposure. Perhaps the differences seen in tolerance on repeat exposure between QSD/QHCL and Ranitidine is owing to the difference in bitterness. Ranitidine's IC50 of 4mM places it in the mildly aversive category, perhaps this concentration of Ranitidine is not sufficient to induce tolerance in the animals as was suggested to be the case for Salicin by Mura's group as discussed above. This is further supported by Monk et al's work which reported high concentrations of saccharin led to rat neophobia and subsequent attenuated neophobia on repeat exposure in the BATA model but this effect was not seen with a low concentration (Monk *et al.*, 2014).

It is important to understand that some bitter compounds may become tolerated over time and if animals are being kept for many months, it may become necessary to re-establish the IC50 concentration by running a full dose response curve.

4.4.8 Bitter control IC50 fluctuation over time: study impact

QHCL is used as a bitter control because it is a well-established bitter tastant on which there is many human taste data so it has a known EC50 and is a useful benchmark. The risk with fluctuating tolerance to QHCL, is that the concentration chosen to reflect IC50 level may not provide an accurate comparator of aversiveness. It is not clear what is the interplay of ageing and repeated exposure but both are likely to play a role in changes to animal sensitivity (figure 4.20).

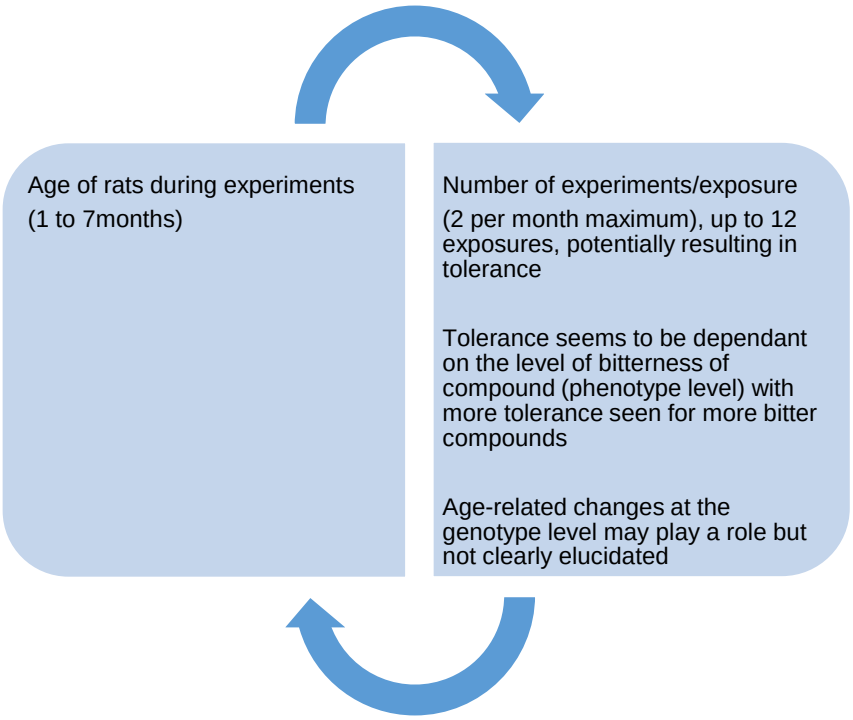


Figure 4.20 The interplay of rodent ageing and repeated exposure to bitter tastants.

Given this fluctuation, using the bitter control IC50 as the main comparator can be misleading. However, this should not be a limitation of the BATA model as if a control compound is giving an inaccurate IC50 value, it is possible to understand a novel compound's palatability by comparison with the water control alone.

4.4.9 The effect of age on water consumption

As the rats age it has been found that they drink significantly less water compared to younger animals (McKinley *et al.*, 2006) with differences seen from 6-months of age. This can mean animals require longer water deprivation to be motivated for BATA experimentation. The water deprivation can be extended by 30 minutes to 1 hour, as laid out by the restrictions in the project license. The need for such an extension was seen by some animals

falling asleep in the Davis Rig or general disinterest in the studies when previously they had been actively participating. For this reason, the lick rates to water and the other formulations can fluctuate as the animals age.

Such an effect on water intake began to be seen during the end-of-study life experiment (week 7) where the animals were 7-months old (figure 4.21). The mean water lick at week 7 was statistically different from that at week 1. The only other experimental week that produced a different water lick count to week 1 was week 3.

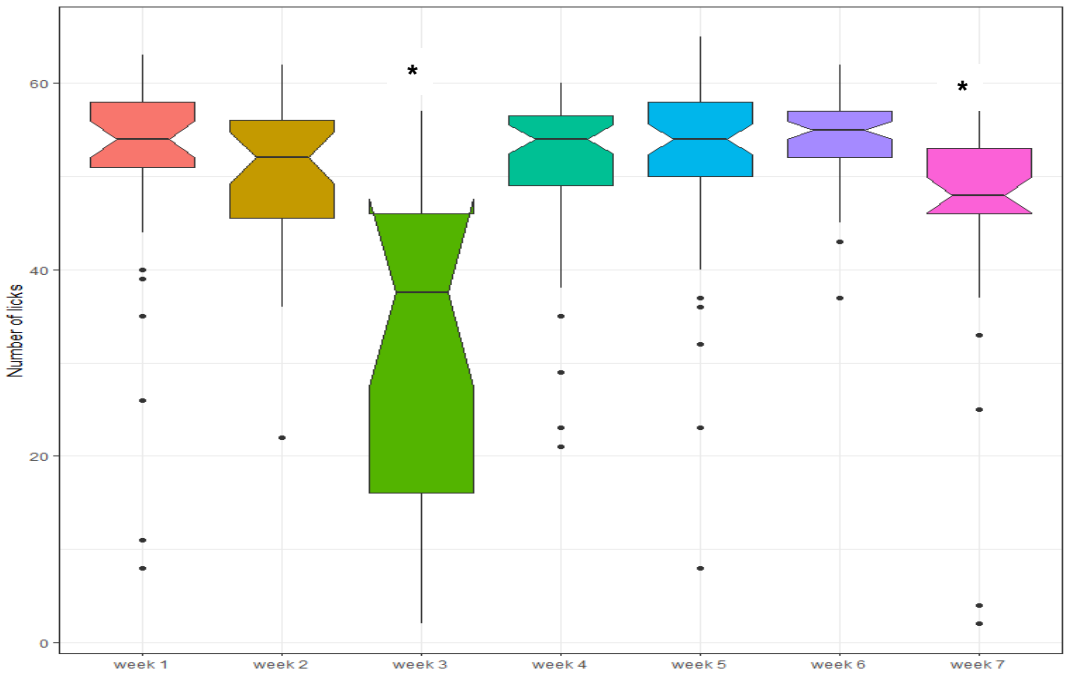


Figure 4.21 Rat lick responses to water over the experimental weeks. Weeks 1-6 were consecutive (with a washout week in between each) with rats being approximately 1-month old at the beginning. Experimental week 7 represents the end-of-study life experiment where the animals are 7-months old. *Denotes difference from week 1

On further evaluation of week 3, it can be seen that the response to water was different over the two test days with a very low response recorded on the first test day (figure 4.22). It was noted that there was training of new technicians being carried out that day so the increased noise was likely a distraction to the animals and responsible for this abnormally low result. The data from day 2 produced during week 3, was statistically comparable to week 1 overall lick rate.

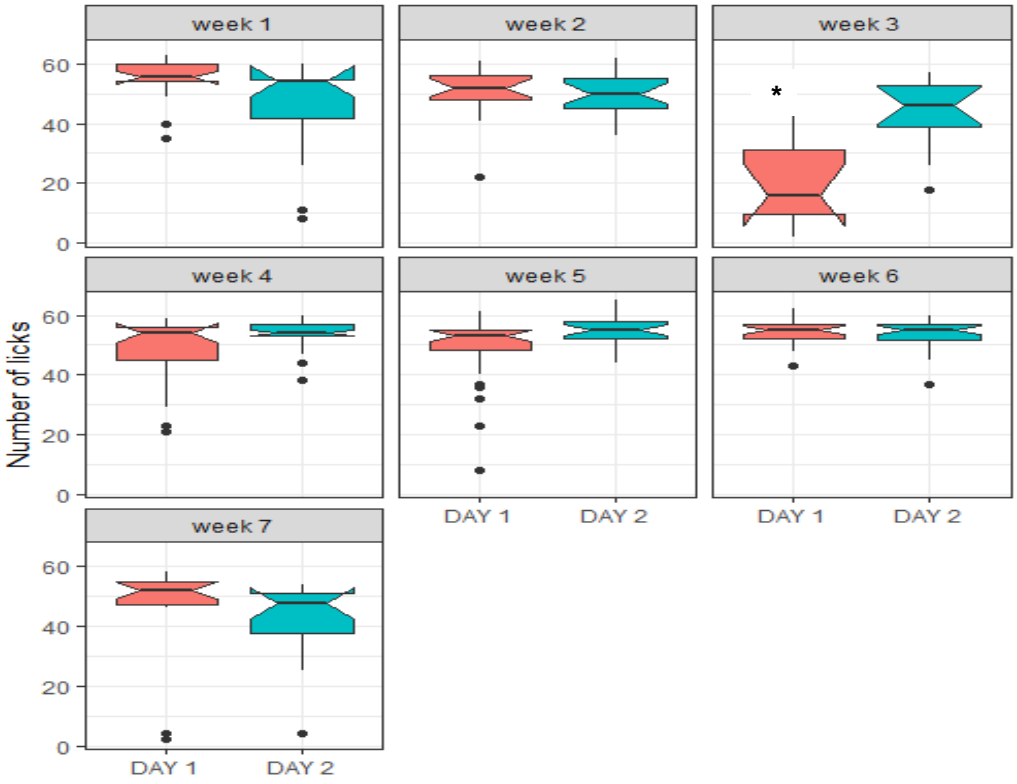


Figure 4.22 Rat lick responses to water over the experimental weeks broken down by day. *Denotes difference according to test day

It is postulated the reason for the reduced water intake over time is due to declining orolingual motor performance (Frutos *et al.*, 2012) which would

affect all test solutions in equal measure; therefore water accurately remains the fully palatable control.

Given the fluctuation seen with bitter-controls over time, and the incomplete understanding surrounding how factors such as age and previous exposure affect this, it is important to compare lick responses to novel compounds with the water control.

4.5 Conclusions

The BATA model was successfully transferred to a new location identified by validation studies using two well established bitter compounds, Caffeine citrate and QSD. This work highlighted the importance of primarily comparing the lick number of test compounds with that of water, and not with bitter control, as the response to the bitter control can alter with exposure and/or ageing for some bitter tastants.

After proof of concept described in chapter 3, on the use of universal bitter-blockers with Quinine HCL, this did not translate to a sufficient decrease in the bitterness of the 2 APIs at the concentrations tested in this chapter (despite one related to Quinine). This suggested they are unable to produce a purely neutral taste but may enable lower levels of other taste-masking sweeteners to be used within paediatric formulations if used in combination.

Chapter 5

Assessing Sorbitol's effect on paediatric gut permeability; a pharmacokinetic study

Taste-masking excipients are used widely in paediatric liquid formulations. Their effect on the paediatric gut and subsequent impact on drug bioavailability has not been adequately assessed. Sorbitol is a polyol that is used at high levels in children's medicines and has been shown to significantly impact the bioavailability of Ranitidine in adults. No work has looked at the effect in children. Children are not mini-adults and have their own unique gut environment so it is necessary to evaluate excipients, and their effects on drugs, in an age-appropriate model. This chapter describes a pharmacokinetic study evaluating the impact of sorbitol on Ranitidine in both juvenile and adult rats in order to fill in the knowledge gaps surrounding this topic. Sorbitol was found to significantly impact drug absorption in both age groups and so should to be used with caution in medicinal formulations.

5.1 Introduction

The bioavailability of a drug represents the fraction of the administered dose which reaches the circulatory system unchanged (Mehrotra *et al.*, 2006). Drug absorption mainly occurs in the small intestine (Murakami, 2017) and is subject to many influencing factors. Factors influencing bioavailability can be patient or formulation specific (Kaushal *et al.*, 2016) and are summarised in figure 5.1. Certain factors can be controlled, for example if the body is in the fed or the fasted state when dosing, whereas others stem from the drug's innate properties and characteristics.

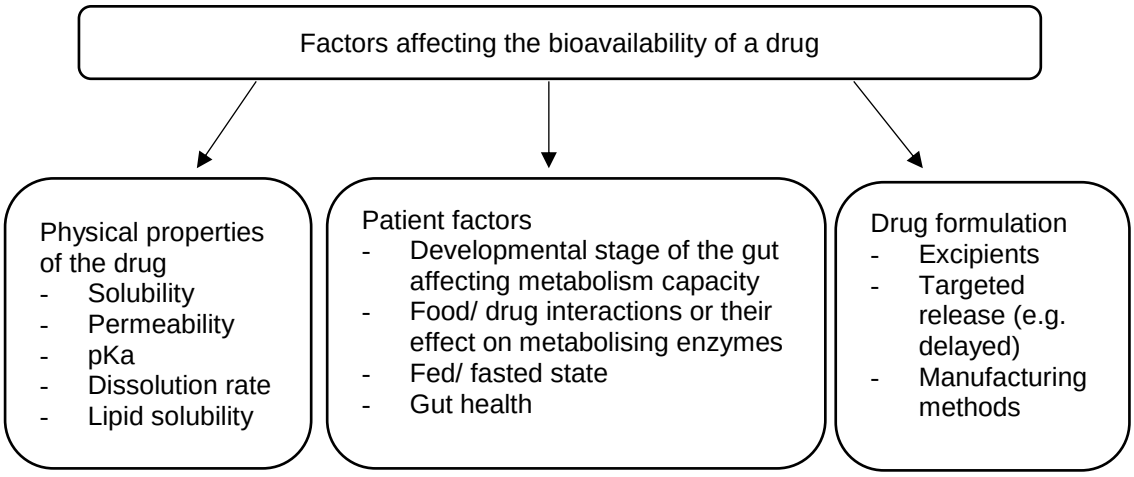


Figure 5.1 Factors affecting the bioavailability of a drug.

5.1.1 The impact of excipients on bioavailability: gut effect

Pharmaceutical excipients frequently make up the majority of a formulation (Katdare and Chaubal, 2006) and, unlike previously thought, are not inert. Excipients can negatively impact the bioavailability of API through acting in the GI tract (Panakanti and Narang, 2012).

Excipients can alter transit time and in doing so affect API absorption. For example, sodium acid pyrophosphate, when added to an effervescent tablet containing Ranitidine HCL, has been shown to reduce small intestine transit time by over 40% and as a consequence, halve the amount of API the body absorbs (Koch *et al.*, 1993). Some taste-masking agents have also shown similar effects on transit time including the polyols mannitol (D. Adkin *et al.*, 1995), sorbitol (Chen *et al.*, 2007) and xylitol (Salminen *et al.*, 1989) which are used routinely in liquid medicinal formulations (Niazi, 2004). If transit time is sped up, then gut residency time is reduced – preventing API for being absorbed.

Alternatively, an excipient can impact drug permeability by acting on intestinal transporters. The two main transporters families in humans can be classified into either the solute carrier (SLC) family or the ATP-binding cassette (ABC) family (Zhang *et al.*, 2016). The former facilitates the influx of small molecules into cells by facilitated diffusion or active transport (Lin *et al.*, 2015). It is the ABC family that mediate the efflux of substrates and reduce drug permeability.

5.1.2 ATP-binding cassette transporters

ABC transporters are a family of trans-membrane proteins which utilise ATP to move substrates (Locher, 2009). They transport a huge variety of, mainly lipid soluble, metabolites, xenobiotics and peptides. Their role is to protect the body from potentially harmful compounds but pose a major obstacle for the uptake of therapeutic drugs from the intestine (Sharom, 2011) (figure 5.2).

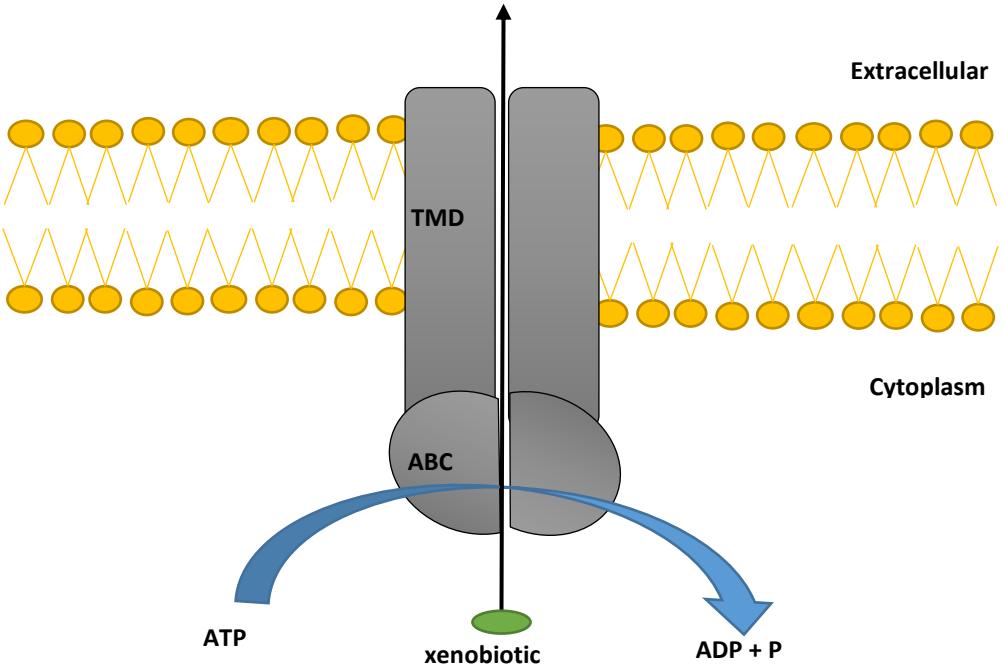


Figure 5.2 A schematic illustration of an ABC exporter. TMD; transmembrane domain, ABD; ATP-binding cassette, ATP; adenosine triphosphate, P; phosphate

There are a large number of ABC transporters, encoded for by different genes which are expressed throughout the human intestine (Drozdzik *et al.*, 2014). Breast cancer resistance protein (BCRP), multidrug resistance associated protein 2 (MRP2) and P-glycoprotein (P-gp) represent important ABC transporters which are found in a number of tissues including the enterocyte membranes (Tucker *et al.*, 2012; Drozdzik *et al.*, 2014).

Excipients which inhibit or induce these transporters can result in a huge change in the amount of drug efflux and thus alter exposure (Matsson *et al.*, 2009). This can be detrimental to a patient as it could lead to adverse drug effects or under dosing. When a drug is a substrate for an ABC transporter, formulators need to know which excipients to be mindful of using. A large number of excipients (mainly surfactants, lubricants and fillers) have been shown to, predominately, inhibit these transporters using preclinical models (Zhang *et al.*, 2016; Katrajkar *et al.*, 2019) with examples given in table 5.1

Table 5.1 Examples of excipients which have been shown to act on ABC transporters. Summarised from Zhang et al and Katrajkar et al. HMPC; Hydroxypropylmethyl cellulose, PEG; Polyethylene glycol, TPGS; d-α-tocopheryl-polyethylene glycol-1000-succinate

ABC transporter	Excipient
PGP	
Inhibitors	Acconon E, Brij-35, Cremophor® EL, Cremophor® RH 40, HPMC, Imwitor 742, Labrasol, Miglyol, 1-Monoolein, 1-Monostearin, PEG 40 stearate, PEG 400, Phosphatidylcholine, Pluronic® F127, Pluronic® F68, Pluronic® P123, Polysorbate 20, Polysorbate 40, Polysorbate 80, Pregelatinized starch, Sodium alginate, Sodium lauryl sulfate, Softigen 767, TPGS 1000,TPGS 800,Tween® 20, Vitamin E TPGS, Vitamin E TPGS, Xanthan gum
BCRP	
Inhibitors	Brij 30, Cremophor® EL, Pluronic® P85, Span 20, Tween® 20
Inducers	Oleic acid
MRP2	
Inhibitors	Brij-35, Capmul® MCM, Cremophor® EL, Cremophor® RH 40, LabrafacLipophile® WL 1349,Labrasol®, Maisine® 35-1, 1-Monoplamitin,1-Monoolein, PEG 2000, PEG 400, Pluronic® F127, Transcutol®, Tween® 80, Vitamin E TPGS 1000

Some work has investigated the effects of food additives, including a selection of colouring agents and sweeteners, for their effect on BCRP, MRP2 and P-gp (Sjöstedt *et al.*, 2017). Using an in vitro assay this work found Neohesperidin dihydrochalcone, a sweetener, had an inhibitory effect on BCRP transport. They also found a number of the food colorants tested inhibited at least one transporter. Acesulfame K, advantame, aspartame, cyclamate, neotame, saccharin, stevioside and sucralose had no effect on transporter action although they were only assessed at low concentrations. Moreover, the authors acknowledge the limitation of this work being the levels at which the

sweeteners were tested (each was tested at 50 μM). These sweeteners are widely used in liquid medicines (Al Humaid, 2018) and are found in the diet, for example some soft drinks contain very high concentrations, correlating to up to 0.55, 1.23, and 1.16 mM for aspartame, acesulfame K, and cyclamate respectively (Sjöstedt *et al.*, 2017). Therefore, in reality much higher levels may be present in the body which could act on transporters.

None of the excipients investigated by Sjöstedt et al or by Zhang et al. were evaluated in paediatric models.

5.1.3 The impact of age

As discussed in chapter 1, children are not mini adults and have their own unique gut environment according to their developmental stage (Khan *et al.*, 2022). However, as previously eluded to, significant knowledge gaps remain regarding the GI development of children (Batchelor, Fotaki and Klein, 2014; Wollmer *et al.*, 2022). As reported by Wollmer et al (Wollmer *et al.*, 2022), historical data detailing the ontogeny of GI characteristics in children is incomplete and may draw incorrect conclusions. This is due to a number of reasons; 1) the quality and/or age of the information, 2) data from healthy and sick children has been mixed together, 3) data from groups of patients were mixed with data from individual patients, 4) data was generated from just one child 5) study techniques are not fully reported so may be inappropriate and affect the outcome. Wollmer's group produced an updated review on paediatric GI physiology collating all the known information and assessing it with a critical eye. This work highlighted a number of gaps in knowledge, citing 'gastric and intestinal fluid composition and physicochemical properties, intestinal fluid volume and pH, pressure conditions along the entire GI tract,

colonic motility patterns, GI transit times of an orally administered dosage form' as a few of major concern. The work found the lack of data for infant and toddlers to be a particular issue.

Moreover, there are many unknowns surrounding transporters in young children. Johnson and Thomson (Johnson and Thomson, 2008) found that P-gp increases to adult levels by 2 years old whereas Fakhoury et al (Fakhoury *et al.*, 2005) found P-gp expression in the intestine was not influenced by age. In the published works entitled 'How Much Do We Know About Drug Handling by SLC and ABC Drug Transporters in Children? (Nigam and Bhatnagar, 2013)' the authors simply concluded 'we don't know enough'. It is likely that GI transporter function matures with age, as has been shown in other organ systems (Martel *et al.*, 1998; Matsuoka *et al.*, 1999) but this remains unclear. Owing to these gaps in knowledge surrounding infant GI physiology, in silico physiologically based pharmacokinetic (PBPK) modelling is challenging for this age group.

Moreover, the literature reports that excipient mediated effects on drug bioavailability differ according to sex (Afonso-Pereira *et al.*, 2016). This work showed that the excipient PEG 400 enhanced the bioavailability of Ranitidine in male but not in female rats and concluded this was likely due to sex differences in GI intestinal transporters. This sex effect suggests other patient specific variables may result in differences in how an excipient affects API bioavailability. This poses the question; are there excipient mediated effects which differ between adults and juveniles?

5.1.4 Polyols affecting the gut

Polyols, also known as sugar alcohols, are used extensively in paediatric formulations as fillers and sweeteners (Grembecka, 2018; Rouaz *et al.*, 2021) and as such need to be explored for their effect in the paediatric gut.

Existing Biopharmaceutical Classification System (BCS) based biowaiver guidelines name a number of excipients which may affect oral drug absorption and polyols are on this list (Metry and Polli, 2022). Polyols are osmotically active (Yamane *et al.*, 2021), promoting water secretion into the GI lumen which increases GI fluid volumes resulting in diluted intraluminal solute. This leads to a smaller concentration gradient across the intestinal epithelial membrane and slower absorption. The increased GI fluid volume increases GI motility and shortens transit time so poorly permeable drugs are insufficiently absorbed (D. A. Adkin, Davis, *et al.*, 1995; D. A. Adkin, Gowland, *et al.*, 1995; D. Adkin *et al.*, 1995). Both baseline GI fluid volume and GI transit time are factors which may differ for children compared to adults (Wollmer *et al.*, 2022) and so polyols may affect drugs differently depending on the age of the patient. No studies have evaluated taste-masking excipients for use in paediatric medicines in relevant paediatric models. It has been assumed that excipient effect on bioavailability in adults will be the same in children.

5.1.5 Sorbitol

Sorbitol (figure 5.3) is a polyol of particular interest. A recent review evaluating troublesome excipients for use in children, featured sorbitol in a list of the ten most important excipients to prioritize for investigation (Rouaz *et al.*, 2021). This is owing to 1) the frequency with which it is used in liquid formulations as a bulking agent and non-nutritive sweetener 2) the concerns associated with

its use (e.g. its negative effect on drug absorption (Chen *et al.*, 2007)) 3) the limited safety information available in relation to children.

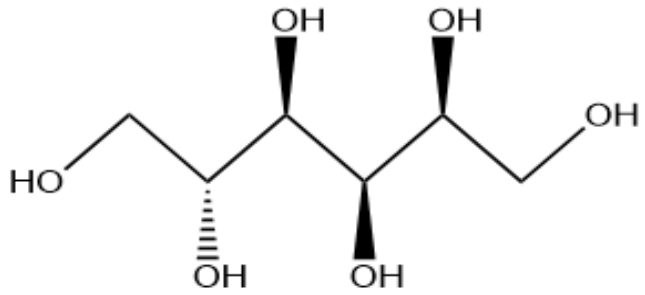


Figure 5.3 The structure of sorbitol

There is limited data available on the relevant thresholds of polyols in children although it is known 140mg/kg/day P.O could lead to GI symptoms (Arthur and Burgess, 2017). However, these levels are routinely surpassed in medicinal practice for example, if a 1-year old patient of 9kg is prescribed 250mg amoxicillin three times per day (containing 125mg amoxicillin and 800mg sorbitol per 5mL) they will be receiving 4.8g (533mg/kg) of sorbitol per day. Sorbitol is also found at high quantities in Ranitidine formulations as listed in the electronic medicines compendium (EMC) summarised in table 5.2. ADVANZ Pharma's formulation contains 3000mg sorbitol and 150mg Ranitidine/ 10mL, if a 3-year-old child experiences reflux and receives 10mg/kg/day, this equates to 3g sorbitol and 150mg Ranitidine, although there is no dose given for a 1-year-old, an off-license prescription would expose that child to 1.8g of sorbitol.

Table 5.2 Sorbitol contents of Ranitidine liquid formulations. Dose of sorbitol based on 1-year old child (9kg) receiving 10mg/kg/day Ranitidine

Drug formulation	**Excipients**	**Dose of sorbitol**
Ranitidine 150mg/10ml Oral Solution – ADVANZ pharma	Sorbitol, ethanol, disodium hydrogen phosphate dehydrate, sodium dihydrogen phosphate dihydrate, hydroxyethyl cellulose, peppermint flavour, saccharin sodium, purified water	• Contains 300mg/mL sorbitol • 1-year-old receives 1.8g sorbitol per day
Ranitidine 150mg/10ml Oral Solution – Rosemont Pharmaceuticals Limited	Ethanol, sorbitol, sodium, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate dihydrate, saccharin sodium, ethanol garden mint flavour, purified water.	• Contains 140mg/mL sorbitol • 1-year-old receives 840mg sorbitol per day
Ranitidine 300mg/10ml Oral Solution – Creo Pharma Limited	Sorbitol, ethanol, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate monohydrate, hydroxyethylcellulose, tutti frutti flavour, sodium saccharin, sucralose, sodium hydroxide, purified water	• Contains 110mg/mL sorbitol • 1-year-old receives 660mg sorbitol per day
Zantac syrup 150mg/10mL – GlaxoSmithKline UK	Ethanol, propyl hydroxybenzoate, butyl hydroxybenzoate, sorbitol, hydroxypropyl methylcellulose, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate anhydrous, sodium chloride, saccharin sodium, mint flavour, purified water	• Contains 70mg/mL sorbitol • 1-year-old receives 420mg sorbitol per day

Dose dependent osmotic effects of sorbitol on Ranitidine's PK have been reported when high doses of the sugar alcohol are present (Chen *et al.*, 2007). Figure 5.4 (drawn using PK-Sim software version 10) presents data from Chen et al (Chen *et al.*, 2007) showing the dose dependent effect of sorbitol on the bioavailability of Ranitidine in humans. The Ranitidine C_{max} is approximately halved when 5g of sorbitol is present in the formulation.

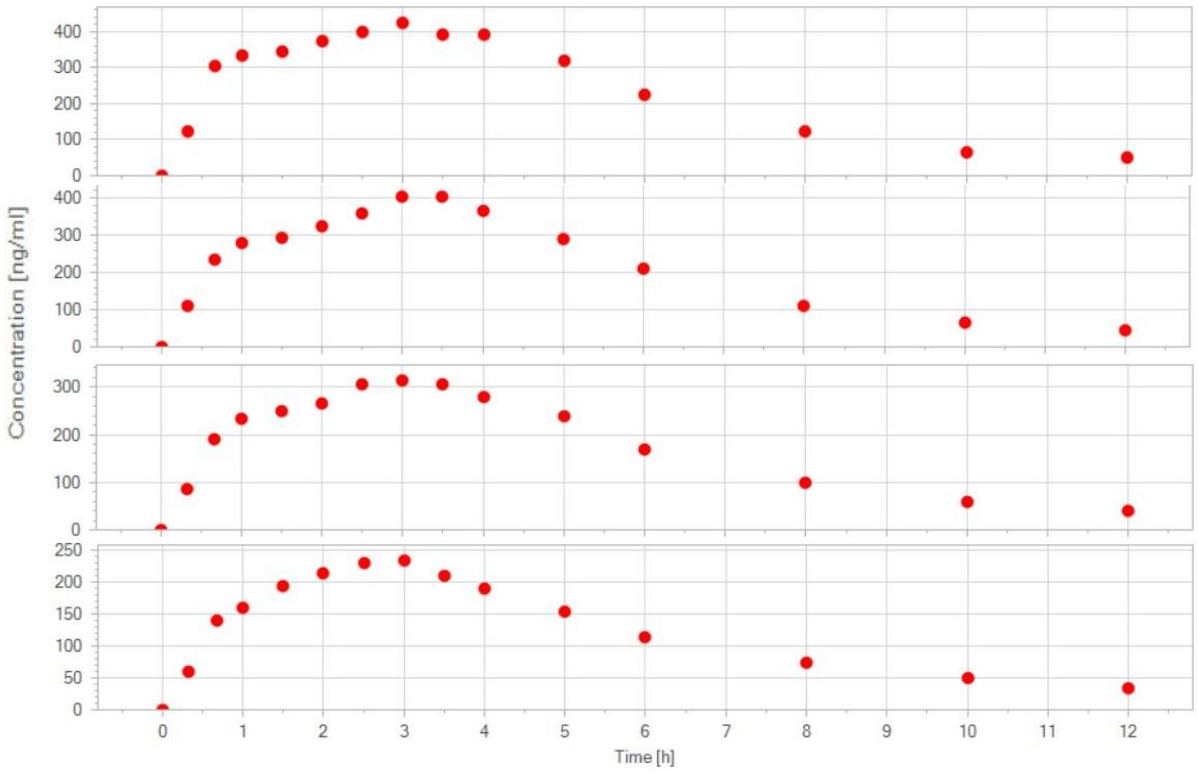


Figure 5.4 Mean plasma concentrations of Ranitidine in humans after 150mg oral dose. Top is Ranitidine alone, second down is Ranitidine + 1.25g sorbitol, third down is Ranitidine + 2.5g sorbitol and forth down is Ranitidine + 5g sorbitol as reported in Chen et al 2007.

An ongoing clinical trial investigating sorbitol's effect on the class III drug Lamivudine in adults is using 3.2, 10.2 and 13.4g of sorbitol to represent relevant concentrations for patients who are on multiple medications (ClinicalTrials.gov, 2017). Although this sorbitol exposure is based on adult doses of formulations, it is not unlikely children who are taking multiple medications and/or getting sorbitol from their diet, would reach the levels featured in this study. This cumulative effect is a cause for concern (as discussed in chapter 1).

Children have a unique gut environment and need to be looked at as a distinct patient population. With children potentially exposed to such high levels of sorbitol its effect on API needs to be explored. Therefore, this work investigated a high but clinically relevant dose of 3 grams of sorbitol which a child could ingest. Since sorbitol has been shown to have a significant effect on Ranitidine in adults, this drug was chosen to be evaluated in this work.

5.2 Aims and objectives

The aim of this work was to identify if there is an age-related effect of sorbitol on the bioavailability of Ranitidine. Sorbitol is known to impact the bioavailability of Ranitidine in adults and so the objective of this work was to carry out a pharmacokinetic study using juvenile and adult animals for comparison. Understanding how excipients, such as sorbitol, influence drug bioavailability in children would improve how the scientific community selects excipients when formulating paediatric medicines.

5.3 Materials and methods

5.3.1 PK study

The PK study took place over two days, the first assessing juvenile animals and the second assessing adults animals.

5.3.1.1 PK study materials

Ranitidine hydrochloride and sorbitol were purchased from Sigma-Aldrich (Gillingham, UK). Oral dosing tubes (16G x 75mm, 18G x 50mM and 20G x 38mm) and winged infusion needles for blood collection were purchased from

VetTech Solutions (Congleton, UK). Lithium heparin coated anti-coagulant blood tubes were supplied by Vetlab Supplies Ltd (Pulborough, UK).

5.3.1.2 Animals

16 male Wistar rats were supplied by Charles River, of which 8 weighed 100-120g (approx. age 4-5 weeks; juvenile, this age was chosen due to the license limitation that the animals must be post-weaning age) and 8 weighed 280-300g (approx. age 12 weeks; adult) on arrival. Animals were randomised into groups of four per age group and ear marked for identification. They were housed in a room maintained at 21±2 °C with 55±15% humidity and with a 12:12 h light/dark cycle. Animals had free access to food and water throughout the study. The animals were not fasted for this PK study. This is because the juvenile rats were representing infants who are likely to be in the postprandial state at any given time due to the high frequency of food intake. Furthermore, there has been no effect of food seen for the absorption of Ranitidine (GlaxoSmithKline, 2004) and so it was unnecessary to fast the animals.

Wistar male rats were investigated in this study since previous work has shown there to be an excipient mediated effect, of PEG, on the bioavailability of Ranitidine in this strain in males but not females (Afonso-Pereira *et al.*, 2016; Mai *et al.*, 2019).

In this study the juvenile rat represents a young child of around 1-year-old. Weaning age in humans is around 6 months and in rats is around 3 weeks, since rats have a much more accelerated childhood compared to humans this

study used rats of 4-5 weeks to represent the chosen age group (Sengupta, 2013).

5.3.1.3 Experimental groups

There were 2 test groups and 2 control groups; 1 test and 1 control group for juvenile and adult rats. The experimental unit was one rat. There were 4 rats in each experimental group, this was calculated using a power analysis on the NC3R's Experimental Design Assistant (EDA) (EDA, 2015) based on the effect size difference for control Ranitidine vs Ranitidine + max dose of excipient in previous work using this strain of rat (Afonso-Pereira *et al.*, 2016).

Treatment was randomised with 2 animals per cage receiving Ranitidine hydrochloride alone and 2 receiving Ranitidine hydrochloride + sorbitol. The technician administering the solution was blinded, with the solution labelled by cage and rat, not by contents.

5.3.1.4 Test solutions

The day before each study day, 2 solutions were made up in deionised water; Ranitidine hydrochloride alone and Ranitidine hydrochloride + sorbitol. The animals either received 61.7mg/kg Ranitidine hydrochloride or 61.7mg/kg Ranitidine hydrochloride + 1852mg/kg sorbitol. This was calculated using a published equation to generate animal dose from human dose, accounting for differences in body surface area (Nair and Jacob, 2016) (figure 5.5)

Based on human dose for 1-year-old (10kg), receiving 3g sorbitol from medicine(s) and 150mg Ranitidine hydrochloride

HED (mg/kg) = AD (mg/kg) x (Animal Km ÷ Human Km)

Km is the BSA conversion factor which is 37 for humans and 6 for wistar rats

10mg/kg ÷ (6÷37) = 61.7mg/kg Ranitidine

300mg/kg ÷ 0.162= 1851.9mg/kg sorbitol

Example dosing; for 100g rat (approx. 4-5 week old), 185.2mg sorbitol (1.8g/kg) and 6.2mg Ranitidine (61.7mg/kg) and for 300g rat (approx. 12 weeks old), 555.6mg sorbitol (1.8g/kg) and 18.6mg Ranitidine (61.7mg/kg).

Figure 5.5 Demonstration of how the animal dose was reached from human equivalent dose (HED) based on the equation published in Nair and Jacob 2016. BSA; body surface area, AD; animal dose.

The volume dosing limit according to the project license was 10mL/ kg. Animals were weighed one day prior to their study in order to make stock solutions. For the juvenile animals, each stock solution was made up so the heaviest animal could receive 1mL to receive their dose. The other juvenile animals received between 0.91 and 0.95mL depending on their weight.

For the adult animals, each stock solution was made up so the heaviest animal received 3mL to receive their dose, the other animals received between 2.7 and 3mL depending on their weight.

5.3.1.5 Ethical statement

The animal study was approved by the School's Ethical Review Committee and all procedures were carried out in accordance with the Home Office standards under ASPA (Project Licence number P4AF0DB91).

5.3.1.6 Study design

On the morning of the test day the animals were placed into a warming cabinet for around 10 minutes and then placed into a restrainer and bled via

the tail vein. This was to ascertain their time 0 baseline. Then each animal was dosed via oral gauvage with either 61.7mg/kg Ranitidine or 61.7mg/kg Ranitidine + 1.9g/kg sorbitol and bled 1, 2, 2.5, 3, 4 and 6 hours post dose using the warming cabinet and restrainer each time. These time points were chosen as Ranitidine's peak plasma concentration occurs between 2-3 hours post oral administration (Chen *et al.*, 2007). At each time point approximately 100µL blood was taken, this is in line with project license limits detailing a maximum daily collection of 15% total blood volume. Blood was collected into anticoagulant cubes and immediately stored on ice.

Blood samples were centrifuged at 10000 rpm (930*g*) for 10 minutes on a Centrifuge 5804R (Eppendorf AG, 22331Hamburg, Germany) within 8 hours of sampling. The supernatant (plasma) was collected and placed into a 1.5 mL Eppendorf tube, and immediately frozen at −20 °C prior to analysis.

The experimental design is summarised in figure 5.6 drawn using EDA software.

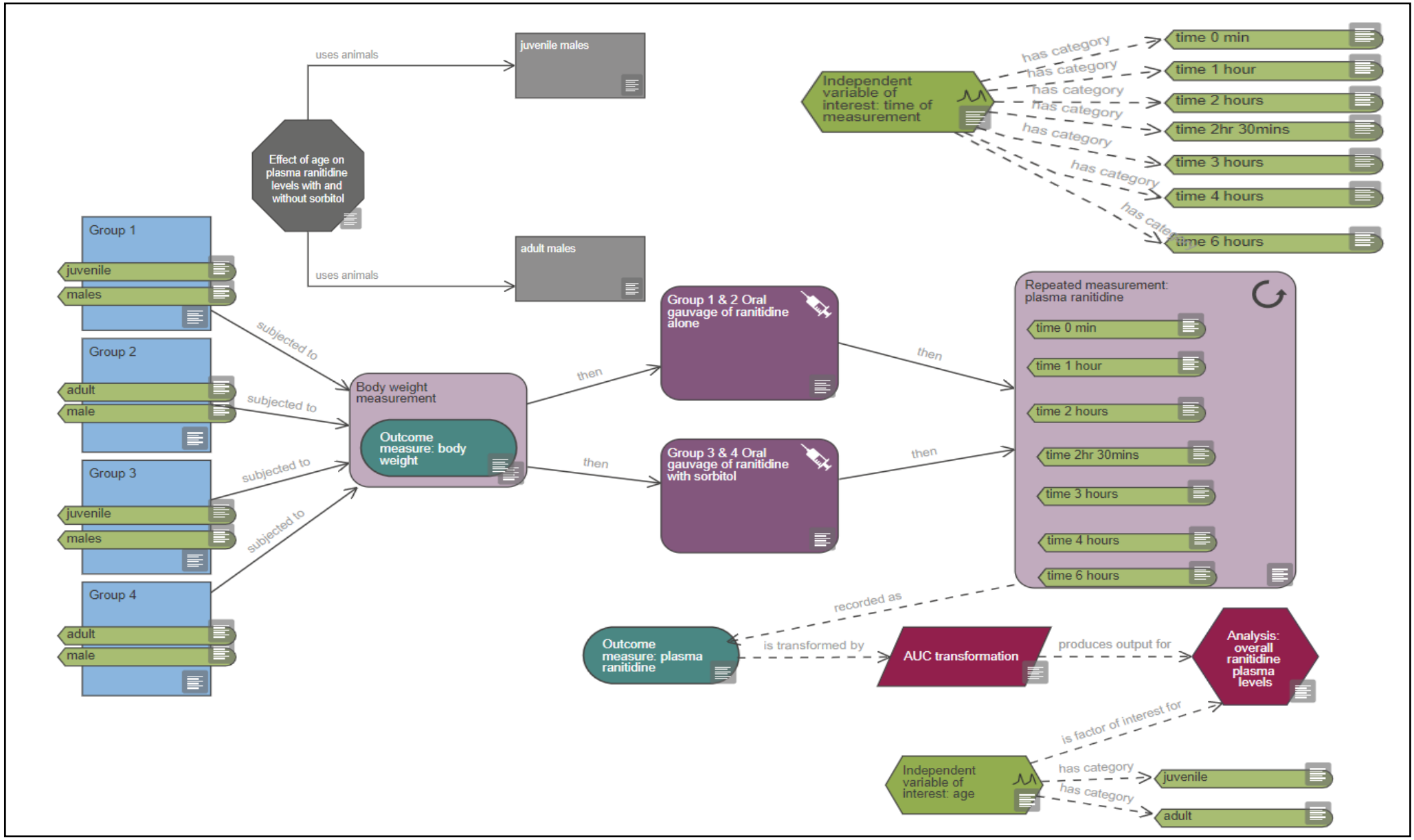


Figure 5.6 Experimental design of the PK study summarised using EDA software

5.3.2 Blood analysis

5.3.2.1 HPLC materials

Acetonitrile, sodium acetate and acetic acid were supplied by Sigma-Aldrich (Gillingham, UK). The column used was a 5µm Luna SCX (250 mm × 4.6 mm I.D.) with a guard column fitted with a filter cartridge (SCX 4 x 3.0mm), both were purchased from Phenomenex (Macclesfield, UK).

5.3.2.2 Blood processing

The plasma samples were thawed and 50µL was mixed with 50µL acetonitrile to precipitate the plasma proteins. This was vortex-mixed for one minute and 100µL HPLC grade water was added and vortex-mixed again. These samples were then centrifuged at 4 °C for 10 min at 10000 rpm. The resulting supernatant was subjected to HPLC-UV analysis using a previously validated method by Ashiru et al (Ashiru, Patel and Basit, 2007).

5.3.2.3 HPLC

The HPLC system used was a Hewlett–Packard 1050 Series high-performance liquid chromatography (HPLC) system, equipped with UV detector set at 320nm for the detection of Ranitidine. The chromatographic data was collected using PC/Chrom software (H&A Scientific Co., UK).

The flow rate was set to 2mL/min with a 40µL injection volume and the temperature was set to 50°C. Analysis for each sample was complete within 15 minutes. The mobile phase used for analysis was a mixture of 20:80 acetonitrile:0.1 M sodium acetate buffered with acetic acid until pH=5.0.

5.3.2.4 Calibration curve

Calibration standards were prepared with blank rat plasma samples spiked with drug subjected to the above-mentioned treatment (n=3).

5.3.2.5 Statistical analysis

All results were expressed as mean ± standard deviation (SD). The results were analyses by a three-way ANOVA to assess the impact of age on the different treatment groups over time. Tukey's post hoc testing was used on OriginPro 9.0. A minimum *p*-value of 0.05 was used as the significance level for the tests. The cumulative area under the plasma concentration vs time curve (AUC_{0-360}) was calculated using the integration method with OriginPro 9.0.

5.4 Results and discussion

The calibration samples (n=3 for each concentration) prepared in blank plasma gave an R^2 value of 0.9969 (figure 5.7)

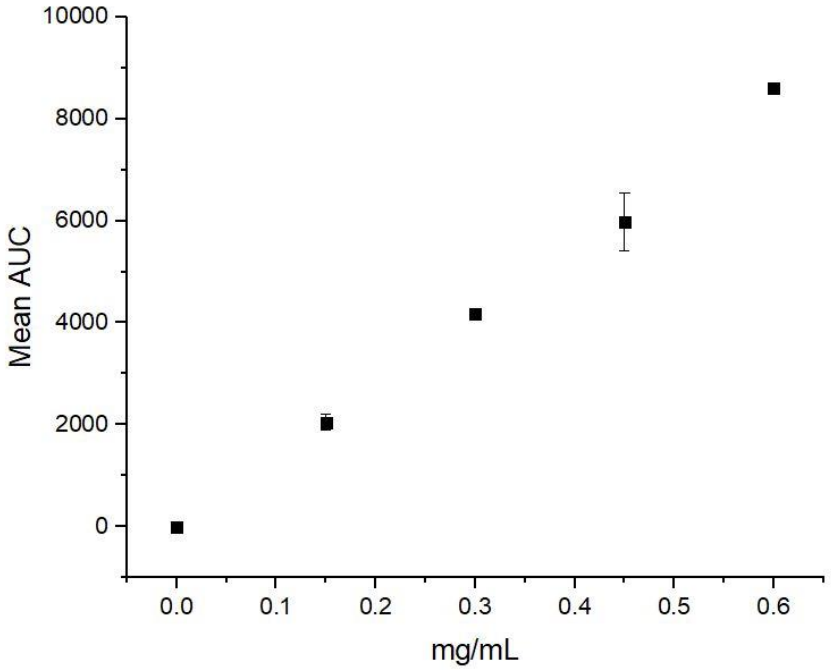


Figure 5.7 HPLC calibration curve generated using Ranitidine spiked rat plasma ± SD, n=3 per concentration. $R^2 = 0.9969$

5.4.1 Adult animals

In the adult rats, the blood concentration of Ranitidine significantly differed between the two treatment groups at the 2 and 2.5-hour time points (figure 5.8)

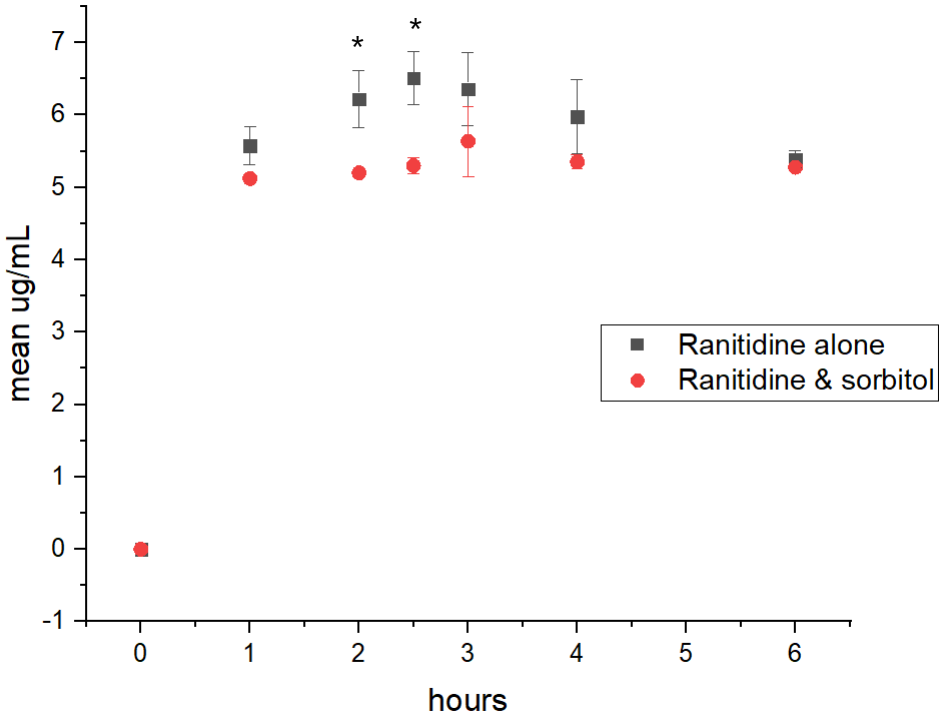


Figure 5.8 Ranitidine concentration vs time profile in the adult rats ± SD. * denotes significant difference to the corresponding time point in the test group

In the control group, receiving Ranitidine alone, the T_{max} was 2.5-hours. This blood concentration was statistically different from the concentration at 1-hour as Ranitidine is continuing to be absorbed. The 2, 2.5 and 3 hour concentrations are not significantly different indicating that the Ranitidine concentration was no longer rising between these time points in the control group.

When sorbitol was present in the formulation, although the highest blood concentration was achieved at 3 hours, this was statistically the same as the 1-hour time point. This means the peak Ranitidine concentration was reached quicker in the presence of the polyol. Sorbitol also reduced the C_{max} itself from 6.51 in the control group to 5.64, although this was a non-significant

difference. The conclusions drawn here is that the absorption of Ranitidine was sped up in the presence of sorbitol but less was absorbed overall. This reflects the published effect of sorbitol on Ranitidine in human adults (Chen *et al.*, 2007).

5.4.2 Juvenile animals

5.4.2.1 Unexpected death

One of the juvenile animals, receiving Ranitidine alone, became overly stressed when in the restrainer for blood collection. This led to his 2.5-hour bleed being abandoned for his welfare as he was exhibiting signs of being in shock. He was checked by a named animal welfare officer (NACWO) who gave him water and placed him in the warming cabinet where he seemed to fully recover and was therefore cleared to continue the study with a 3-hour bleed taken successfully. However, during the 4-hour time-point, once being placed into the restrainer, he immediately became stressed and died. A post-mortem was carried out and no abnormalities were seen. The 4-hour bleed was obtained from this animal via cardiac puncture. For this reason, n = 3 for the juvenile 2.5 and 6-hour Ranitidine alone time points.

5.4.2.2 Juvenile results

In the juvenile rats, the blood concentration of Ranitidine significantly differed between the two treatment groups at the 3 and 4-hour time points (figure 5.9)

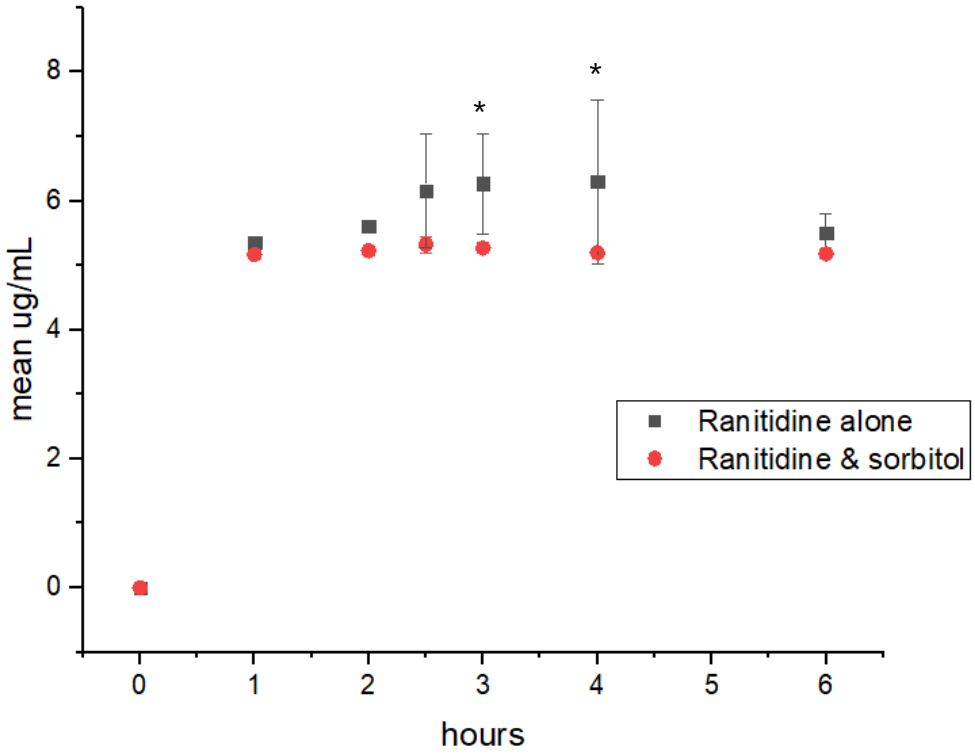


Figure 5.9 Ranitidine concentration vs time profile in the juvenile rats ± SD. * Denotes significant difference to the corresponding time point in the test group

In the control group, receiving Ranitidine alone, the T_{max} was 3 hours; this blood concentration was statistically different from the concentration at 1-hour as Ranitidine was continuing to be absorbed. The 2, 2.5 and 3 hour concentrations were not significantly different indicating that the Ranitidine concentration was no longer rising between these time points in the control group.

When sorbitol was present in the formulation, the peak Ranitidine blood concentration was achieved at 2.5 hours, this was statistically the same as the 1-hour time point. As with the adults, this means the peak Ranitidine concentration was reached quicker in the presence of the polyol. Again,

sorbitol reduced the C_{max} itself from 6.23 in the control group to 5.32, this difference was found to be significant. The conclusions drawn here is that in juvenile animals, the absorption of Ranitidine is sped up in the presence of sorbitol but less is absorbed overall – just as it is in adults.

5.4.3 Comparing the data sets

In order to understand if age plays a role on sorbitol's effect on Ranitidine bioavailability it was necessary to compare the two data sets. There was no significant difference seen between the two age groups at any of the time points (figure 5.10).

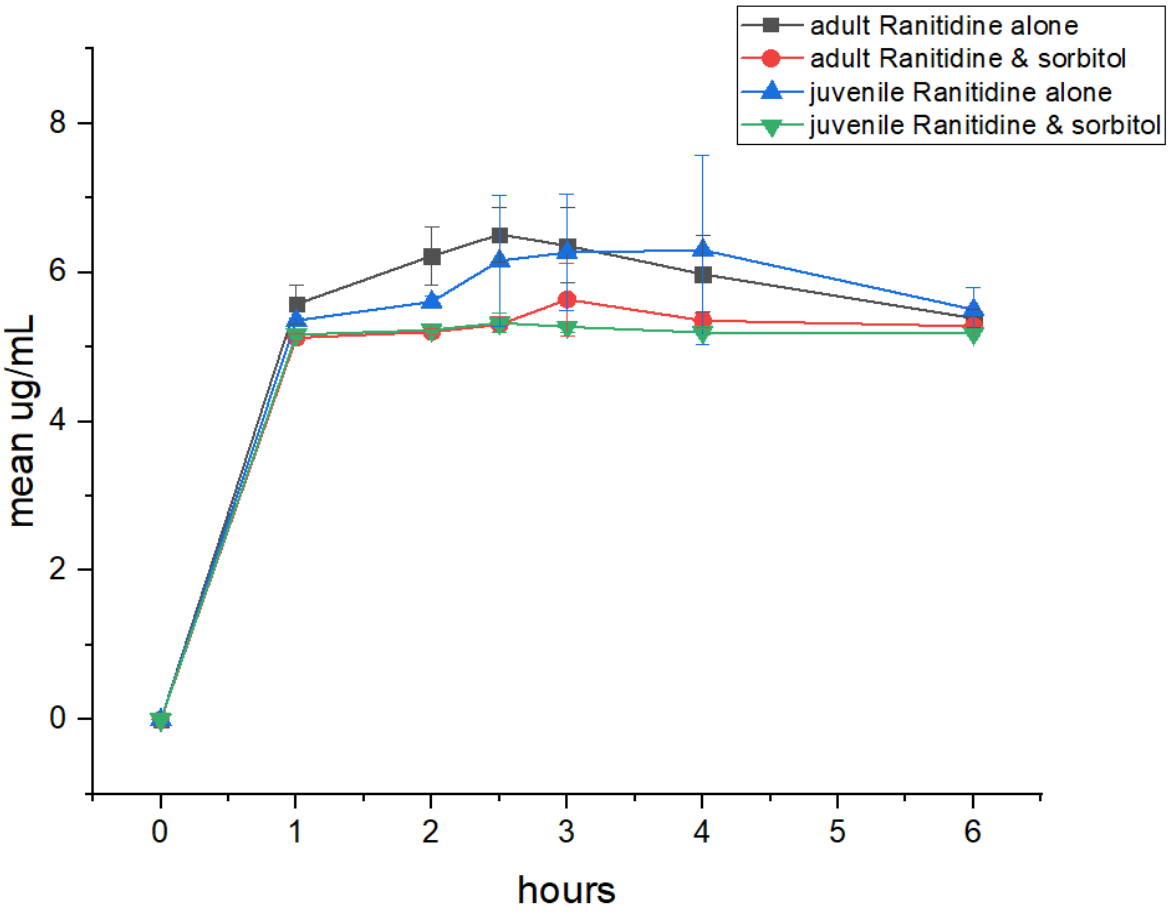


Figure 5.10 Sorbitol's effect on Ranitidine blood concentration (µg/mL ± SD) in juvenile and adult animals

The AUC_{0-360} for each of the four experimental groups is plotted in figure 5.11. There was no significant difference found between the treatment groups due to age ($p<0.05$).

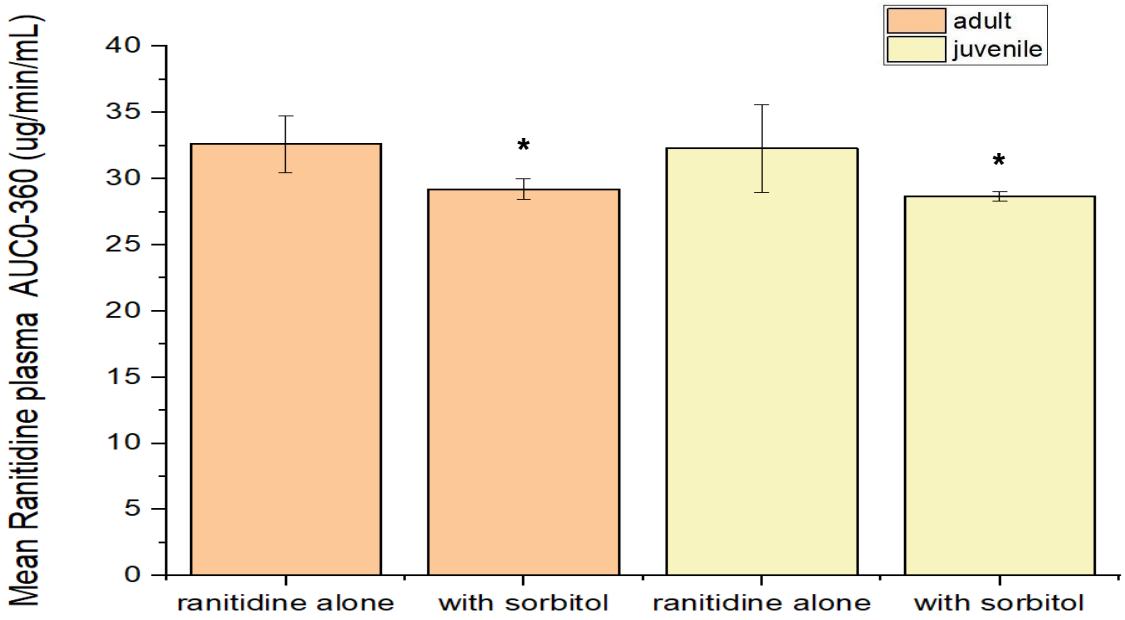


Figure 5.11 AUC_{0-360} of the experimental groups ± SD. *Denotes significant difference from respective control

Age was not found to be a factor in how sorbitol affected Ranitidine absorption; there was no difference between the C_{max} of the control groups or the C_{max} of the sorbitol groups depending on age. The only significant factor found in this PK study was treatment group; i.e if sorbitol was present. The results are summarised in table 5.3.

Table 5.3 PK study summary; there was no difference between the effect of sorbitol on Ranitidine in the juvenile and adult rats. The Ranitidine alone/ With sorbitol $AUC_{0\text{-}360}$ (ug/min/mL) and C_{max} (µL/mL) show no age difference

	Ranitidine alone	**With sorbitol**
Adult		
$AUC_{0\text{-}360}$ (µg/min/mL)	32.6 ± 2.2	29.2 ± 0.8
% difference $AUC_{0\text{-}360}$ vs control	n/a	10.4 %
C_{max} (µL/mL)	6.5	5.6
% difference C_{max} vs control	n/a	13.8 % (non-significant)
Juvenile		
$AUC_{0\text{-}360}$ (µg/min/mL)	32.3 ± 3.3	28.7 ± 0.4
% difference $AUC_{0\text{-}360}$ vs control	n/a	11.2 %
C_{max} (µL/mL)	6.23	5.32
% difference C_{max} vs control	n/a	14.6 %

5.4.4 Sorbitol's GI effect

Sorbitol is known to reduce small intestinal transit time, osmotically increase GI fluid volumes and enhance GI motility (D. A. Adkin, Davis, *et al.*, 1995; D. A. Adkin, Gowland, *et al.*, 1995; D. Adkin *et al.*, 1995) – actions resulting in GI side effects. Sorbitol is slowly absorbed by the small intestine via passive diffusion whilst most enters the colon for fermentation, producing short chain fatty acids (Islam and Sakaguchi, 2006). It is when there is a high concentration of these short chain fatty acids that patients can experience osmotic diarrhoea. Sorbitol's GI effects also impact drug permeability as demonstrated in this work. The increased GI fluid coupled with enhanced motility led to Ranitidine reaching the distal regions of the gut quickly, a place where absorption is more limited; Ranitidine is primarily absorbed in the small

bowel (Willia *et al.*, 1992). Sorbitol's effect on Ranitidine is significant due to the drug's poor permeability, sorbitol has less of an effect on other, more permeable drugs for example Metoprolol (Chen *et al.*, 2007).

Sorbitol is commonly found in liquid formulations and is also present in sweeties and juices that children may consume. It is possible that the efficacy of a medicine can be affected by the co-administration of sorbitol from either the diet or other medicines (or both) that a child is taking. Sorbitol has no limit to it's ADI as it is deemed a safe excipient. Whilst it's safety is not being called into question, here it is shown that sorbitol impacts the PK of the drug and does so in both adults and children. Therefore, the dose should be limited for effects on bioavailability independent of age and it's use at high levels should be viewed with caution. This is important for paediatric populations who are most likely to be administered liquid dosage forms (Van Riet-Nales *et al.*, 2013). Further work is needed to understand at what concentration sorbitol begins to have a negative effect on API gut permeability, specifically in paediatric models. This would allow for sorbitol to be used in a more informed manner within formulations, hopefully reducing the overall amount children are exposed to.

5.4.5 Other excipients of interest

It is conceivable that other common place excipients may be able to negatively influence the bioavailability of drugs. Owing to COVID laboratory closures in place at UCL other excipients of interest were unable to be investigated during this PhD. One such excipient is sucralose. The effect of sucralose on the intestinal environment is a hotly debated topic. An initial paper by Abou-Donia et al (Abou-Donia *et al.*, 2008) cited sucralose's

negative impact on faecal microflora, faecal pH, expression levels of P-gp and various CYP enzymes. This work concluded that the sweetener will impact the bioavailability of orally administered drugs. This was followed by a rebuttal paper from an 'expert panel' stating the work to be 'deficient in several critical areas and that its results cannot be interpreted as evidence that…sucralose, produced adverse effects' (Brusick *et al.*, 2009). This was then refuted by the original authors who published another paper to clarify their findings and discredit the expert panel for having 'undeclared conflicts of interest' (Schiffman and Abou-Donia, 2012). Other published research report sucralose to have a negative impact on the gut (Suez *et al.*, 2014; Sylvetsky *et al.*, 2016; Ruiz-Ojeda *et al.*, 2019; Shil and Chichger, 2021) and has led to a clinical trial, The SweetMeds study (ClinicalTrials.gov, 2018), to investigate further the impact of sucralose on drug absorption and metabolism. Although this study is sponsored by the National Institute of Child Health and Human Development, this work is looking at the effect in adults only. If Abou-Donia's findings are correct and sucralose impacts transporter levels in the gut, then it is even more important to assess the effect of age. The information surrounding transporter levels in young children (Nigam and Bhatnagar, 2013; Khalil and Läer, 2014) is sparse and so it cannot be assumed that the adult situation reflects the paediatric. If sucralose impacts API bioavailability differently in children then in adults, further work would be needed to elucidate the mechanisms behind this. For example, to understand if the excipient is acting on efflux transporters and if so, what differences exist in transporter levels between children at different stages of development. A more holistic

picture is needed in order to better inform how excipients are used within formulations for children.

5.5 Conclusions

Excipients can negatively impact the bioavailability of a drug by direct action in the gut. Such effects of excipients have been demonstrated in humans (Chen *et al.*, 2007) but the effect of excipients in the immature gut had not been evaluated until now. Children have their own unique gut environment with many differences to adults known (Batchelor, Fotaki and Klein, 2014; Khan *et al.*, 2022) however, there are also many gaps in knowledge (Wollmer *et al.*, 2022) making in silico modelling difficult for this age group. This work investigated the effect of sorbitol on Ranitidine's PK profile in juvenile animals. Sorbitol significantly impacted the bioavailability of Ranitidine in both age groups and there was no difference in the excipient's effect according to age; the treatment groups had statistically comparable $AUC_{0\text{-}360}$ and C_{max} independent of age.

Historically taste-masking excipients, such as sorbitol, have been used by formulators at high levels to achieve improved palatability and patient compliance. This is an important goal but not the only one; 100% patient compliance to a formulation containing excipients which decrease API blood concentration by 50% is no better than a child spitting out half of the medicine due to poor palatability. It is important to understand the effects taste-masking excipients at high concentrations are having in children and consciously act to lower them. One way of doing this is to employ other taste-masking strategies

in combination. For example, the bitter-blockers explored in chapters 3 and 4 could be evaluated alongside sorbitol to enable the polyol to be used at lower levels whilst still achieving an acceptably palatable formulation.

Chapter 6

General discussion, conclusions and future work

The research laid out in this thesis highlights how taste-masking excipients, crucial to palatable liquid formulations for children, can affect the bioavailability of API. This chapter provides a general discussion of the work with experimental findings overviewed and future work outlined.

6.1 The importance of taste-masking paediatric formulations

Children require palatable medicines as poor taste is known to be a major barrier to compliance in young patients (American Academy of Pediatrics, 2000; Milne, 2005; Nordenmalm *et al.*, 2019). Liquid formulations are routinely prescribed to children (Rashed *et al.*, 2021) owing to swallowability issues with solid alternatives. However, liquid formulations are challenging to taste-mask.

A recent review by Rouaz et al (Rouaz *et al.*, 2021) compiled a number of paediatric formulations and showed a worrying trend of exposing children to excipients that are not recommended for this age group and using excipients, including those used for taste-masking such as sorbitol and aspartame, at inappropriately high concentrations. Traditionally, to achieve palatability, artificial sweeteners have been used with the aim of overwhelming the bitter taste of a drug with sweetness. However sweeteners have limited efficacy when an API is particularly soluble or aversive (Sohi, Sultana and Khar, 2004; Walsh *et al.*, 2014) and there are health concerns associated with their cumulative use which can occur when children take multiple medicines (Pawar and Kumar, 2002; Abou-Donia *et al.*, 2008; Hampton, 2008; Swithers

and Davidson, 2008; Tandel, 2011; Suez *et al.*, 2014; Sylvetsky *et al.*, 2016; Pearlman, Obert and Casey, 2017; Mossavar-Rahmani *et al.*, 2019; Ruiz-Ojeda *et al.*, 2019; Shil and Chichger, 2021). Similarly, polyols have historically been used at high levels within paediatric formulations as bulking agents but in combination with artificial sweeteners to provide sweetness (Rouaz *et al.*, 2021) in so called sugar free preparations which should rather be called sucrose free preparations considering they do contains some sort of sugars. When used at high levels, polyols have shown a negative effect on Ranitidine HCL absorption (Chen *et al.*, 2007). For these reasons, other taste-masking excipients are required to reduce the reliance on sweeteners and/or enable lower quantities to be used within paediatric formulations. Taste-neutral approaches such as bitter-receptor antagonists and cyclodextrins/maltodextrins are endorsed by the EMA (EMA, 2013) and offer an alternative way to improve patient compliance relying less on the hedonic response of the patients (pleasantness).

6.2 Rationale for investigating a maltodextrin and a cyclodextrin in the BATA model

Cyclodextrins and maltodextrins can molecularly encapsulate API and are used within formulations for many different purposes. They can sequester API out of a formulation, resulting in a taste-neutral effect, and, due to their excellent per os safety profile, could become a productive means of taste-masking liquid medicines. In order to use such dextrins as a taste-masking strategy it is necessary to have a means of accurately assessing their taste masking efficacy preclinically. The BATA platform is a recognised taste-

assessment model (Clapham *et al.*, 2021) which has shown good translatability to humans for API (Devantier *et al.*, 2008; Clapham *et al.*, 2012; Rudnitskaya *et al.*, 2013; Noorjahan, Amrita and Kavita, 2014; Mohamed-Ahmed *et al.*, 2016; Soto *et al.*, 2018) but has not been thoroughly assessed for use with excipients including cyclodextrins or maltodextrins. A primary aim of this work was to understand if the BATA model is a predictable way to assess the taste-masking ability of a promising pea maltodextrin and HP- β-CD by comparing BATA data with human panel data.

Without validating pre-clinical models in this way, their predictability remains unknown.

6.3 Rationale for investigating bitter-blockers indirect impact on drug bioavailability

Bitter-blockers offer a taste-masking strategy that work independently from the API, targeting TAS2 receptors directly. They act to neutralise bitterness at the source. Many bitter drugs interact with multiple TAS2 receptors (Dagan-Wiener *et al.*, 2019) so it is unlikely that a bitter-blocking excipient will entirely prevent bitter perception however, it may dampen down the intensity of the taste enough to facilitate ingestion and improve patient compliance.

The aims of this work included to first thoroughly review the current knowledge of bitter-blocking agents and, with the use of a novel data mining methodology, identify promising compounds. Secondly, the aim of this work was to assess the three most promising bitter-blockers for their utility as potential excipients in paediatric medicines by evaluating their efficacy

against, and potential interaction with Quinine HCL. Lastly this work aimed to assess the efficacy of the three promising bitter-blockers with two model paediatric drugs which require taste-masking strategies.

The purpose of this work was to further understanding on the applicability of bitter-blockers within paediatric formulations.

6.4 Rationale for investigating sorbitol's direct impact on drug bioavailability

Sorbitol has been highlighted as an excipient of concern for use in children's medicines (Rouaz *et al.*, 2021). It is known to negatively impact API absorption in adults (Chen *et al.*, 2007) yet little is known about its effect, and safety in children (Rouaz *et al.*, 2021). Despite this it is used at high levels within many formulations. As discussed within this thesis, children are not mini-adults and need to be viewed as an individual patient population. Therefore, the aim of this work was to measure the effect sorbitol has on the bioavailability of Ranitidine in the juvenile gut in order to better inform the levels with which it is used as an excipient within paediatric formulations.

6.5 Overview of the implications of this work

The main findings of the research described in this thesis are summarised below.

- The effect taste-masking excipients can have on the bioavailability of a drug in children by direct action in the gut or by indirect action,

improving palatability, is described. This work highlighted the need for new and improved taste-masking strategies for children. The importance of further understanding taste-neutral methods using non-traditional excipients such as bitter-blockers and maltodextrins/ cyclodextrins is discussed.

- The use of the BATA model to predict the taste-masking ability of a pea maltodextrin and HP- β-CD was evaluated. Historical outcomes in rodents were compared to outcomes from a human panel, both assessing these dextrins with Ranitidine and Sildenafil. This work furthered understanding of how the BATA model can be used with excipients and discussed limitations of evaluating compounds with characteristics which are new to the animals which may result in neophobia. In this work, the animals showed neophobia to the dextrin vehicles (in particular the cyclodextrin) suggesting some aspect was unfamiliar to the animals. The pH of the solutions was acceptable (pH 5-8 for the HP-β-CD solutions) and the solutions were non-viscous (confirmed by the ease of licking from the spouts on the second day). However, they were very sticky. The surface tension of a solution can give an indication as to its 'stickiness' (Adhikari *et al.*, 2007) this can be measured using a tensiometer. It would be useful to measure the surface tension of the dextrin vehicles at 25% w/v, as used in this work, to give an understanding of what values can lead to neophobia. Future vehicles can then also be assessed for their surface tension and if comparable values are noted the animals can be pre-exposed to them, as discussed in chapter 2, to avoid a potential neophobic response

which may affect the BATA experiment. Further, this work found that the maltodextrin successfully taste-masked Sildenafil in rats but did not do so in humans. Literature evidence suggests that rodents may have receptors which can convey the taste of polysaccharides in a way humans cannot (Sclafani, 2004) and that maltodextrins are appealing to the animals (once they are familiar with them). This can result in taste-masking being offered because of rat preference to the vehicle which is not seen in humans, potentially limiting the use of maltodextrins within the BATA, but this is likely to depend on concentrations used going forward.

- Review of the scientific literature for bitter-blockers identified sporadic available information for many of the compounds. There was a lack of uniformity in assessing their taste-masking efficacy and they had not been assessed at a range of concentrations to understand at what level they can become effective, this made it difficult to assess their potential as excipients. A novel data mining methodology was developed to address this considering factors such as usability, efficacy and safety to generate a score so the compounds could be quantitatively compared to identify which show potential as excipients for liquid formulations and should be evaluated further.

- A standardised assessment of three promising bitter-blockers was carried out in two models of palatability. First, to understand levels at which they could be efficacious, each bitter-blocker was assessed at a range of concentrations in the e-tongue alongside Quinine HCL. Then

they were evaluated in the BATA model to understand both their palatability as standalone compounds and with bitter API; Quinine HCL, Ranitidine HCL and PQP were the model drugs chosen. This work showed the bitter-blockers offered some limited taste-masking. These excipients may be best used in combination with other taste-masking agents enabling the latter to be used at lower levels – something which is sorely needed in paediatric formulations.

- The BATA model was successfully established at a new location. This was done by comparative testing using validation studies which were compared with historical data. This work demonstrated that the BATA model can be set-up in a new laboratory with new animals and produce translatable results

- This work assessed how IC50 values of bitter controls can change over time in the BATA animals. The interplay between tolerance on repeat exposure to a bitter control (which may be depend on the intensity of the compound's bitterness) and age-related decline in sensitivity to bitterness is unclear. Thus, this work highlighted the importance of using water as the primary comparator when assessing novel formulations for aversiveness.

- The effect of sorbitol on the bioavailability of Ranitidine in juvenile and adult animals was compared. This was in order to understand if a difference is seen depending on the developmental stage of the GIT. The key finding here was that sorbitol negatively effected the

bioavailability of Ranitidine in the same way independent of age. It is necessary to lower the levels of polyols utilised within liquid formulations suggesting the need for a dual approach to taste-masking in order to still deliver acceptable palatability – a gap which could potentially be filled with bitter-blockers.

6.6 Updated literature review

Since the literature review 'Bitter-blockers as a taste masking strategy: A systematic review towards their utility in pharmaceuticals' was written, there have been some updates in the knowledge of bitter-blockers which could inform the future work generated from this thesis. An updated literature review generated few relevant new papers pertaining to the three bitter-blockers investigated in this PhD. However, it did offer more information on some other compounds discussed in the initial paper as well as highlighting some novel bitter-blockers.

6.6.1. Recently uncovered bitter-blockers

Novel peptide sequences acting as in vitro TAS2R antagonists have been identified from rainbow trout nebulin (Yu *et al.*, 2022) as well as hen (Xu *et al.*, 2019) and beef (Zhang *et al.*, 2019) protein hydrolysate. The dipeptide Trp-Trp has since been shown to inhibit TAS2R14,16,43 and 46 in HEK cells (Ojiro *et al.*, 2021) and lysine has been trialled as a bitter-blocker for food products but was found to be ineffective in a human panel (Filho *et al.*, 2020). Also derivatives of amino acids, the advanced glycation end-products (AGEs); glyoxal-derived lysine dimer (GOLD) and carboxymethyllysine (CML) have

demonstrated in vitro antagonism at TAS2R4. However, at similar concentrations, GOLD and CML act as agonists at TASR20 (Jaggupilli *et al.*, 2019) so have limited use.

Applying the scoring system drawn up in the initial systematic review (discussed in chapter 3), none of these bitter-blocking compounds would have scored highly on the criteria to warrant further investigation since they have not demonstrated efficacy in a human panel.

6.6.2 Further work carried out on bitter-blockers mentioned in the original review

The amino acids derivatives, Gamma Aminobutyric acid (GABA) and *N*α,*N*α-bis (carboxymethyl)-l-lysine (BCML), were discussed in the review as having limited potential as excipients. At the time of the review they had only shown in vitro efficacy against 1mM Quinine (Pydi *et al.*, 2014), they have since been explored in vivo but did not improve the bitter perception of 0.01 – 3mM Quinine HCL in mice (Masamoto *et al.*, 2020). Also previously discussed was Probenecid, a gout medication. This compound was used as a rinse to taste-mask but now has been tested within formulations as an excipient (Masamoto *et al.*, 2020). Probenecid at 1mM was found to improve lick response in mice to Denatonuim and Phenylthiourea but not Quinine HCL However, the use of Probenecid is not recommended for children under 2-years old and the drug has been shown to potently inhibit the SLC transporter organic anion transporter 3 (at μM levels) which could be detrimental to substrate API bioavailability (Zhou *et al.*, 2020) and is therefore not promising as an excipient.

Flavanones, such as 6-methylflavone have also been further investigated since the original review (when only in vitro efficacy had been demonstrated) and have now been shown to block the bitterness of Tenofovir in human panels (Schwiebert *et al.*, 2021). In this work 8 of 16 participants had reduced perception of bitterness to the drug when 6-methylflavone was used either as a pre-rinse or mixed with Tenofovir. The authors found the drug activates TAS2R 39,1,8 and 14 with 6-methylflavone antagonising TAS2R39, they suggest a combination approach with other bitter-blockers (for example of TAS2R1, 8 or 14) may enable bitterness suppression in more people which warrants further investigation.

Also discussed in the review was 3-(pyrazol-4-yl) imidazolidine-2,4-diones. However, at the time of writing their efficacy had only been demonstrated in cell lines. A paper published after the original review was written, demonstrates two such compounds, namely S6821 and S7958, have been shown to reduce the perceived bitterness of coffee in a human sensory panel (Fotsing *et al.*, 2020). These compounds are GRAS in adults and so, with this updated information, could be pursued further. However, they are not available as ‘off-the-shelf’ products and are tied to patents (Karanewsky *et al.*, 2011) making them less desirable as excipients.

6.7 Future work

Potential future work has been suggested within the relevant chapters and are summarised here with other areas which warrant further exploration.

- The human panel presented in chapter 2 showed only one of the two model API was successfully taste masked (and only at the EC50 level) by HP- β –CD and the maltodextrin so it is difficult to draw firm conclusions as to the predictability of the BATA. Future work needs to identify other API which can be successfully taste-masked by these dextrins in humans. Selection of API needs to be based around those which are likely to form inclusion complexes, for example drugs with poor solubility. Furthermore, API chosen need to have a well understood safety profile so they can be assessed in human panels. Phase solubility studies could be used to indicate if complexes form but, as discussed in chapter 2, taste-masking ability needs to be confirmed in vivo. Once API are selected, they could be assessed using humans to see where dextrins offer benefit and then successful formulations could be carried forward to the BATA to see if there is good correlation with predicted efficacy. Furthermore, the levels at which these dextrins are used needs to be optimised to balance taste-masking efficacy (for therapeutic levels of drug) with solubility enhancement and vehicle acceptability. Once an appropriate level of dextrin is established for taste-masking in humans then further work can be done to assess the translatability of the BATA. If lower levels of maltodextrin are found to be of benefit in humans, then further BATA work should be carried out to see if the rats' inherent preference for the compound is still evident and if false predictions of taste-masking are still seen. Moreover, independent of the concentrations of HP- β –CD/maltodextrin used going forward, it would be important to pre-

expose naïve animals to the vehicle prior to using it within formulations to assess if it could prevent neophobic responses. The BATA study in chapter 2 could then be repeated, in particular for HP- β –CD in formulation with Ranitidine which was not taste-masked in the rats likely owing to the substantial neophobic response. Outcomes could then be re-compared to the human panel which showed taste-masking was achieved when the cyclodextrin was in high excess. This would allow for better understanding of the translatability of the model.

- In order to better direct the use of bitter-blockers, a pressing research need is to elucidate which TAS2Rs sodium gluconate, sodium acetate and NaAMP act on. This can be done using an in vitro calcium flux assay using human embryonic kidney (HEK) cells expressing various TAS2Rs and measuring intracellular calcium levels via a calcium activated florescent dye (Greene *et al.*, 2011). To transfect HEK cells with the appropriate plasmids and to carry this out for all 25 human TAS2Rs would be time-consuming but is possible as the mRNA sequence of each is known (Roudnitzky *et al.*, 2015). Alternatively, since all three bitter-blockers contain sodium, efforts could be placed on evaluating more closely which TAS2Rs are inhibited by sodium. It is known sodium itself blocks bitterness. This has been shown using NaCL, at concentrations containing 3mg/mL sodium, in food products (Wise, Damani and Breslin, 2019) although foods are likely to be inherently less bitter than API. For a paediatric liquid formulation of 2mL where sodium gluconate is used at 300mM (as shown to be

effective in literature human panels against Quinine), this would contain approximately 14mg sodium (7mg/mL). For 100mM sodium acetate and 20mM NaAMP (as shown to be effective in literature) in the same formulation would contain 23mg (11.5mg/mL) and 1mg (0.5mg/mL) sodium respectively. It is likely sodium present in all three bitter-blockers is contributing to the taste-masking although at present the exact mechanism is unknown.

- Furthermore, when planning the taste-masking strategies of API, it would be useful to carry out an in vitro assay to determine which TAS2Rs are activated. This dual approach would enable bitter-blockers and API to be matched for use within formulation and give a more structured approach to taste-masking. This would offer an alternative, targeted, strategy to reduce the reliance on sweetening excipients. However, this would be a huge undertaking and require massive development investment. Therefore , such investigation would be best prioritised for life-saving medicines, for example to treat malaria (Unitaid, 2020), rather than for over-the-counter medications. The risk of not taking a life-saving medicine for long periods of time, if refused by the child due to unacceptable palatability, could have devastating consequences. This targeted approach could begin to address patient acceptability and work towards improving this issue.

- The three bitter-blockers evaluated in chapters 3 + 4 were unable to provide an acceptably palatable formulation of PQP or Ranitidine at

the therapeutic dose. Sodium gluconate and NaAMP did very slightly improve the perceived palatability of PQP but this was not sufficient due to the aversiveness of the compound. This poses the idea that bitter-blockers may prove useful in combination with other taste-masking strategies, such as sweeteners, to enable lower concentrations of the latter to be used. This could reduce the likelihood of children being exposed to concentrations which have been shown to be detrimental to health (discussed in chapter 1) and/ or have potential adverse impacts on drug bioavailability (discussed in chapter 5). Future work, which could not be carried out due to COVID delays, include;

- 'Sweet and salty' approach using bitter-blockers in combination with polyols or artificial sweeteners to see if the concentration of sweetener required to taste-mask therapeutic levels of bitter API can be lowered. Overall sodium gluconate was the most promising bitter-blocker tested in the BATA, improving the lick rate of IC90 Quinine, 4mM Ranitidine, therapeutic PQP and being well accepted itself with 300mM being as palatable as water. Thus, sodium gluconate should be focused on with regards to elucidating TAS2R activity as discussed above. Sodium gluconate also has a pleasantly salty taste to children (Mennella *et al.*, 2014). Sodium gluconate should be assessed alongside API with liquid formulations containing artificial sweeteners and/or high amounts of, polyols, such as sorbitol, with the aim of eliminating the former and reducing the levels of the latter. For example, a) Amoxicillin; Kent

Pharma's oral suspension contains 50mg/mL API, 160mg/mL sorbitol and an undisclosed concentration of sodium saccharin (an artificial sweetener) b) Ranitidine hydrochloride; ADVANZ Pharma's oral solution contains 15mg/mL API, 300mg/mL sorbitol and an undisclosed concentration of sodium saccharin. Previous work has shown 100mg/mL sorbitol is well tolerated by the BATA rats (Ruiz *et al.*, 2019) and so it can be successfully evaluated in the BATA. This work could be carried out over two BATA studies, one for each drug, in combination with 300mM sodium gluconate (the highest concentration which was fully palatable to the animals) without the use of saccharin. For example,

  - 50mg/mL Amoxicillin could be assessed as a suspension with 160, 120, 80, 40 and 0 mg/mL sorbitol with and without 300mM sodium gluconate. However, suspensions are a complex dosage form to assess in the BATA model due to the incomplete understanding of how the lick numbers relate to taste of the API when the particles can be perceived as gritty to the animals.
  - 15mg/mL Ranitidine could be assessed as a solution with 300, 225, 150, 75 and 0 mg/mL sorbitol with and without 300mM sodium gluconate.

- To further develop the BATA model, it would be useful to design a methodology to define a bitter index in the model. A bitter-index method has been described in humans (WHO, 1998) where the bitter properties

of compounds can be determined by comparing the threshold bitter concentration with that of dilute QHCL. QHCL solutions are given at increasing concentrations, to swirl in the mouth for 30 seconds, spit out and wait for 1-minute. The threshold bitter concentration is the lowest concentration which continues to provoke a biter sensation after 30 seconds. Test solutions are then assessed in the same way by administering a stock solution at decreasingly diluted volumes (starting with the most dilute) to find the threshold bitter concentration. This gives a bitterness value which can be expressed in units equivalent to the bitterness of a solution containing 1g of QHCL in 2000mL by this equation:

$$\frac{2000 \times \text{QHCL in threshold concentration (mg)}}{\text{Concentration of API stock (mg/mL)} \times \text{volume of stock which achieved threshold concentration (mL)}}$$

A similar method could be developed in the BATA with a range of dilute QHCL solutions being given to the animals to determine the lowest concentration which results in detectable bitterness, perhaps this could be represented by the first concentration that gives statistically different lick rate to water, then novel API could also be assessed at a range of concentrations to determine the bitter threshold and the concentration required for this could be compared to QHCL. However, if the aversiveness of an API was not known, then it would not be practical to assess the range of concentrations required to elucidate an accurate bitterness threshold. Instead, various inhibitory concentrations (with respect to water) could be determined for QHCL, such as IC10, IC20,

IC30 and beyond. Then when other API are assessed for aversiveness at the therapeutic level for example, the level of aversion (with respect to water) could then be correlated to the concentration of QHCL which evokes the same response. This would be useful for API with previously unknown bitterness levels to quantify the bitterness using a well-known comparator.

- As discussed in chapter 5, it is conceivable that other common place taste-masking excipients may be able to negatively influence the bioavailability of drugs. It has been reported that sucralose at FDA approved doses elevated expression of P-gp and CYP enzymes in male rats (Abou-Donia *et al.*, 2008) to levels previously associated with reductions in bioavailability of API (Dürr *et al.*, 2000). Sucralose is currently being investigated for effects in adults (ClinicalTrials.gov, 2018) but should be investigated for such effects in children. It is especially important to investigate a potential age dependent effect of sucralose as there are gaps in knowledge surrounding how transporter levels differ in juveniles and adults so it cannot be assumed the adult situation reflects the paediatric. Future work, which could not be carried out due to COVID delays, include;
    - Running another PK study using the model detailed in chapter 5, using high but clinically relevant concentrations of sucralose to understand if it alters the bioavailability of API and if it does so differently depending on age.

- If the PK study showed there to be a difference in API bioavailability according to age this could be investigated further using Ussing chamber apparatus comparing juvenile and adult intestinal sections to see if there is a difference in drug permeability in the presence of sucralose. This could be further explored by quantifying P-gp, and other transporters, using western blot methods to compare levels during different developmental stages of the gut.

- An updated literature review inspired more work to be carried out with bitter blockers. Before future work is carried out with novel bitter-blockers it would be important to ascertain their optimal concentration. This would include assuring that they have a safe toxicological profile in children for the concentrations at which they would be used and that the expected levels required have no inherent aversive taste.
  Future work ideas here include;
  - Evaluating the bitter-blockers 6-methylflavone and S6821/ S7958 in more detail for their efficacy against model bitter API. For example, to see at what levels they can become effective and where their use can offer benefit. It would be useful to evaluate combinations of e.g. S6821 (acting on TAS2R8) with 6-methylflavone (acting on TAS2R39) on the taste masking of Tenofovir to see if the dual approach offers better palatability to more people than was seen in Schwiebert et al study using 6-methylflavone alone.
  - Although the TAS2Rs inhibited by the three bitter-blockers investigated in this thesis are unknown, NaAMP successfully taste-

masked IC50 Quinine HCL (chapter 3), it would be interesting to use NaAMP in combination with 6-methylflavone to see if more improvement can be gained in the rats (perhaps at a lower concentration of NaAMP) since Quinine HCL is known to activate a number of bitter-receptors including TAS2R39 (Dagan-Wiener *et al.*, 2019).

- Work has shown that temperature effects the intensity of the taste of sodium acetate and sodium gluconate with maximal response achieved at 35-39 degrees (Lu, Breza and Contreras, 2016). Humans may experience temperature playing a role in the perceived sweetness of food and drink. For example, sugar filled fizzy drinks may taste sweeter when drank at room temperature rather than chilled suggesting warming consumables can increase their sweetness whereas cooling them supresses this (Lemon, 2015). This anecdotal evidence is supported by laboratory experiments which have shown perceived concentration of sucrose decreases on cooling (Bartoshuk *et al.*, 1982). Electrophysiological studies in both rats and mice have shown that warming can enhance gustatory responses to sucrose in fibres of the chorda tympani branch of the facial nerve which in partly responsible for supplying both taste and sensation to the tongue (Breza, Curtis and Contreras, 2006). Importantly increased temperature did not intensify bitter stimuli (Lu, Breza and Contreras, 2016). In humans, the heterodimeric receptor TAS1R2-TAS1R3 recognises sweet tasting sugars and proteins via a large Venus flytrap domain which

oscillates between an open and closed formation and facilitates the binding of sweet ligands (Chun, Zhang and Liu, 2012). Activation of the receptor leads to dissociation of the G-protein and release of intracellular calcium and ATP exocytosis which then activate receptors on afferent fibres ultimately resulting in taste perception (Mahalapbutr *et al.*, 2019). Polyols are also agonists for these receptors and primarily act on the Venus flytrap domain of the TAS1R2 monomer, forming a strong hydrogen bond with the E302 amino acid residue. The stronger the binding affinity of a polyol to the TAS1R2, the sweeter tasting the molecule (Mahalapbutr *et al.*, 2019). This evidence suggests that giving pre-warmed solutions containing either polyols or bitter-blockers in formulation with bitter API may improve the efficacy of taste-masking and enable lower concentrations to be used. Before exploring this further with bitter API it would be important to know the stability of the drug under such conditions. Moreover, the practicality around assessing these temperatures preclinically in the BATA model is challenging. It would be very hard to keep the solutions at this temperature whilst they are presented to the animals. Perhaps evaluating the effect of temperature could be incorporated into a human panel, where the drug's stability allows, and the solutions could be pre-warmed prior to assessment for half the panellists, to see if any benefit is offered compared with the participants receiving room temperature solutions.

6.8 Conclusions

The need for effective taste-masking excipients for use in children's medicines is clear. Current strategies rely on sweeteners. The risks surrounding sweeteners are not completely understood in children but there is certainly evidence to suggest they can be detrimental to both health (particularly when children are exposed to high levels from multiple medications) and to API absorption, an effect that has been demonstrated in adults and now in juvenile models. There are known differences between the adult and juvenile GI environment and yet assessing taste-masking excipients for effect on API in age-appropriate models has seldom been done. Alternative excipients must be sought to offer better options to formulators to improve palatability and facilitate better paediatric acceptability. Furthermore, palatability evaluation of such excipients with API must occur early on in drug development to allow for taste-masking strategies to be optimised before the drug meets the patient. Therefore, it is important to have well validated preclinical means of assessment.

The outcomes of this research serve to enhance the understanding of bitter-blockers as a taste-neutral approach to taste-masking, highlighting compounds of interest and assessing them in formulation with bitter API. Moreover, this work has furthered knowledge surrounding the use of the preclinical BATA model with a cyclodextrin and maltodextrin in order to increase understanding of when/how this platform could be used effectively. Further, this work has assessed the effect of sorbitol, a sweetening agent, on API bioavailability in a relevant paediatric model. Although the same effect of

the polyol was seen in both juvenile and adult models, the importance of treating children as an independent patient population remains.

Improving the knowledge of how taste-masking excipients can impact the bioavailability of drugs in children is a step towards better formulated medicines and improved paediatric healthcare.

Scientific communications

Journal articles

Andrews D, Salunke S, Bennett J, Cram A, Basit A, Tuleu C. 2020. *Taste-modifiers as a bitter masking strategy: a review*. European Journal of Pharmaceutics and Biopharmaceutics. Elsevier, 158, pp. 35–51. doi: 10.1016/J.EJPB.2020.10.017

Ives R, Andrews D, Lennon M, Clarkson J, Hill M, Whelan G, Wassell E. submitted 2019, undergoing changes per reviewers' comments. *Enhanced Tower Caging Improves Rat Learning on the Brief Access Taste Aversion Assay.* Nature Scientific Reports

Salunke S, Alessandrini E, Amin D, Kaneria N, Andrews D, Liu J, Tuleu C. Accepted May 2020. *Can one teach old drugs new tricks? Reformulating to Repurpose Chloroquine and Hydroxychloroquine.* Journal of Pharmacology & Pharmaceutical Research

Oral presentations

Andrews D, Salunke S, Basit A, and Tuleu C (2020). *Na Salts as Bitter-Blocking Excipients for Children?* Pharmalliance symposium October 2020

Poster presentations

Andrews D, Ernest T, Ives R, Edwards A (2018). *Palatability and Manufacturability Assessment of Dispersible Tablets Prepared Using Co-Processed Excipients with Different Active Pharmaceutical Ingredients.* EuPFI Conference: Formulating Better Medicines for Children

Andrews D, Salunke S, Basit A, Tuleu C (2019). *Taste-modifiers as a bitter masking strategy for children.* EuPFI Conference: Formulating Better Medicines for Children

Andrews D, Ives R, Rutherford L, Tuleu C (2020). *'Na Salts as Taste-modifying Excipients for Children; BATA Findings'.* EuPFI Conference: Formulating Better Medicines for Children

Andrews D, Salunke S, Basit A, Tuleu C (2021). *Applicability of Sodium Salts as Bitter-blocking Excipients: a BATA case study with 2 bitter paediatric drugs*

EuPFI Conference: Formulating Better Medicines for Children

References

Abou-Donia, M. B. *et al.* (2008) 'Splenda Alters Gut Microflora and Increases Intestinal P-Glycoprotein and Cytochrome P-450 in Male Rats', *Journal of Toxicology and Environmental Health, Part A*, 71(21), pp. 1415–1429. doi: 10.1080/15287390802328630.

Abou-Hamdan, A. *et al.* (2000) 'High-Pressure Studies as a Novel Approach in Determining Inclusion Mechanisms: Thermodynamics and Kinetics of the Host−Guest Interactions for α-Cyclodextrin Complexes'. American Chemical Society. doi: 10.1021/JA993139M.

Adhikari, B. *et al.* (2007) 'Effect of surface tension and viscosity on the surface stickiness of carbohydrate and protein solutions', *Journal of Food Engineering*. Elsevier, 79(4), pp. 1136–1143. doi: 10.1016/J.JFOODENG.2006.04.002.

Adkin, D. *et al.* (1995) 'The effects of pharmaceutical excipients on small intestinal transit.', *British Journal of Clinical Pharmacology*. Wiley/Blackwell (10.1111), 39(4), pp. 381–387. doi: 10.1111/j.1365-2125.1995.tb04466.x.

Adkin, D. A., Gowland, P., *et al.* (1995) 'Echo-Planar Magnetic Resonance Imaging to Assess Water Volume in the Distal Small Bowel', *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists*. Pharm Res, 12(8), pp. 1134–1139. doi: 10.1023/A:1016251624220.

Adkin, D. A., Davis, S. S., *et al.* (1995) 'The Effect of Different Concentrations of Mannitol in Solution on Small Intestinal Transit: Implications for Drug Absorption', *Pharmaceutical Research*. Kluwer Academic Publishers-Plenum Publishers, 12(3), pp. 393–396. doi: 10.1023/A:1016256619309.

Afonso-Pereira, F. *et al.* (2016) 'Sex differences in excipient effects: Enhancement in ranitidine bioavailability in the presence of polyethylene glycol in male, but not female, rats', *International Journal of Pharmaceutics*. Elsevier B.V., 506(1–2), pp. 237–241. doi: 10.1016/j.ijpharm.2016.04.022.

Ahmad, R. and Dalziel, J. E. (2020) 'G Protein-Coupled Receptors in Taste Physiology and Pharmacology', *Frontiers in Pharmacology*. Frontiers Media S.A., 11, p. 1771. doi: 10.3389/FPHAR.2020.587664/BIBTEX.

Al-kasmi, B. *et al.* (2017) 'Mechanical microencapsulation: The best technique in taste masking for the manufacturing scale - Effect of polymer encapsulation on drug targeting', *Journal of Controlled Release*. Elsevier, 260, pp. 134–141. doi: 10.1016/J.JCONREL.2017.06.002.

Al-Kasmi, B. *et al.* (2018) 'Structural and in vitro in vivo evaluation for taste masking', *Expert Opinion on Drug Delivery*, 15(11), pp. 1105–1116. doi: 10.1080/17425247.2018.1535590.

Alam, M. A., Al-Jenoobi, F. I. and Al-mohizea, A. M. (2012) 'Everted gut sac model as a tool in pharmaceutical research: limitations and applications', *Journal of Pharmacy and Pharmacology*. John Wiley & Sons, Ltd, 64(3), pp. 326–336. doi: 10.1111/j.2042-7158.2011.01391.x.

American Academy of Pediatrics (2000) *Periodic Survey #44. Patient Compliance With Prescription Regimens*. Available at: http://www.aap.org/research/periodicsurvey/ps44bexs.htm (Accessed: 22 February 2022).

Andrews, D. *et al.* (2021) 'Bitter-blockers as a taste masking strategy: A systematic review

towards their utility in pharmaceuticals', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier, 158, pp. 35–51. doi: 10.1016/J.EJPB.2020.10.017.

de Angelis, I. and Turco, L. (2011) 'Caco-2 cells as a model for intestinal absorption', *Current Protocols in Toxicology*. John Wiley and Sons Inc., (SUPPL.47). doi: 10.1002/0471140856.tx2006s47.

Arey, L. B., Tremaine, M. J. and Monzingo, F. L. (1935) 'The numerical and topographical relations of taste buds to human circumv allate papillae throughout the life span', *The Anatomical Record*, 64(1), pp. 9–25. doi: 10.1002/AR.1090640103.

Arima, H. *et al.* (2001) 'Contribution of P-glycoprotein to the Enhancing Effects of Dimethyl-β-cyclodextrin on Oral Bioavailability of Tacrolimus', *Journal of Pharmacology and Experimental Therapeutics*, 297(2), pp. 547–555.

Arima, H. *et al.* (2004) 'Contribution of Cholesterol and Phospholipids to Inhibitory Effect of Dimethyl-β-Cyclodextrin on Efflux Function of P-Glycoprotein and Multidrug Resistance-Associated Protein 2 in Vinblastine-Resistant Caco-2 Cell Monolayers', *Pharmaceutical Research 2004 21:4*. Springer, 21(4), pp. 625–634. doi: 10.1023/B:PHAM.0000022409.27896.D4.

Arima, H., Higashi, T. and Motoyama, K. (2012) 'Improvement of the bitter taste of drugs by complexation with cyclodextrins: Applications, evaluations and mechanisms', *Therapeutic Delivery*. Future Science Ltd London, UK , 3(5), pp. 633–644. doi: 10.4155/TDE.12.28/ASSET/IMAGES/LARGE/FIGURE15.JPEG.

Arthur, S. and Burgess, A. (2017) 'How to identify and manage "problem" excipients in medicines for children', *The Pharmaceutical Journal*.

Arvidson, K. (1979) 'Location and variation in number of taste buds in human fungiform papillae', *European Journal of Oral Sciences*. John Wiley & Sons, Ltd, 87(6), pp. 435–442. doi: 10.1111/J.1600-0722.1979.TB00705.X.

Ashiru, D. A. I., Patel, R. and Basit, A. W. (2007) 'Simple and universal HPLC-UV method to determine cimetidine, ranitidine, famotidine and nizatidine in urine: Application to the analysis of ranitidine and its metabolites in human volunteers', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. J Chromatogr B Analyt Technol Biomed Life Sci, 860(2), pp. 235–240. doi: 10.1016/j.jchromb.2007.10.029.

Bartoshuk, L. M. *et al.* (1982) 'Effects of temperature on the perceived sweetness of sucrose', *Physiology and Behavior*. Physiol Behav, 28(5), pp. 905–910. doi: 10.1016/0031-9384(82)90212-8.

Basit, A. W. *et al.* (2001) 'The effect of polyethylene glycol 400 on gastrointestinal transit: implications for the formulation of poorly-water soluble drugs.', *Pharmaceutical research*, 18(8), pp. 1146–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11587486 (Accessed: 17 May 2018).

Batchelor, H. K., Fotaki, N. and Klein, S. (2014) 'Paediatric oral biopharmaceutics: Key considerations and current challenges', *Advanced Drug Delivery Reviews*. Elsevier, pp. 102–126. doi: 10.1016/j.addr.2013.10.006.

Batchelor, H. K. and Marriott, J. F. (2015) 'Formulations for children: problems and solutions.', *British journal of clinical pharmacology*. Wiley-Blackwell, 79(3), pp. 405–18. doi: 10.1111/BCP.12268.

BeMiller, J. (2003) 'Dextrins', in Caballero, B. (ed.) *Encyclopedia of Food Sciences and*

Nutrition. 2nd edn. Available at: https://www.sciencedirect.com/referencework/9780122270550/encyclopedia-of-food-sciences-and-nutrition (Accessed: 19 November 2021).

Bergeron, R. J. *et al.* (1977) 'Disposition requirements for binding in aqueous solution of polar substrates in the cyclohexaamylose cavity', *Journal of the American Chemical Society*. American Chemical Society, 99(15), pp. 5146–5151. doi: 10.1021/ja00457a040.

Blazek-Welsh, A. I. and Rhodes, D. G. (2001) 'Maltodextrin-based proniosomes', *AAPS PharmSci*. Springer New York, 3(1), pp. 1–8. doi: 10.1208/ps030101.

Breslin, P. A. S. and Beauchamp, G. K. (1995) 'Suppression of bitterness by sodium: Variation among bitter taste stimuli', *Chemical Senses*, 20(6), pp. 609–623. doi: 10.1093/chemse/20.6.609.

Breslin, P. and Spector, A. (2008) 'Mammalian taste perception', *Current Biology*, 18(4). Available at: https://www.cell.com/current-biology/pdf/S0960-9822(07)02370-6.pdf (Accessed: 9 March 2022).

Breza, J. M., Curtis, K. S. and Contreras, R. J. (2006) 'Temperature modulates taste responsiveness and stimulates gustatory neurons in the rat geniculate ganglion', *Journal of Neurophysiology*. J Neurophysiol, 95(2), pp. 674–685. doi: 10.1152/jn.00793.2005.

Brusick, D. *et al.* (2009) 'Expert Panel report on a study of Splenda in male rats', *Regulatory Toxicology and Pharmacology*. Academic Press Inc., 55(1), pp. 6–12. doi: 10.1016/j.yrtph.2009.06.013.

Bryson, S. P. (2014) 'Patient-centred, administration friendly medicines for children - an evaluation of children's preferences and how they impact medication adherence', *International journal of pharmaceutics*. Int J Pharm, 469(2), pp. 257–259. doi: 10.1016/J.IJPHARM.2014.04.069.

Buggins, T. R., Dickinson, P. A. and Taylor, G. (2007) 'The effects of pharmaceutical excipients on drug disposition', *Advanced drug delivery reviews*. Adv Drug Deliv Rev, 59(15), pp. 1482–1503. doi: 10.1016/J.ADDR.2007.08.017.

Cabras, T. *et al.* (2012) 'Responsiveness to 6-n-Propylthiouracil (PROP) Is Associated with Salivary Levels of Two Specific Basic Proline-Rich Proteins in Humans', *PLoS ONE*. Edited by W. Meyerhof. Public Library of Science, 7(2), p. e30962. doi: 10.1371/journal.pone.0030962.

Cayman Chemical (2022) *Sodium Acetate Stock Solution (3 M, pH 5.2)*. Available at: https://www.caymanchem.com/product/600228 (Accessed: 3 December 2018).

Chay, S. K. *et al.* (2018) 'Evaluation of the taste-masking effects of (2-hydroxypropyl)-β-cyclodextrin on ranitidine hydrochloride; a combined biosensor, spectroscopic and molecular modelling assessment', *RSC Advances*. The Royal Society of Chemistry, 8(7), pp. 3564–3573. doi: 10.1039/C7RA11015D.

Chemical Book (2019) *Sodium acetate*. Available at: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB1230044.htm (Accessed: 16 March 2022).

Chen, A. W., Resurreccion, A. V. A. and Paguio, L. P. (1996) 'Age Appropriate Hedonic Scales To Measure Food Preferences In Young Children', *Journal of Sensory Studies*. John Wiley & Sons, Ltd, 11(2), pp. 141–163. doi: 10.1111/J.1745-459X.1996.TB00038.X.

Chen, M. L. *et al.* (2007) 'A modern view of excipient effects on bioequivalence: Case study

of sorbitol', *Pharmaceutical Research*, 24(1), pp. 73–80. doi: 10.1007/s11095-006-9120-4.

Chun, L., Zhang, W. H. and Liu, J. F. (2012) 'Structure and ligand recognition of class C GPCRs', *Acta Pharmacologica Sinica*. Nature Publishing Group, 33(3), p. 312. doi: 10.1038/APS.2011.186.

Cid-Samamed, A. *et al.* (2022) 'Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications', *Food Chemistry*. Elsevier, 384, p. 132467. doi: 10.1016/J.FOODCHEM.2022.132467.

Clapham, D. *et al.* (2012) 'Assessing taste without using humans: Rat brief access aversion model and electronic tongue', *International Journal of Pharmaceutics*, 435(2), pp. 137–139. doi: 10.1016/j.ijpharm.2012.05.056.

Clapham, D. *et al.* (2021) 'Proposed Tool to Compare and Assess the Applicability of Taste Assessment Techniques for Pharmaceuticals', *Journal of Pharmaceutical Sciences*. Elsevier. doi: 10.1016/J.XPHS.2021.09.002.

ClinicalTrials.gov (2017) *A Phase IV, Open Label, 4-period Cross-over Study to Investigate a Drug Interaction Between Lamivudine and Sorbitol Oral Solutions in Healthy Volunteers*. Available at: https://clinicaltrials.gov/ct2/show/study/NCT02634073 (Accessed: 5 February 2020).

ClinicalTrials.gov (2018) *Effects of Sucralose on Drug Absorption and Metabolism (The SweetMeds Study)*. Available at: https://clinicaltrials.gov/ct2/show/results/NCT03407079 (Accessed: 26 January 2022).

Cocorocchio, M. *et al.* (2016) 'Bitter tastant responses in the amoeba Dictyostelium correlate with rat and human taste assays', *ALTEX*, 33(3), pp. 225–236. doi: 10.14573/altex.1509011.

Coupland, J. N. and Hayes, J. E. (2014) 'Physical Approaches to Masking Bitter Taste: Lessons from Food and Pharmaceuticals', *Pharmaceutical research*. NIH Public Access, 31(11), p. 2921. doi: 10.1007/S11095-014-1480-6.

Cram, A. *et al.* (2009) 'Challenges of developing palatable oral paediatric formulations', *International Journal of Pharmaceutics*. Elsevier, 365(1–2), pp. 1–3. doi: 10.1016/J.IJPHARM.2008.09.015.

Cravotto, G. *et al.* (2006) 'Cyclodextrins as Food Additives and in Food Processing', *Current Nutrition & Food Science*, 2(4), pp. 343–350. doi: 10.2174/157340106778699485.

Dagan-Wiener, A. *et al.* (2019) 'Bitterdb: Taste ligands and receptors database in 2019', *Nucleic Acids Research*. Oxford University Press, 47(D1), pp. D1179–D1185. doi: 10.1093/nar/gky974.

Dass, C. R. and Jessup, W. (2000) 'Apolipoprotein A-I, Cyclodextrins and Liposomes as Potential Drugs for the Reversal of Atherosclerosis. A Review', *Journal of Pharmacy and Pharmacology*, 52(7), pp. 731–761. doi: 10.1211/0022357001774606.

Davies, E. H. and Tuleu, C. (2008) 'Medicines for Children: A Matter of Taste', *The Journal of Pediatrics*. Mosby, 153(5), pp. 599-604.e2. doi: 10.1016/J.JPEDS.2008.06.030.

Davis, H. J. and Phipps, R. J. (2017) 'Harnessing non-covalent interactions to exert control over regioselectivity and site-selectivity in catalytic reactions', *Chemical Science*. Royal Society of Chemistry, 8(2), pp. 864–877. doi: 10.1039/C6SC04157D.

Dawson, L. A. (2019) 'What factors affect adherence to medicines?', *Archives of Disease in Childhood - Education and Practice*. Royal College of Paediatrics and Child Health, 104(1), pp.

49–52. doi: 10.1136/ARCHDISCHILD-2017-312820.

Depoortere, I. (2014) 'Taste receptors of the gut: emerging roles in health and disease', *Gut*, 63(1), pp. 179–190. doi: 10.1136/gutjnl-2013-305112.

Devantier, H. R. *et al.* (2008) 'Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion assay', *Behavioural Pharmacology*, 19(7), pp. 673–682. doi: 10.1097/FBP.0b013e3283123cd6.

DPP project (2021) *Developing Paediatric Primaquine*. Available at: https://dpp-project.org/ (Accessed: 20 March 2022).

Drewnowski, A. *et al.* (1997) 'Nontasters, Tasters, and Supertasters of 6-n-Propylthiouracil (PROP) and Hedonic Response to Sweet', *Physiology & Behavior*. Elsevier, 62(3), pp. 649–655. doi: 10.1016/S0031-9384(97)00193-5.

Drewnowski, A. *et al.* (2012) 'Sweetness and Food Preference', *The Journal of Nutrition*. Oxford University Press (OUP), 142(6), pp. 1142S-1148S. doi: 10.3945/jn.111.149575.

Drozdzik, M. *et al.* (2014) 'Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine', *Molecular Pharmaceutics*. American Chemical Society, 11(10), pp. 3547–3555. doi: 10.1021/mp500330y.

Dürr, D. *et al.* (2000) 'St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4', *Clinical pharmacology and therapeutics*. Clin Pharmacol Ther, 68(6), pp. 598–604. doi: 10.1067/MCP.2000.112240.

Dwaine Reuber, M. (1978) 'Carcinogenicity of saccharin.', *Environmental Health Perspectives*. National Institute of Environmental Health Sciences, 25, p. 173. doi: 10.1289/EHP.7825173.

EDA (2015) *Improving the design of animal experiments: Introducing the Experimental Design Assistant (EDA) | NC3Rs*. Available at: https://www.nc3rs.org.uk/news/improving-design-animal-experiments-introducing-experimental-design-assistant-eda (Accessed: 14 March 2022).

EFSA (2013) 'Conclusion on the peer review of the pesticide risk assessment of the active substance maltodextrin', *EFSA Journal*. Wiley-Blackwell Publishing Ltd, 11(1). doi: 10.2903/J.EFSA.2013.3007.

EMA (2001) 'Note for guidance on clinical investigation of medicinal products in the paediatric populations CPMP/ICH/2711/99'. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-1.pdf (Accessed: 2 March 2022).

EMA (2006) *Reflection paper: formulations of choice for the paediatric population EMEA/CHMP/PEG/194810/2005*. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003782.pdf.

EMA (2013) *Guideline on pharmaceutical development of medicines for paediatric use. EMA/CHMP/QWP/805880/2012*. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/07/WC500147002.pdf (Accessed: 5 June 2020).

EMA (2014) 'Background review for cyclodextrins used as excipients'. Available at: www.ema.europa.eu/contact (Accessed: 10 November 2021).

EMA (2017) *Cyclodextrins used as excipients. EMA/CHMP/333892/2013.*

EMA (2018) *Nonclinical Safety Testing in Support of Development of Paediatric pharmaceuticals. EMA/CHMP/ICH/616110/2018*. Available at: http://www.ich.org/products/guidelines/safety/safety-single/article/nonclinical-safety-testing-in-support-of-development-of-paediatric-medicines.html (Accessed: 30 August 2018).

EMC (2017) *Ranitidine 150mg/10ml Oral Solution - Summary of Product Characteristics (SmPC)*. Available at: https://www.medicines.org.uk/emc/product/3371/smpc (Accessed: 10 March 2022).

EMC (2021) *Revatio 10 mg/ml powder for oral suspension - Summary of Product Characteristics (SmPC)*. Available at: https://www.medicines.org.uk/emc/medicine/27153/SPC/Revatio+10+mg+ml+powder+for+oral+suspension/ (Accessed: 10 March 2022).

Eurofins (2021) *Method Transfer*. Available at: https://www.eurofins.com/biopharma-services/product-testing/services/biopharma-product-testing-services/method-development/method-transfer/ (Accessed: 5 January 2022).

Fakhoury, M. *et al.* (2005) 'Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age', *Drug Metabolism and Disposition*. American Society for Pharmacology and Experimental Therapeutics, 33(11), pp. 1603–1607. doi: 10.1124/dmd.105.005611.

FDA (2004) *Agency Response Letter GRAS Notice No. GRN 000144. Office of Food Additive Safety, Center 464 for Food Safety and Applied Nutrition, Food and Drug Administration.* Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153976.htm.

FDA (2006) *Guidance for Industry Nonclinical Safety Evaluation of Pediatric Drug Products*. Available at: http://www.fda.gov/cder/guidance/index.htm (Accessed: 8 March 2022).

FDA (2016) *Food for human consumption. Direct food substances affirmed as generally recoginized as safe*. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=184.1444 (Accessed: 24 February 2022).

FDA (2018) *Food for human consumption - FDA; Code of Federal Regulations*. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.6757 (Accessed: 10 July 2018).

FDA (2020) *FDA Requests Removal of All Ranitidine Products (Zantac) from the Market | FDA*. Available at: https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidine-products-zantac-market (Accessed: 20 March 2022).

FDA (2022) *Inactive Ingredient Search for Approved Drug Products*. Available at: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm (Accessed: 23 February 2022).

Fifere, A. *et al.* (2012) 'Theoretical study on β-cyclodextrin inclusion complexes with propiconazole and protonated propiconazole.', *Beilstein journal of organic chemistry*. Beilstein-Institut, 8, pp. 2191–201. doi: 10.3762/bjoc.8.247.

Filho, E. R. T. *et al.* (2020) 'Dynamic aspects of salt reduction in tomato sauce by use of flavor enhancers and a bitter blocker':, *https://doi.org/10.1177/1082013220913361*. SAGE

PublicationsSage UK: London, England, 26(6), pp. 549–559. doi: 10.1177/1082013220913361.

Finger, T. E. (2005) 'Cell Types and Lineages in Taste Buds', *Chemical Senses*. Narnia, 30(Supplement 1), pp. i54–i55. doi: 10.1093/chemse/bjh110.

Forestell, C. A. (2017) 'Flavor Perception and Preference Development in Human Infants', *Annals of Nutrition and Metabolism*. Karger Publishers, 70(Suppl. 3), pp. 17–25. doi: 10.1159/000478759.

Fotsing, J. R. *et al.* (2020) 'Discovery and Development of S6821 and S7958 as Potent TAS2R8 Antagonists', *Journal of Medicinal Chemistry*. American Chemical Society, 63(9), pp. 4957–4977. doi: 10.1021/ACS.JMEDCHEM.0C00388.

Frutos, M. G. S. *et al.* (2012) 'Feed efficiency, food choice, and food reward behaviors in young and old Fischer rats', *Neurobiology of Aging*. Elsevier, 33(1), pp. 206.e41-206.e53. doi: 10.1016/J.NEUROBIOLAGING.2010.09.006.

Fuke, S. and Shimizu, T. (1993) 'Sensory and preference aspects of umami', *Trends in Food Science & Technology*. Elsevier, 4(8), pp. 246–251. doi: 10.1016/0924-2244(93)90139-2.

Furrer, P. (2013) 'The central role of excipients in drug formulation', *European Pharmaceutical Review*, 18(2), pp. 67–70. Available at: https://www.europeanpharmaceuticalreview.com/article/18434/the-central-role-of-excipients-in-drug-formulation-2/ (Accessed: 24 August 2021).

Gaillard, D. and Stratford, J. M. (2016) 'Measurement of Behavioral Taste Responses in Mice: Two-Bottle Preference, Lickometer, and Conditioned Taste-Aversion Tests', *Current Protocols in Mouse Biology*. John Wiley & Sons, Ltd, 6(4), pp. 380–407. doi: 10.1002/cpmo.18.

Gao, X. *et al.* (2008) 'Nonparametric multiple comparison procedures for unbalanced one-way factorial designs', *Journal of Statistical Planning and Inference*. North-Holland, 138(8), pp. 2574–2591. doi: 10.1016/J.JSPI.2007.10.015.

Gaudette, N. J. and Pickering, G. J. (2012) 'Optimizing the orosensory properties of model functional beverages: the influence of novel sweeteners, odorants, bitter blockers, and their mixtures on (+)-catechin.', *Journal of food science*, 77(6), pp. S226-32. doi: 10.1111/j.1750-3841.2012.02707.x.

Gillette, M. (1990) 'Sensory Evaluation: Analytical and Affective Testing', *Perfumer & Flavorist*, 15.

Gilsenan, M. B. (2011) 'Additives in Dairy Foods | Safety', *Encyclopedia of Dairy Sciences: Second Edition*. Academic Press, pp. 55–60. doi: 10.1016/B978-0-12-374407-4.00005-4.

Ginsberg, G., Hattis, D. and Sonawane, B. (2004) 'Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants', *Toxicology and Applied Pharmacology*. Academic Press Inc., 198(2), pp. 164–183. doi: 10.1016/J.TAAP.2003.10.010.

GlaxoSmithKline (2004) *ZANTAC prescribing information*. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/20251scf015_zantac_lbl.pdf (Accessed: 20 January 2022).

Gore-Langton, J. K. *et al.* (2015) 'Measures of the aversive effects of drugs: A comparison of conditioned taste and place aversions', *Pharmacology Biochemistry and Behavior*. Elsevier Inc., 134, pp. 99–105. doi: 10.1016/j.pbb.2015.05.002.

Greene, T. A. *et al.* (2011) 'Probenecid Inhibits the Human Bitter Taste Receptor TAS2R16 and Suppresses Bitter Perception of Salicin', *PLoS ONE*. Edited by H. Matsunami, 6(5), p. e20123. doi: 10.1371/journal.pone.0020123.

Grembecka, M. (2015) 'Natural sweeteners in a human diet', *Roczniki Panstwowego Zakladu Higieny*. Rocz Panstw Zakl Hig, 66(3), pp. 195–202. Available at: https://pubmed.ncbi.nlm.nih.gov/26400114/ (Accessed: 22 February 2022).

Grembecka, M. (2018) 'Sugar Alcohols as Sugar Substitutes in Food Industry', *Reference Series in Phytochemistry*. Springer, Cham, pp. 547–573. doi: 10.1007/978-3-319-27027-2_23.

Gupta, H. *et al.* (2010) 'E-Tongue: A Tool for Taste Evaluation', *Recent Patents on Drug Delivery & Formulation*, 4(1), pp. 82–89. doi: 10.2174/187221110789957309.

Hampton, T. (2008) 'Sugar substitutes linked to weight gain', *JAMA*. JAMA, 299(18), pp. 2137–2138. doi: 10.1001/JAMA.299.18.2137.

Handbook of Pharmaceutical Excipients (2006) *Handbook of Pharmaceutical Excipients*, *Fifth edition*. Edited by R. Rowe, P. Sheskey, and S. Owen. Pharmaceutical Press.

Hanghøj, S. and Boisen, K. A. (2014) 'Self-reported barriers to medication adherence among chronically ill adolescents: a systematic review', *The Journal of adolescent health : official publication of the Society for Adolescent Medicine*. J Adolesc Health, 54(2), pp. 121–138. doi: 10.1016/J.JADOHEALTH.2013.08.009.

Haraguchi, T. *et al.* (2016) 'Usefulness and limitations of taste sensors in the evaluation of palatability and taste-masking in oral dosage forms', *Asian Journal of Pharmaceutical Sciences*. Elsevier, 11(4), pp. 479–485. doi: 10.1016/J.AJPS.2016.03.001.

Health.gov (2015) *Executive Summary - 2015-2020 Dietary Guidelines - health.gov*. Available at: https://health.gov/dietaryguidelines/2015/guidelines/executive-summary/ (Accessed: 16 February 2019).

Henney, J., Taylor, C. and Boon, C. (2010) *Strategies to Reduce Sodium Intake in the United States*. Washington, D.C.: National Academies Press. doi: 10.17226/12818.

Hikaru, F. and Akiko, K. (2003) 'Pharmaceutical formulations comprising sodium laurylsulfate as bitterness masking agent'. American Pharmaceutical Association. Available at: https://patents.google.com/patent/WO2004075900A2/en (Accessed: 16 February 2019).

Hofman, D. L., van Buul, V. J. and Brouns, F. J. P. H. (2015) 'Nutrition, Health, and Regulatory Aspects of Digestible Maltodextrins', *https://doi.org/10.1080/10408398.2014.940415*. Taylor & Francis, 56(12), pp. 2091–2100. doi: 10.1080/10408398.2014.940415.

Hollister, E. B. *et al.* (2015) 'Structure and function of the healthy pre-adolescent pediatric gut microbiome', *Microbiome*. BioMed Central, 3, p. 36. doi: 10.1186/S40168-015-0101-X.

Home office (2013) *Animal testing and research: guidance for the regulated community*. Available at: https://www.gov.uk/guidance/research-and-testing-using-animals (Accessed: 25 February 2022).

Home Office (2014) *Code of Practice for the housing and care of animals used in scientific procedures*, *Animals (Scientific Procedures) Act 1986*. Available at: https://www.gov.uk/government/publications/code-of-practice-for-the-housing-and-care-of-animals-bred-supplied-or-used-for-scientific-purposes (Accessed: 7 January 2022).

Al Humaid, J. (2018) 'Sweetener content and cariogenic potential of pediatric oral medications: A literature.', *International journal of health sciences*. Qassim University, 12(3),

pp. 75–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29896075 (Accessed: 11 October 2019).

International Pharmaceutical Excipients Council (2008) *Qualification of Excipients for Use in Pharmaceuticals*. Available at: file:///C:/Users/DANIEL~1.AND/AppData/Local/Temp/ExcipientQualificationGuide.pdf (Accessed: 16 October 2019).

Inui-Yamamoto, C. *et al.* (2017) 'Taste preference changes throughout different life stages in male rats', *PLOS ONE*. Public Library of Science, 12(7), p. e0181650. doi: 10.1371/JOURNAL.PONE.0181650.

Islam, M. S. and Sakaguchi, E. (2006) 'Sorbitol-based osmotic diarrhea: Possible causes and mechanism of prevention investigated in rats', *World Journal of Gastroenterology : WJG*. Baishideng Publishing Group Inc, 12(47), p. 7635. doi: 10.3748/WJG.V12.I47.7635.

Ives, R., Tuleu, C. and Bachmanov, A. (2018) 'Rat Brief Access Taste Aversion Test Predicts Human Perception of Bitter Medicines: Comparison of Data from Two Laboratories. Presented at 10th EuPFI Conference, London – EuPFI'. Available at: http://www.eupfi.org/past-conferences/10th-eupfi-conference/ (Accessed: 13 January 2022).

Jaggupilli, A. *et al.* (2019) 'Advanced Glycation End-Products Can Activate or Block Bitter Taste Receptors', *Nutrients 2019, Vol. 11, Page 1317*. Multidisciplinary Digital Publishing Institute, 11(6), p. 1317. doi: 10.3390/NU11061317.

Johnson, T. N. and Thomson, M. (2008) 'Intestinal metabolism and transport of drugs in children: The effects of age and disease', *Journal of Pediatric Gastroenterology and Nutrition*, pp. 3–10. doi: 10.1097/MPG.0b013e31816a8cca.

Juluri, A. *et al.* (2016) 'Taste Masking of Griseofulvin and Caffeine Anhydrous Using Kleptose Linecaps DE17 by Hot Melt Extrusion', *AAPS PharmSciTech*. Springer New York LLC, 17(1), pp. 99–105. doi: 10.1208/S12249-015-0374-1/FIGURES/8.

Jungbunzlauer (2022) *Facial Moisturizer with Sodium Lactate, Sodium Gluconate and Citrofol*. Available at: https://www.personalcaremagazine.com/formulation-details/1423/facial-moisturizer-with-sodium-lactate-sodium-gluconate-and-citrofol-ai?companyid=2672 (Accessed: 16 October 2019).

Kalra, A., Bhat, P. and Kaur, I. P. (2021) 'Deciphering molecular mechanics in the taste masking ability of Maltodextrin: Developing pediatric formulation of Oseltamivir for viral pandemia', *Carbohydrate Polymers*. Elsevier, 260, p. 117703. doi: 10.1016/J.CARBPOL.2021.117703.

Karanewsky, D. *et al.* (2011) 'Compounds that inhibit (block) bitter taste in composition and use thereof'. United States Patent.

Katdare, A. and Chaubal, M. V (2006) 'Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems'. Available at: www.informa.com (Accessed: 14 March 2022).

Katrajkar, K. *et al.* (2019) 'Shedding light on interaction of so called inactive ingredients (excipients) with permeability-glycoprotein', *Journal of Drug Delivery Science and Technology*. Editions de Sante, pp. 531–552. doi: 10.1016/j.jddst.2019.05.022.

Kaushal, N. *et al.* (2016) 'Study of regulatory requirements for the conduct of bioequivalence studies in US, Europe, Canada, India, ASEAN and SADC countries: Impact on generic drug

substitution', *Journal of Applied Pharmaceutical Science*, 6(04), pp. 206–222. doi: 10.7324/JAPS.2016.60430.

Kaushik, D. and Dureja, H. (2014) 'Recent patents and patented technology platforms for pharmaceutical taste masking.', *Recent patents on drug delivery & formulation*, 8(1), pp. 37–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24499438 (Accessed: 7 March 2019).

Kearns, G. L. *et al.* (2003) 'Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children', *New England Journal of Medicine*. Edited by A. J. J. Wood. Massachusetts Medical Society , 349(12), pp. 1157–1167. doi: 10.1056/NEJMra035092.

Keast, R. S. J. and Breslin, P. A. S. (2002) 'Modifying the bitterness of selected oral pharmaceuticals with cation and anion series of salts', *Pharmaceutical Research*. Springer, 19(7), pp. 1019–1026. doi: 10.1023/A:1016474607993.

Keeley, A. *et al.* (2017) 'Are Rats Supertasters?' Presented at EuPFI 9th Annual Conference 'Formulating Better Medicines for Children'.

Keeley, A. *et al.* (2019) 'In Vitro Dissolution Model Can Predict the in Vivo Taste Masking Performance of Coated Multiparticulates', *Molecular Pharmaceutics*. American Chemical Society, 16(5), pp. 2095–2105. doi: 10.1021/ACS.MOLPHARMACEUT.9B00060/ASSET/IMAGES/ACS.MOLPHARMACEUT.9B00060.SOCIAL.JPEG_V03.

Khalil, F. and Läer, S. (2014) 'Physiologically based pharmacokinetic models in the prediction of oral drug exposure over the entire pediatric age range-sotalol as a model drug', *The AAPS journal*. AAPS J, 16(2), pp. 226–239. doi: 10.1208/S12248-013-9555-6.

Khan, D. *et al.* (2022) 'Paediatric specific dosage forms: Patient and formulation considerations', *International Journal of Pharmaceutics*. Elsevier, 616, p. 121501. doi: 10.1016/J.IJPHARM.2022.121501.

Klinkesorn, U. *et al.* (2004) 'Stability and rheology of corn oil-in-water emulsions containing maltodextrin', *Food Research International*. Elsevier, 37(9), pp. 851–859. doi: 10.1016/J.FOODRES.2004.05.001.

Koch, K. M. *et al.* (1993) 'Effect of sodium acid pyrophosphate on ranitidine bioavailability and gastrointestinal transit time.', *Pharmaceutical research*, 10(7), pp. 1027–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8397394 (Accessed: 17 May 2018).

Koldovsky, O., Johnson, S. M. and Koldovsky, O. (1988) 'In vitro intestinal processing of prostaglandin F2 alpha in rats: developmental aspects', *Journal of pediatric gastroenterology and nutrition*. J Pediatr Gastroenterol Nutr, 7(5), pp. 755–761. doi: 10.1097/00005176-198809000-00022.

Korteja¨rvi, H. *et al.* (2005) *Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Ranitidine Hydrochloride*, *J Pharm Sci*. doi: 10.1002/jps.20392.

Kroeze, J. H. A. and Bartoshuk, L. M. (1985) 'Bitterness suppression as revealed by split-tongue taste stimulation in humans', *Physiology & Behavior*. Elsevier, 35(5), pp. 779–783. doi: 10.1016/0031-9384(85)90412-3.

Kuhn, C. *et al.* (2004) 'Bitter taste receptors for saccharin and acesulfame K', *Journal of Neuroscience*. Society for Neuroscience, 24(45), pp. 10260–10265. doi: 10.1523/JNEUROSCI.1225-04.2004.

Labib, G. (2015) 'Novel levocetirizine HCl tablets with enhanced palatability: synergistic

effect of combining taste modifiers and effervescence technique', *Drug Design, Development and Therapy*, 9, p. 5135. doi: 10.2147/DDDT.S92245.

Lajoinie, A. *et al.* (2014) 'Solid oral forms availability in children: a cost saving investigation', *British Journal of Clinical Pharmacology*. Wiley-Blackwell, 78(5), p. 1080. doi: 10.1111/BCP.12442.

Lander, A. and Newman, J. (2013) 'Paediatric anatomy', *Surgery (Oxford)*. Elsevier, 31(3), pp. 101–105. doi: 10.1016/J.MPSUR.2013.01.002.

Latha, R. S. and Lakshmi, P. K. (2012) 'Electronic tongue: An analytical gustatory tool.', *Journal of advanced pharmaceutical technology & research*. Wolters Kluwer -- Medknow Publications, 3(1), pp. 3–8. doi: 10.4103/2231-4040.93556.

Lawless, H. and Heymann, H. (2010) 'Principles of Good Practice', in *Sensory Evaulation of Food*. New York, NY: Springer New York, p. 58.

Lemon, C. H. (2015) 'Perceptual and Neural Responses to Sweet Taste in Humans and Rodents', *Chemosensory Perception*. Springer New York LLC, 8(2), pp. 46–52. doi: 10.1007/s12078-015-9177-8.

Lenhart, A. and Chey, W. D. (2017) 'A Systematic Review of the Effects of Polyols on Gastrointestinal Health and Irritable Bowel Syndrome.', *Advances in nutrition (Bethesda, Md.)*. American Society for Nutrition, 8(4), pp. 587–596. doi: 10.3945/an.117.015560.

Lin, L. *et al.* (2015) 'SLC transporters as therapeutic targets: emerging opportunities', *Nature Reviews Drug Discovery 2015 14:8*. Nature Publishing Group, 14(8), pp. 543–560. doi: 10.1038/nrd4626.

Liu, B. *et al.* (2011) 'Molecular Mechanism of Species-Dependent Sweet Taste toward Artificial Sweeteners', *Journal of Neuroscience*. Society for Neuroscience, 31(30), pp. 11070–11076. doi: 10.1523/JNEUROSCI.0791-11.2011.

Liu, F. *et al.* (2014) 'Patient-Centered Pharmaceutical Design to Improve Acceptability of Medicines: Similarities and Differences in Paediatric and Geriatric Populations', *Drugs*. Springer, 74(16), p. 1871. doi: 10.1007/S40265-014-0297-2.

Locher, K. P. (2009) 'Structure and mechanism of ATP-binding cassette transporters', *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society, pp. 239–245. doi: 10.1098/rstb.2008.0125.

Loftsson, T., Hreinsdóttir, D. and Másson, M. (2007) 'The complexation efficiency', *Journal of Inclusion Phenomena and Macrocyclic Chemistry*. Springer, 57(1–4), pp. 545–552. doi: 10.1007/S10847-006-9247-2/FIGURES/2.

Lu, B., Breza, J. M. and Contreras, R. J. (2016) 'Temperature Influences Chorda Tympani Nerve Responses to Sweet, Salty, Sour, Umami, and Bitter Stimuli in Mice', *Chemical Senses*. Oxford Academic, 41(9), pp. 727–736. doi: 10.1093/CHEMSE/BJW082.

Lu, H. and Rosenbaum, S. (2014) 'Developmental Pharmacokinetics in Pediatric Populations', *The Journal of Pediatric Pharmacology and Therapeutics : JPPT*. Pediatric Pharmacology Advocacy Group, 19(4), p. 262. doi: 10.5863/1551-6776-19.4.262.

Lu, P. *et al.* (2017) 'Extraoral bitter taste receptors in health and disease.', *The Journal of general physiology*. The Rockefeller University Press, 149(2), pp. 181–197. doi: 10.1085/jgp.201611637.

Lvovskaya, S. and Smith, D. P. (2013) 'A spoonful of bitter helps the sugar response go

down', *Neuron*. NIH Public Access, pp. 612–614. doi: 10.1016/j.neuron.2013.07.038.

Mahalapbutr, P. *et al.* (2019) 'Atomistic mechanisms underlying the activation of the G protein-coupled sweet receptor heterodimer by sugar alcohol recognition', *Scientific Reports 2019 9:1*. Nature Publishing Group, 9(1), pp. 1–11. doi: 10.1038/s41598-019-46668-w.

Mai, Y. *et al.* (2019) 'Sex-Dependence in the Effect of Pharmaceutical Excipients: Polyoxyethylated Solubilising Excipients Increase Oral Drug Bioavailability in Male but not Female Rats.', *Pharmaceutics*. Multidisciplinary Digital Publishing Institute (MDPI), 11(5). doi: 10.3390/pharmaceutics11050228.

Malode, S. and Gudsoorkar, V. (2014) 'Taste Masking : Overview of Taste Assessment Approaches in the development of oral pharmaceutical formulations', *Indo American Journal of Pharmaceutical Research*, 4(01), pp. 204–211. doi: 10.1044/1980-iajpr.01322.

Manor, P. C. and Saenger, W. (1974) 'Topography of cyclodextrin inclusion complexes. III. Crystal and molecular structure of cyclohexaamylose hexahydrate, the water dimer inclusion complex', *Journal of the American Chemical Society*. American Chemical Society, 96(11), pp. 3630–3639. doi: 10.1021/ja00818a042.

Margolskee, R. and Ming, D. (2003) 'Inhibitors of the bitter taste response'. Available at: https://patentimages.storage.googleapis.com/f1/fd/02/f75894eee2c319/US6540978.pdf (Accessed: 26 July 2018).

Martel, F. *et al.* (1998) 'Postnatal development of organic cation transport in the rat liver', *Pharmacological Research*. Academic Press, 37(2), pp. 131–136. doi: 10.1006/phrs.1997.0283.

Martínez-García, M. A. *et al.* (2002) 'Analysis of the Stability of Stored Adenosine 5'-monophosphate used for Bronchoprovocation', *Pulmonary Pharmacology & Therapeutics*, 15(2), pp. 157–160. doi: 10.1006/pupt.2001.0334.

Masamoto, M. *et al.* (2020) 'Effects of bitter receptor antagonists on behavioral lick responses of mice', *Neuroscience Letters*. Elsevier, 730, p. 135041. doi: 10.1016/J.NEULET.2020.135041.

Matsson, P. *et al.* (2009) 'Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs', *Pharmaceutical Research*. Pharm Res, 26(8), pp. 1816–1831. doi: 10.1007/s11095-009-9896-0.

Matsuoka, Y. *et al.* (1999) 'Developmental expression of P-glycoprotein (multidrug resistance gene product) in the rat brain', *Journal of Neurobiology*. J Neurobiol, 39(3), pp. 383–392. doi: 10.1002/(SICI)1097-4695(19990605)39:3<383::AID-NEU5>3.0.CO;2-4.

McEwan, J. A. and Lyon, D. H. (2003) 'Sensory Rating and Scoring Methods', in *Encyclopedia of Food Sciences and Nutrition*. Academic Press, pp. 5148–5152. doi: 10.1016/B0-12-227055-X/01064-6.

McKinley, M. J. *et al.* (2006) 'Differential effects of aging on fluid intake in response to hypovolemia, hypertonicity, and hormonal stimuli in Munich Wistar rats', *Proceedings of the National Academy of Sciences of the United States of America*, 103(9), pp. 3450–3455. doi: 10.1073/PNAS.0511099103/ASSET/3BC37D4C-7E1A-4F47-A513-BFC230884DBE/ASSETS/GRAPHIC/ZPQ0060611730005.JPEG.

Mclaughlin, S. K., Mckinnon, P. J. and Margolskee, R. F. (1992) 'Gustducin is a taste-cell-specific G protein closely related to the transducins'.

McOmber, M. E., Ou, C. N. and Shulman, R. J. (2010) 'Effects of Timing, Sex, and Age on Site-Specific Gastrointestinal Permeability Testing in Children and Adults', *Journal of pediatric gastroenterology and nutrition*. NIH Public Access, 50(3), p. 269. doi: 10.1097/MPG.0B013E3181AA3AA9.

Mehrotra, N. *et al.* (2006) 'The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy', *International Journal of Impotence Research 2007 19:3*. Nature Publishing Group, 19(3), pp. 253–264. doi: 10.1038/sj.ijir.3901522.

Mennella, J. A. *et al.* (2013) 'The Bad Taste of Medicines: Overview of Basic Research on Bitter Taste', *Clinical Therapeutics*. Elsevier, 35(8), pp. 1225–1246. doi: 10.1016/j.clinthera.2013.06.007.

Mennella, J. A. *et al.* (2014) 'Age-Related Differences in Bitter Taste and Efficacy of Bitter Blockers', *PLoS ONE*. Edited by M. Behrens, 9(7), p. e103107. doi: 10.1371/journal.pone.0103107.

Mennella, J. A. and Beauchamp, G. K. (2008) 'Optimizing oral medications for children.', *Clinical therapeutics*. NIH Public Access, 30(11), pp. 2120–32. doi: 10.1016/j.clinthera.2008.11.018.

Mennella, J. A. and Bobowski, N. K. (2015) 'The sweetness and bitterness of childhood: Insights from basic research on taste preferences', *Physiology and Behavior*. Elsevier Inc., 152(0 0), pp. 502–507. doi: 10.1016/j.physbeh.2015.05.015.

Mennella, J. A., Pepino, M. Y. and Beauchamp, G. K. (2003) 'Modification of bitter taste in children.', *Developmental psychobiology*. NIH Public Access, 43(2), pp. 120–7. doi: 10.1002/dev.10127.

Metry, M. and Polli, J. E. (2022) 'Evaluation of Excipient Risk in BCS Class I and III Biowaivers', *AAPS Journal*. Springer Science and Business Media Deutschland GmbH, 24(1), pp. 1–11. doi: 10.1208/S12248-021-00670-1/TABLES/1.

Meyerhof, W. *et al.* (2010) 'The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors', *Chem. Senses*, 35, pp. 157–170. doi: 10.1093/chemse/bjp092.

Milne, C. (2005) 'The pediatric studies initiative.', in Mathieu, M. (ed.) *New Drug Development: A Regulatory Overview.* 8th ed. Waltham, Mass: PAREXEL Internation, pp. 339–362.

Ming, D., Ninomiya, Y. and Margolskee, R. F. (1999) 'Blocking taste receptor activation of gustducin inhibits gustatory responses to bitter compounds', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 96(17), pp. 9903–9908. doi: 10.1073/pnas.96.17.9903.

Miyoshi, M. A., Abe, K. and Emori, Y. (2001) 'IP(3) receptor type 3 and PLCbeta2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells.', *Chemical senses*, 26(3), pp. 259–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11287386 (Accessed: 24 April 2019).

Mohamed-Ahmed, A. H. A. *et al.* (2016) 'Non-human tools for the evaluation of bitter taste in the design and development of medicines: a systematic review', *Drug Discovery Today*. Elsevier Current Trends, 21(7), pp. 1170–1180. doi: 10.1016/J.DRUDIS.2016.05.014.

Mojet, J., Christ-Hazelhof, E. and Heidema, J. (2001) 'Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes?', *Chemical senses*. Chem Senses, 26(7), pp. 845–860. doi: 10.1093/CHEMSE/26.7.845.

Monk, K. J. *et al.* (2014) 'Licking Microstructure Reveals Rapid Attenuation of Neophobia', *Chemical Senses*. Oxford University Press, 39(3), p. 203. doi: 10.1093/CHEMSE/BJT069.

Mortensen, A. *et al.* (2016) 'Re-evaluation of β-cyclodextrin (E 459) as a food additive', *EFSA Journal*. Wiley, 14(12). doi: 10.2903/J.EFSA.2016.4628.

Mossavar-Rahmani, Y. *et al.* (2019) 'Artificially sweetened beverages and stroke, coronary heart disease, and all-cause mortality in the women's health initiative', *Stroke*. Lippincott Williams and Wilkins, 50(3), pp. 555–562. doi: 10.1161/STROKEAHA.118.023100.

Münster, M. *et al.* (2017) 'Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations', *International journal of pharmaceutics*. Int J Pharm, 529(1–2), pp. 310–318. doi: 10.1016/J.IJPHARM.2017.06.084.

Mura, E. *et al.* (2018) 'Innate and acquired tolerance to bitter stimuli in mice'. doi: 10.1371/journal.pone.0210032.

Murakami, T. (2017) 'Absorption sites of orally administered drugs in the small intestine', *http://dx.doi.org/10.1080/17460441.2017.1378176*. Taylor & Francis, 12(12), pp. 1219–1232. doi: 10.1080/17460441.2017.1378176.

Nader, A. M. *et al.* (2016) 'Effect of Gastric Fluid Volume on the In Vitro Dissolution and In Vivo Absorption of BCS Class II Drugs: a Case Study with Nifedipine', *The AAPS journal*. AAPS J, 18(4), pp. 981–988. doi: 10.1208/S12248-016-9918-X.

Nair, A. B. and Jacob, S. (2016) 'A simple practice guide for dose conversion between animals and human', *Journal of Basic and Clinical Pharmacy*. Wolters Kluwer -- Medknow Publications, 7(2), p. 27. doi: 10.4103/0976-0105.177703.

Narukawa, M. *et al.* (2017) 'Participation of the peripheral taste system in aging-dependent changes in taste sensitivity', *Neuroscience*. Pergamon, 358, pp. 249–260. doi: 10.1016/J.NEUROSCIENCE.2017.06.054.

National Cancer Institute (2016) *Artificial Sweeteners and Cancer - National Cancer Institute*. Available at: https://www.cancer.gov/about-cancer/causes-prevention/risk/diet/artificial-sweeteners-fact-sheet (Accessed: 24 February 2022).

Neath, K. N. *et al.* (2010) 'Increased liking for a solution is not necessary for the attenuation of neophobia in rats', *Behavioral neuroscience*. NIH Public Access, 124(3), p. 398. doi: 10.1037/A0019505.

Niazi, S. K. (2004) 'Handbook of Pharmaceutical Manufacturing Formulations: Liquid Products'.

Nicolas, J. M. *et al.* (2017) 'Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions', *Biopharmaceutics & Drug Disposition*. Wiley-Blackwell, 38(3), p. 209. doi: 10.1002/BDD.2052.

Nieuwlaat, R. *et al.* (2014) 'Interventions for enhancing medication adherence', *The Cochrane database of systematic reviews*. Cochrane Database Syst Rev, 2014(11). doi: 10.1002/14651858.CD000011.PUB4.

Nigam, S. K. and Bhatnagar, V. (2013) 'How Much Do We Know About Drug Handling by SLC and ABC Drug Transporters in Children?', *Clinical Pharmacology & Therapeutics*. John Wiley & Sons, Ltd, 94(1), pp. 27–29. doi: 10.1038/CLPT.2013.82.

Noorjahan, A., Amrita, B. and Kavita, S. (2014) '*In vivo* evaluation of taste masking for developed chewable and orodispersible tablets in humans and rats', *Pharmaceutical*

Development and Technology, 19(3), pp. 290–295. doi: 10.3109/10837450.2013.778870.

Nordenmalm, S. *et al.* (2019) 'Children's views on taking medicines and participating in clinical trials', *Archives of Disease in Childhood*. BMJ Publishing Group Ltd, 104(9), pp. 900–905. doi: 10.1136/ARCHDISCHILD-2018-316511.

Nunn, T. and Williams, J. (2005) 'Formulation of medicines for children', *British Journal of Clinical Pharmacology*. Wiley-Blackwell, 59(6), p. 674. doi: 10.1111/J.1365-2125.2005.02410.X.

O'Mahony, M. and Rousseau, B. (2003) 'Discrimination testing: a few ideas, old and new', *Food Quality and Preference*. Elsevier, 14(2), pp. 157–164. doi: 10.1016/S0950-3293(02)00109-X.

Ohkubo, S. and Nakahata, N. (2007) 'The Role of Lipd Rafts in Trimeric G Protein-Mediated Signal Transduction', *ChemInform*. Wiley, 38(19). doi: 10.1002/CHIN.200719265.

Ojiro, I. *et al.* (2021) 'Trp-Trp acts as a multifunctional blocker for human bitter taste receptors, hTAS2R14, hTAS2R16, hTAS2R43, and hTAS2R46', *Bioscience, Biotechnology, and Biochemistry*. Oxford Academic, 85(6), pp. 1526–1529. doi: 10.1093/BBB/ZBAB061.

Okada, S. (2015) 'The taste system of small fish species', *Bioscience, Biotechnology, and Biochemistry*. Japan Society for Bioscience Biotechnology and Agrochemistry, 79(7), pp. 1039–1043. doi: 10.1080/09168451.2015.1023251.

Al Omari, M. M. *et al.* (2006) 'Sildenafil/cyclodextrin complexation: Stability constants, thermodynamics, and guest–host interactions probed by 1H NMR and molecular modeling studies', *Journal of Pharmaceutical and Biomedical Analysis*. Elsevier, 41(3), pp. 857–865. doi: 10.1016/J.JPBA.2006.01.055.

Ono, N. *et al.* (2011) 'Reduction of Bitterness of Antihistaminic Drugs by Complexation with β-Cyclodextrins', *Journal of Pharmaceutical Sciences*, 100(5), pp. 1935–1943. doi: 10.1002/jps.22417.

Østergaard, G. and Knudsen, I. (1998) 'The applicability of the ADI (Acceptable Daily Intake) for food additives to infants and children', *Food additives and contaminants*. Food Addit Contam, 15 Suppl(SUPPL.), pp. 63–74. doi: 10.1080/02652039809374617.

Ouranidis, A. *et al.* (2021) 'Sildenafil 4.0—Integrated Synthetic Chemistry, Formulation and Analytical Strategies Effecting Immense Therapeutic and Societal Impact in the Fourth Industrial Era', *Pharmaceuticals*. Multidisciplinary Digital Publishing Institute (MDPI), 14(4). doi: 10.3390/PH14040365.

Palmer, R. K. *et al.* (2013) 'A High Throughput In Vivo Assay for Taste Quality and Palatability', *PLoS ONE*. Edited by J. I. Glendinning. Public Library of Science, 8(8), p. e72391. doi: 10.1371/journal.pone.0072391.

Panakanti, R. and Narang, A. S. (2012) 'Impact of excipient interactions on drug bioavailability from solid dosage forms', *Pharmaceutical research*. Pharm Res, 29(10), pp. 2639–2659. doi: 10.1007/S11095-012-0767-8.

Parikh, A., Agarwal, S. and Raut, K. (2014) 'A review on applications of maltodextrin in pharmaceutical industry', *International Journal of Pharmacy and Biological Sciences*, 4(4), pp. 67–74. Available at: https://www.semanticscholar.org/paper/A-REVIEW-ON-APPLICATIONS-OF-MALTODEXTRIN-IN-Parikh-Agarwal/c74b58a29ccff435f2908fc428eb7dfb274ce432 (Accessed: 3 March 2022).

Pavlov, G. M. *et al.* (2010) 'Hydrodynamic properties of cyclodextrin molecules in dilute solutions.', *European biophysics journal : EBJ*. Springer, 39(3), pp. 371–9. doi: 10.1007/s00249-008-0394-9.

Pawar, S. and Kumar, A. (2002) 'Issues in the formulation of drugs for oral use in children: role of excipients', *Paediatric drugs*. Paediatr Drugs, 4(6), pp. 371–379. doi: 10.2165/00128072-200204060-00004.

Pearlman, M., Obert, J. and Casey, L. (2017) 'The Association Between Artificial Sweeteners and Obesity', *Current Gastroenterology Reports*. Current Medicine Group LLC 1, 19(12), pp. 1–8. doi: 10.1007/S11894-017-0602-9/TABLES/1.

Pedrosa, A. and Serrano, M. (2000) 'Solubilities of Sodium Gluconate in Water and in Aqueous Solutions of Ethanol and Methanol', *J. Chem. Eng. Data*. American Chemical Society, 45(3), pp. 461–463. doi: 10.1021/JE990305X.

Pein, M. *et al.* (2014) 'Taste-masking assessment of solid oral dosage forms–A critical review', *International Journal of Pharmaceutics*. Elsevier, 465(1–2), pp. 239–254. doi: 10.1016/J.IJPHARM.2014.01.036.

Pereira, A. G. *et al.* (2021) 'Main Applications of Cyclodextrins in the Food Industry as the Compounds of Choice to Form Host–Guest Complexes', *International Journal of Molecular Sciences*. Multidisciplinary Digital Publishing Institute (MDPI), 22(3), pp. 1–23. doi: 10.3390/IJMS22031339.

Peryam, D. R. and Girardot, N. F. (1952) 'Advanced taste test method', *Food Engineering*, 24(194), pp. 58–61.

Podrażka, M. *et al.* (2017) 'Electronic Tongue-A Tool for All Tastes?', *Biosensors*. Multidisciplinary Digital Publishing Institute (MDPI), 8(1). doi: 10.3390/bios8010003.

Popescu, C. *et al.* (2012) 'NSAIDs Taste Masking by New Maltodextrins (KLEPTOSE® Linecaps)'. Available at: https://www.roquette.com/-/media/contenus-gbu/pharma/scientific-posters/roquette-pharma-case-study-nsaids-taste-masking-by-new-maltodextrins-kleptose-linecaps.pdf.

Popper, R. and Kroll, J. J. (2011) 'Consumer testing of food products using children', in *Developing Children's Food Products*. Woodhead Publishing, pp. 163–187. doi: 10.1533/9780857091130.3.163.

Prasad, B. *et al.* (2016) 'Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics', *Clinical pharmacology and therapeutics*. Clin Pharmacol Ther, 100(4), pp. 362–370. doi: 10.1002/CPT.409.

Preis, M. *et al.* (2014) 'A comparative study on solubilizing and taste-masking capacities of hydroxypropyl-β-cyclodextrin and maltodextrins with high amylose content', *Sensors and Actuators B: Chemical*. Elsevier, 193, pp. 442–450. doi: 10.1016/J.SNB.2013.12.005.

Preis, M., Pein, M. and Breitkreutz, J. (2021) 'Development of a Taste-Masked Orodispersible Film Containing Dimenhydrinate', *Pharmaceutics*, 4, pp. 551–562. doi: 10.3390/pharmaceutics4040551.

Prescott, F. J. *et al.* (1953) 'Gluconic Acid and Its Derivatives', *Industrial & Engineering Chemistry*. American Chemical Society (ACS), 45(2), pp. 338–342. doi: 10.1021/ie50518a030.

Putseys, J. A., Lamberts, L. and Delcour, J. A. (2010) 'Amylose-inclusion complexes: Formation, identity and physico-chemical properties', *Journal of Cereal Science*. Academic

Press, 51(3), pp. 238–247. doi: 10.1016/J.JCS.2010.01.011.

Pydi, S. P. *et al.* (2014) 'Amino acid derivatives as bitter taste receptor (T2R) blockers.', *The Journal of biological chemistry*, 289(36), pp. 25054–66. doi: 10.1074/jbc.M114.576975.

Radeloff, M. A. and Beck, R. H. F. (2013) 'Polyols – more than sweeteners', *Sugar Industry*. Verlag Dr. Albert Bartens KG, pp. 226–234. doi: 10.36961/SI14095.

Raffaini, G. and Ganazzoli, F. (2020) 'Understanding Surface Interaction and Inclusion Complexes between Piroxicam and Native or Crosslinked β-Cyclodextrins: The Role of Drug Concentration', *Molecules*. Multidisciplinary Digital Publishing Institute (MDPI), 25(12). doi: 10.3390/MOLECULES25122848.

Ranbaxy Limited (2020) 'Clarithromycin oral suspension patient information leaflet'. Available at: https://www.medicines.org.uk/emc/files/pil.4360.pdf (Accessed: 24 February 2022).

Rashed, A. N. *et al.* (2021) 'Feasibility of developing children's Pill School within a UK hospital', *Archives of disease in childhood*. Arch Dis Child, 106(7), pp. 705–708. doi: 10.1136/ARCHDISCHILD-2020-319154.

Reed-Knight, B., Lewis, J. D. and Blount, R. L. (2011) 'Association of disease, adolescent, and family factors with medication adherence in pediatric inflammatory bowel disease', *Journal of pediatric psychology*. J Pediatr Psychol, 36(3), pp. 308–317. doi: 10.1093/JPEPSY/JSQ076.

Van Riet-Nales, D. A. *et al.* (2013) 'Acceptability of different oral formulations in infants and preschool children', *Archives of Disease in Childhood*. BMJ Publishing Group Ltd, 98(9), pp. 725–731. doi: 10.1136/ARCHDISCHILD-2012-303303.

Robino, A. *et al.* (2014) 'A population-based approach to study the impact of PROP perception on food liking in populations along the Silk Road', *PloS one*. PLoS One, 9(3). doi: 10.1371/JOURNAL.PONE.0091716.

Rouaz, K. *et al.* (2021) 'Excipients in the Paediatric Population: A Review', *Pharmaceutics*, 13(3), p. 387. doi: 10.3390/pharmaceutics13030387.

Roudnitzky, N. *et al.* (2015) 'Receptor Polymorphism and Genomic Structure Interact to Shape Bitter Taste Perception', *PLoS Genetics*. Public Library of Science, 11(9). doi: 10.1371/journal.pgen.1005530.

Royal College of Paediatrics and Child Health (2021) *Medicine safety alert - ranitidine (withdrawn)* . Available at: https://www.rcpch.ac.uk/news-events/news/medicine-safety-alert-ranitidine-withdrawn (Accessed: 20 March 2022).

Rudnitskaya, A. *et al.* (2013) 'Assessment of bitter taste of pharmaceuticals with multisensor system employing 3 way PLS regression', *Analytica Chimica Acta*, 770, pp. 45–52. doi: 10.1016/j.aca.2013.02.006.

Ruiz-Ojeda, F. J. *et al.* (2019) 'Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials.', *Advances in nutrition (Bethesda, Md.)*. American Society for Nutrition, 10(suppl_1), pp. S31–S48. doi: 10.1093/advances/nmy037.

Ruiz, F. *et al.* (2019) 'Sex differences in medicine acceptability: A new factor to be considered in medicine formulation', *Pharmaceutics*. MDPI AG, 11(8). doi: 10.3390/pharmaceutics11080368.

Rupesh, S. and Nayak, U. (2006) 'Genetic sensitivity to the bitter taste of 6-n propylthiouracil: A new risk determinant for dental caries in children', *Journal of Indian*

Society of Pedodontics and Preventive Dentistry. Medknow Publications, 24(2), p. 63. doi: 10.4103/0970-4388.26018.

Salminen, E. K. *et al.* (1989) 'Xylitol vs glucose: effect on the rate of gastric emptying and motilin, insulin, and gastric inhibitory polypeptide release', *The American Journal of Clinical Nutrition*, 49(6), pp. 1228–1232. doi: 10.1093/ajcn/49.6.1228.

Santer, M. *et al.* (2014) 'Treatment non-adherence in pediatric long-term medical conditions: systematic review and synthesis of qualitative studies of caregivers' views', *BMC pediatrics*. BMC Pediatr, 14(1). doi: 10.1186/1471-2431-14-63.

Saokham, P. *et al.* (2018) 'Solubility of Cyclodextrins and Drug/Cyclodextrin Complexes', *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*. Multidisciplinary Digital Publishing Institute (MDPI), 23(5). doi: 10.3390/MOLECULES23051161.

Sarah French, A. *et al.* (2015) 'Dual mechanism for bitter avoidance in drosophila', *Journal of Neuroscience*. Society for Neuroscience, 35(9), pp. 3990–4004. doi: 10.1523/JNEUROSCI.1312-14.2015.

Schiffman, S. S. and Abou-Donia, M. B. (2012) 'Sucralose revisited: Rebuttal of two papers about Splenda safety', *Regulatory Toxicology and Pharmacology*. Academic Press Inc., 63(3), pp. 505–508. doi: 10.1016/J.YRTPH.2012.05.002.

Schulze, J. D. R. *et al.* (2006) 'Impact of formulation excipients on human intestinal transit', *Journal of Pharmacy and Pharmacology*, 58(6), pp. 821–825. doi: 10.1211/jpp.58.6.0012.

Schwartz, D. D. and Axelrad, M. E. (2015) 'Healthcare Partnerships for Pediatric Adherence'. Cham: Springer International Publishing (SpringerBriefs in Public Health). doi: 10.1007/978-3-319-13668-4.

Schwiebert, E. *et al.* (2021) 'Inhibition of Bitter Taste from Oral Tenofovir Alafenamide', *Molecular Pharmacology*. American Society for Pharmacology and Experimental Therapeutics, 99(5), pp. 319–327. doi: 10.1124/MOLPHARM.120.000071.

Sclafani, A. (1986) 'Carbohydrate Taste, Appetite, and Obesity: An Overview I', *Neuroscience & Biobehavioral Reviews*, pp. 131–153.

Sclafani, A. (2004) 'The sixth taste?', *Appetite*. Academic Press, 43(1), pp. 1–3. doi: 10.1016/J.APPET.2004.03.007.

Sengupta, P. (2013) 'The laboratory rat: Relating its age with human's', *International Journal of Preventive Medicine*. Wolters Kluwer -- Medknow Publications, pp. 624–630.

Senopsys (2018) *The Role of Sweeteners in Taste Masking*. Available at: https://www.senopsys.com/taste-masking-blog/role-sweeteners-taste-masking/ (Accessed: 23 February 2022).

du Sert, N. P. *et al.* (2020) 'Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0', *PLOS Biology*. Public Library of Science, 18(7), p. e3000411. doi: 10.1371/JOURNAL.PBIO.3000411.

Sharafi, M., Hayes, J. E. and Duffy, V. B. (2013) 'Masking Vegetable Bitterness to Improve Palatability Depends on Vegetable Type and Taste Phenotype.', *Chemosensory perception*. NIH Public Access, 6(1), pp. 8–19. doi: 10.1007/s12078-012-9137-5.

Sharom, F. J. (2011) 'The P-glycoprotein multidrug transporter', *Essays In Biochemistry*, 50(1), pp. 161–178. doi: 10.1042/bse0500161.

Shigemura, N. *et al.* (2013) 'Angiotensin II Modulates Salty and Sweet Taste Sensitivities', *Journal of Neuroscience*, 33(15), pp. 6267–6277. doi: 10.1523/JNEUROSCI.5599-12.2013.

Shil, A. and Chichger, H. (2021) 'Artificial Sweeteners Negatively Regulate Pathogenic Characteristics of Two Model Gut Bacteria, E. coli and E. faecalis', *International Journal of Molecular Sciences 2021, Vol. 22, Page 5228*. Multidisciplinary Digital Publishing Institute, 22(10), p. 5228. doi: 10.3390/IJMS22105228.

Shin, Y. K. *et al.* (2012) 'Age-Related Changes in Mouse Taste Bud Morphology, Hormone Expression, and Taste Responsivity', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*. Oxford University Press, 67A(4), p. 336. doi: 10.1093/GERONA/GLR192.

Sigma-Aldrich (2022) *Adenosine 5'-monophosphate sodium salt from yeast, ≥99% | 5'-Adenylic acid | Sigma-Aldrich*. Available at: https://www.sigmaaldrich.com/catalog/product/sigma/a1752?lang=en&region=GB (Accessed: 6 January 2020).

Silvius, J. R. (2003) 'Role of cholesterol in lipid raft formation: Lessons from lipid model systems', *Biochimica et Biophysica Acta - Biomembranes*. Elsevier, 1610(2), pp. 174–183. doi: 10.1016/S0005-2736(03)00016-6.

Sjöstedt, N. *et al.* (2017) 'Interaction of Food Additives with Intestinal Efflux Transporters', *Molecular Pharmaceutics*. American Chemical Society, 14(11), pp. 3824–3833. doi: 10.1021/acs.molpharmaceut.7b00563.

Smith, J. C. (2001) 'The history of the "Davis Rig".', *Appetite*, 36(1), pp. 93–98. doi: 10.1006/APPE.2000.0372.

Soetewey Antoine (2020) *Outliers detection in R*. Available at: https://statsandr.com/blog/outliers-detection-in-r/ (Accessed: 14 June 2022).

Sohi, H., Sultana, Y. and Khar, R. K. (2004) 'Taste Masking Technologies in Oral Pharmaceuticals: Recent Developments and Approaches', *Drug Development and Industrial Pharmacy*. Taylor & Francis, 30(5), pp. 429–448. doi: 10.1081/DDC-120037477.

Soto, J. *et al.* (2015) 'Development of a model for robust and exploratory analysis of the rodent brief-access taste aversion data', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier B.V., 91, pp. 47–51. doi: 10.1016/j.ejpb.2015.01.016.

Soto, J. (2016) *Assessing the feasbility of using an animal model for in vivo taste assessment of pharmaceutical compounds and formulations*. UCL School of Pharmacy.

Soto, J. *et al.* (2018) 'Rats can predict aversiveness of Active Pharmaceutical Ingredients', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier B.V., 133, pp. 77–84. doi: 10.1016/j.ejpb.2018.09.027.

Spector, A. C. and Kopka, S. L. (2002) 'Rats fail to discriminate quinine from denatonium: Implications for the neural coding of bitter-tasting compounds', *Journal of Neuroscience*. Society for Neuroscience, 22(5), pp. 1937–1941. doi: 10.1523/jneurosci.22-05-01937.2002.

Stone, H., Bleibaum, R. and Thomas, H. (2012) 'Descriptive Analysis', in *Sensory Evaluation Practices*. 4th edn. Elsevier, pp. 233–289. doi: 10.1016/B978-0-12-382086-0.00006-6.

Suez, J. *et al.* (2014) 'Artificial sweeteners induce glucose intolerance by altering the gut microbiota', *Nature*. Nature, 514(7521), pp. 181–186. doi: 10.1038/NATURE13793.

Suez, J. *et al.* (2015) 'Non-caloric artificial sweeteners and the microbiome: findings and

challenges', *https://doi.org/10.1080/19490976.2015.1017700*. Taylor & Francis, 6(2), pp. 149–155. doi: 10.1080/19490976.2015.1017700.

Swithers, S. E. and Davidson, T. L. (2008) 'A role for sweet taste: calorie predictive relations in energy regulation by rats', *Behavioral neuroscience*. Behav Neurosci, 122(1), pp. 161–173. doi: 10.1037/0735-7044.122.1.161.

Sylvetsky, A. C. *et al.* (2016) 'Hormonal responses to non-nutritive sweeteners in water and diet soda', *Nutrition & metabolism*. Nutr Metab (Lond), 13(1). doi: 10.1186/S12986-016-0129-3.

Szejtli, J. and Szente, L. (2005) 'Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier, 61(3), pp. 115–125. doi: 10.1016/J.EJPB.2005.05.006.

Szente, L. and Szejtli, J. (2004) 'Cyclodextrins as food ingredients', *Trends in Food Science & Technology*. Elsevier, 15(3–4), pp. 137–142. doi: 10.1016/J.TIFS.2003.09.019.

Tan, V. W. K. *et al.* (2019) 'Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA)', *Food Research International*. Elsevier, 121, pp. 39–47. doi: 10.1016/J.FOODRES.2019.03.019.

Tandel, K. R. (2011) 'Sugar substitutes: Health controversy over perceived benefits', *Journal of Pharmacology & Pharmacotherapeutics*. Wolters Kluwer -- Medknow Publications, 2(4), p. 236. doi: 10.4103/0976-500X.85936.

Temussi, P. A. (2009) 'Sweet, bitter and umami receptors: a complex relationship', *Trends in Biochemical Sciences*. Elsevier Current Trends, 34(6), pp. 296–302. doi: 10.1016/J.TIBS.2009.02.005.

The United States Pharmacopeial Convention (2016a) *Transfer of Analytical Procedures (USP 1224)*. Available at: http://www.ropack.com/wp-content/uploads/c1224.pdf.

The United States Pharmacopeial Convention (2016b) *Validation of Compendial Procedures*. Available at: https://latam-edu.usp.org/wp-content/uploads/2021/08/1225.pdf.

Tiwari, R. V. *et al.* (2017) 'Rat Palatability Study for Taste Assessment of Caffeine Citrate Formulation Prepared via Hot-Melt Extrusion Technology', *AAPS PharmSciTech*, 18(2), pp. 341–348. doi: 10.1208/s12249-015-0447-1.

Toda, J. *et al.* (1985) 'Interaction of cyclodextrins with taste substances', *Proceedings of the 4th International Flavour Conference*, pp. 19–34. Available at: https://agris.fao.org/agris-search/search.do?recordID=US8248478 (Accessed: 9 March 2022).

Tordoff, M. G. (2007) 'Taste solution preferences of C57BL/6J and 129X1/SvJ mice: Influence of age, sex, and diet', *Chemical Senses*, 32(7), pp. 655–671. doi: 10.1093/CHEMSE/BJM034.

Tucker, T. G. H. A. *et al.* (2012) 'Absolute immunoquantification of the expression of ABC transporters P-glycoprotein, breast cancer resistance protein and multidrug resistance-associated protein 2 in human liver and duodenum', *Biochemical Pharmacology*. Biochem Pharmacol, 83(2), pp. 279–285. doi: 10.1016/j.bcp.2011.10.017.

Unitaid (2020) *Innovative Delivery Systems for Technology Landscape*. Available at: https://unitaid.org/assets/Innovative-delivery-systems-for-paediatric-medicines-technology-landscape.pdf (Accessed: 20 March 2022).

Del Valle, E. M. M. (2004) 'Cyclodextrins and their uses: a review', *Process Biochemistry*. Elsevier, 39(9), pp. 1033–1046. doi: 10.1016/S0032-9592(03)00258-9.

Venables, R. *et al.* (2015) 'Determination of formulation factors that affect oral medicines acceptability in a domiciliary paediatric population', *International journal of pharmaceutics*. Int J Pharm, 480(1–2), pp. 55–62. doi: 10.1016/J.IJPHARM.2015.01.023.

Volpe, D. A. (2008) 'Variability in caco-2 and MDCK cell-based intestinal permeability assays', *Journal of Pharmaceutical Sciences*. John Wiley and Sons Inc., pp. 712–725. doi: 10.1002/jps.21010.

Walsh, J. *et al.* (2014) 'Playing hide and seek with poorly tasting paediatric medicines: Do not forget the excipients', *Advanced Drug Delivery Reviews*, 73, pp. 14–33. doi: 10.1016/j.addr.2014.02.012.

Wankar, Jitendra *et al.* (2020) 'Recent Advances in Host–Guest Self-Assembled Cyclodextrin Carriers: Implications for Responsive Drug Delivery and Biomedical Engineering', *Advanced Functional Materials*. John Wiley & Sons, Ltd, 30(44), p. 1909049. doi: 10.1002/ADFM.201909049.

Wee, M., Tan, V. and Forde, C. (2018) 'A comparison of psychophysical dose-response behaviour across 16 sweeteners', *Nutrients*. MDPI AG, 10(11). doi: 10.3390/NU10111632.

Weiffenbach, J. M., Baum, B. J. and Burghauser, R. (1982) 'Taste thresholds: quality specific variation with human aging', *Journal of gerontology*. J Gerontol, 37(3), pp. 373–377. doi: 10.1093/GERONJ/37.3.372.

Whittaker, A. *et al.* (2009) 'Toxic additives in medication for preterm infants.', *Archives of disease in childhood. Fetal and neonatal edition*. BMJ Publishing Group, 94(4), pp. F236-40. doi: 10.1136/adc.2008.146035.

WHO (1998) *Quality control methods for medicinal plant materials World Health Organization Geneva*.

WHO (2007) *Development of Paediatric Medicines: Points to Consider in Formulation. WHO Expert Committee*.

Willia, M. F. *et al.* (1992) 'Influence of gastrointestinal site of drug delivery on the absorption characteristics of ranitidine', *Pharmaceutical research*. Pharm Res, 9(9), pp. 1190–1194. doi: 10.1023/A:1015860007380.

Williams, H. D. *et al.* (2013) 'Strategies to Address Low Drug Solubility in Discovery and Development', *The American Society for Pharmacology and Experimental Therapeutics* , 65(1), pp. 315–499. doi: 10.1124/pr.112.005660.

Wise, P. M., Damani, S. and Breslin, P. A. S. (2019) 'Sodium, but not potassium, blocks bitterness in simple model chicken broths', *Journal of food science and technology*. J Food Sci Technol, 56(6), pp. 3151–3156. doi: 10.1007/S13197-019-03770-1.

Woertz, K. *et al.* (2011a) 'A comparative study on two electronic tongues for pharmaceutical formulation development', *Journal of Pharmaceutical and Biomedical Analysis*. J Pharm Biomed Anal, 55(2), pp. 272–281. doi: 10.1016/j.jpba.2011.02.002.

Woertz, K. *et al.* (2011b) 'Taste sensing systems (electronic tongues) for pharmaceutical applications', *International Journal of Pharmaceutics*. Elsevier, 417(1–2), pp. 256–271. doi: 10.1016/J.IJPHARM.2010.11.028.

Wollmer, E. *et al.* (2022) 'Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines', *Advanced Drug Delivery Reviews*. Elsevier, 181, p. 114084. doi: 10.1016/J.ADDR.2021.114084.

Wooding, S. *et al.* (2006) 'Independent evolution of bitter-taste sensitivity in humans and chimpanzees', *Nature*. Nature Publishing Group, 440(7086), pp. 930–934. doi: 10.1038/nature04655.

Xu, Q. *et al.* (2019) 'Hen protein-derived peptides as the blockers of human bitter taste receptors T2R4, T2R7 and T2R14', *Food Chemistry*. Elsevier, 283, pp. 621–627. doi: 10.1016/J.FOODCHEM.2019.01.059.

Xu, Y. *et al.* (2021) 'An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers', *Advanced Drug Delivery Reviews*. Elsevier, 175, p. 113795. doi: 10.1016/J.ADDR.2021.05.005.

Yamaguchi, S. and Ninomiya, K. (2000) 'Umami and Food Palatability', *The Journal of Nutrition*. Oxford University Press, 130(4), pp. 921S-926S. doi: 10.1093/jn/130.4.921S.

Yamane, M. *et al.* (2021) 'The Provisional No-Effect Threshold of Sugar Alcohols on Oral Drug Absorption Estimated by Physiologically Based Biopharmaceutics Model', *Journal of Pharmaceutical Sciences*. Elsevier B.V., 110(1), pp. 467–477. doi: 10.1016/j.xphs.2020.05.013.

Yu, Z. *et al.* (2022) 'Identification of Oncorhynchus mykiss nebulin-derived peptides as bitter taste receptor TAS2R14 blockers by in silico screening and molecular docking', *Food Chemistry*. Elsevier, 368, p. 130839. doi: 10.1016/J.FOODCHEM.2021.130839.

Zakharova, L. *et al.* (2016) 'Supramolecular strategy of the encapsulation of low-molecular-weight food ingredients', *Encapsulations*. Academic Press, pp. 295–362. doi: 10.1016/B978-0-12-804307-3.00008-9.

Zhang, C. *et al.* (2019) 'Glycated Beef Protein Hydrolysates as Sources of Bitter Taste Modifiers', *Nutrients 2019, Vol. 11, Page 2166*. Multidisciplinary Digital Publishing Institute, 11(9), p. 2166. doi: 10.3390/NU11092166.

Zhang, W. *et al.* (2016) 'The Effects of Pharmaceutical Excipients on Gastrointestinal Tract Metabolic Enzymes and Transporters—an Update', *AAPS Journal*. Springer New York LLC, 18(4), pp. 830–843. doi: 10.1208/s12248-016-9928-8.

Zhou, D. *et al.* (2020) 'Interaction of Organic Anion Transporter 3-Mediated Uptake of Steviol Acyl Glucuronide, a Major Metabolite of Rebaudioside A, with Selected Drugs', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 68(6), pp. 1579–1587. doi: 10.1021/ACS.JAFC.9B05808/ASSET/IMAGES/ACS.JAFC.9B05808.SOCIAL.JPEG_V03.

Zidovetzki, R. and Levitan, I. (2007) 'Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies', *Biochimica et biophysica acta*. NIH Public Access, 1768(6), p. 1311. doi: 10.1016/J.BBAMEM.2007.03.026.

Annexes

1. Ethics approval for human panel discussed in chapter 2

UCL RESEARCH ETHICS COMMITTEE
OFFICE FOR THE VICE PROVOST RESEARCH

16th October 2018

Professor Catherine Tuleu
School of Pharmacy
UCL

Dear Professor Tuleu

Notification of Ethics Approval with Provisos
Project ID/Title: 4612/020: Assessing the taste masking ability of dextrins with model bitter tastants

I am pleased to confirm in my capacity as Joint Chair of the UCL Research Ethics Committee (REC) that your study has been ethically approved by the UCL REC until **16th October 2019** subject to the following provisos which I would be grateful if you could respond to by providing a revised application.

1. It was noted that the Participant Information Leaflet (PIL) was too detailed and technical and should be simplified and proof read before use. It is also variably written in the 3rd person and contains some unnecessary information e.g. about the advertisement email.
2. The Committee asked why, as outlined in Section D5, the data would not be archived for use by other researchers given that the Consent Form states that it will be.
3. The Consent Form should be reformatted before use to include signatures.
4. Section B6: you should make it clear that the information collected so far would need to be used.
5. Section C4 and PIL: There was a discrepancy in relation to payment.
6. Section C5: Please clarify how potential participants will be approached given the text states "An email will
7. Section C8 and PIL: You state that no deception will be used, however the PIL includes a section about why deception is needed. Please clarify.
8. PIL: Change the legal basis for the processing of the data from 'consent' to 'public task' and the payment is mentioned too early.
9. Section E2 and PIL: Is there a way to screen out people who are sensitive so they do not get to the point of vomiting?
10. PIL: It was unclear what participants are allowed to do between tasting sessions. Please clarify.

Ethical approval is also subject to the following conditions:

Notification of Amendments to the Research
You must seek Chair's approval for proposed amendments (to include extensions to the duration of the project) to the research for which this approval has been given. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing an 'Amendment Approval Request Form'
http://ethics.grad.ucl.ac.uk/responsibilities.php

Adverse Event Reporting – Serious and Non-Serious

It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator (ethics@ucl.ac.uk) immediately the incident occurs. Where the adverse incident is unexpected and serious, the Joint Chairs will decide whether the study should be terminated pending the opinion of an independent expert. For non-serious adverse events the Joint Chairs of the Ethics Committee should again be notified via the Ethics Committee Administrator within ten days of the incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Joint Chairs will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Final Report

At the end of the data collection element of your research we ask that you submit a very brief report (1-2 paragraphs will suffice) which includes in particular issues relating to the ethical implications of the research i.e. issues obtaining consent, participants withdrawing from the research, confidentiality, protection of participants from physical and mental harm etc.

In addition, please:

- ensure that you follow all relevant guidance as laid out in UCL's Code of Conduct for Research: http://www.ucl.ac.uk/srs/governance-and-committees/resgov/code-of-conduct-research
- note that you are required to adhere to all research data/records management and storage procedures agreed as part of your application. This will be expected even after completion of the study.

With best wishes for the research.

Yours sincerely

Professor Michael Heinrich
Joint Chair, UCL Research Ethics Committee

Cc: Danielle Andrews

2. **Systematic review discussed in chapter 3**

Bitter-blockers as a taste masking strategy: a systematic review towards their utility in pharmaceuticals.

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Abstract: Acceptable palatability of an oral dosage form is crucial to patient compliance. Excipients can be utilised within a formulation to mask the bitterness of a drug. One such category is the bitter-blockers. This term is used inconsistently within the literature and has historically been used to describe any additive which alters the taste of an unpleasant compound. This review defines a bitter-blocker as a compound which interacts with the molecular pathway of bitterness at a taste-cell level and compiles data obtained from publication screening of such compounds. Here, a novel scoring system is created to assess their potential utility in a medicinal product using factors such as usability, safety, efficacy and quality of evidence to understand their taste-masking ability. Sodium acetate, sodium gluconate and adenosine 5'monophophate each have a good usability and safety profile and are generally regarded as safe and have shown evidence of bitter-blocking in

human sensory panels. These compounds could offer a much needed option to taste-mask particularly aversive medicines where traditional methods alone are insufficient.

Keywords: bitter-blocker**;** taste-modifier; bitter-blocker; excipient; palatability

1 Introduction

Oral dosage forms need to be acceptably palatable for good compliance in any given patient population (Lopez *et al.*, 2015). Palatability is a major component of the 'acceptability' of a formulation, which encompasses a number of attributes the drug possesses, including other organoleptic properties such as smell and mouthfeel. Palatability of a pharmaceutical dosage form is a key influencer in how well patients adhere to their treatment programme (Palmer, 2007). Considerable progress has been made in the furthering of taste masking, not only in the form of excipients but also in novel technologies to deliver a more acceptable product such as the medicated straw which utilises coated beads within a straw for ease of administration(*Pediatric Drug Delivery Systems - Drinking Straw XStraw*, no date) and the use of food and drink as a vehicle to administer liquid formulations (Kytariolos *et al.*, 2013). However, these approaches have limitations; they are not appropriate for all dosage forms and they may require specific storage conditions and be unattractive from a commercial perspective (Lopez *et al.*, 2015).

Techniques are available to taste mask drugs by providing a physical barrier to prevent the bitter active pharmaceutical ingredient (API) interacting with the taste buds, for example, polymer coating or adding cyclodextrins to form inclusion complexes (Del Valle, 2004). This design can limit formulation

options, as encapsulating the API into a solid dosage form can be problematic for the many patient populations that experience problems swallowing medicines (Mennella *et al.*, 2014). Polymer coating may also not be feasible depending on the properties of the API or dose requirements which may make the drug product too large to swallow. The inclusion complex approach also encounters limitations as some compounds will not form interactions with dextrins (Szejtli and Szente, 2005). Alternatively, if inclusion complexes are formed, the encapsulated portion of the API may not be the part that confers bitter taste. These barrier approaches can alter the speed of onset of the drug and can increase production costs of the goods (McGregor, 2007).

The addition of sweeteners and flavouring agents is commonly used to taste mask poorly palatable formulations. This simplistic approach does not always improve the perceived bitterness of highly aversive or highly soluble compounds and these excipients are often used in combination with other taste-masking strategies. This type of taste-masking aims to achieve an 'acceptable' taste profile which can be subjective, especially in children(Mennella and Beauchamp, 2008). Furthermore a number of artificial sweeteners are reported to have an aversive metallic or bitter taste component(Riera *et al.*, 2007) and have shown agonist activity at certain subsets of bitter receptors(Kuhn *et al.*, 2004). An updated approach to taste-masking may be in shifting the aim from a palatable medicinal product to a taste-neutral product by targeting bitter receptors directly. This would overcome the issues associated with personal preferences to flavours and differing perception to sweeteners. This approach would also prevent the creation of an overly palatable and attractive medicine which can lead to

accidental poisoning, especially in children. This taste-neutral approach is supported by current EMA guidelines(EMA, 2013).

Pharmaceuticals can be unpalatable for reasons other than bitterness ; but humans have evolved to recognise bitter tastants as potentially toxic (Wooding *et al.*, 2006) and this causes a major issue with compliance, reducing the oral tolerance for pharmaceuticals. Access to bitter receptor blockers could help create an acceptable product from a previously unpalatable one and thus improve patient compliance. This approach could also reduce industry costs (both financial and time) pre-clinically when tackling the issue of taste-masking. Such bitter-blockers would act at the level of the taste cell in the oral cavity and would act independently of the co-administered compound.

1.1 The human taste pathway

There are five recognised tastes; sweet, sour, umami, bitter and salty. Both sour and salty are mediated by ion channels whereas sweet, umami and bitter tastes are detected by members of two G-protein coupled receptor (GPCR) families; the taste 1 receptor family (TAS1R) and the taste 2 receptor family (TAS2R) (Temussi, 2009). Most bitter tastants are detected by their interaction with TAS2Rs. TAS2Rs are a large family of around 25 GPCRs, many of which can detect a huge variety of bitter molecules. When the receptor is stimulated, the G-protein, gustducin, is activated and stimulates phospholipase C β2 which results in inositoltriphosphate activation mediating a rise in intracellular calcium levels and thus activating transient receptor potential cation channel M5 (TRPM5). The result is membrane depolarisation, generation of an action potential and the release of ATP which then acts on

purinergic receptors activating afferent nerve fibres which in turn activate the appropriate brain centres leading to taste perception (Depoortere, 2014). This pathway is summarised in figure 1.

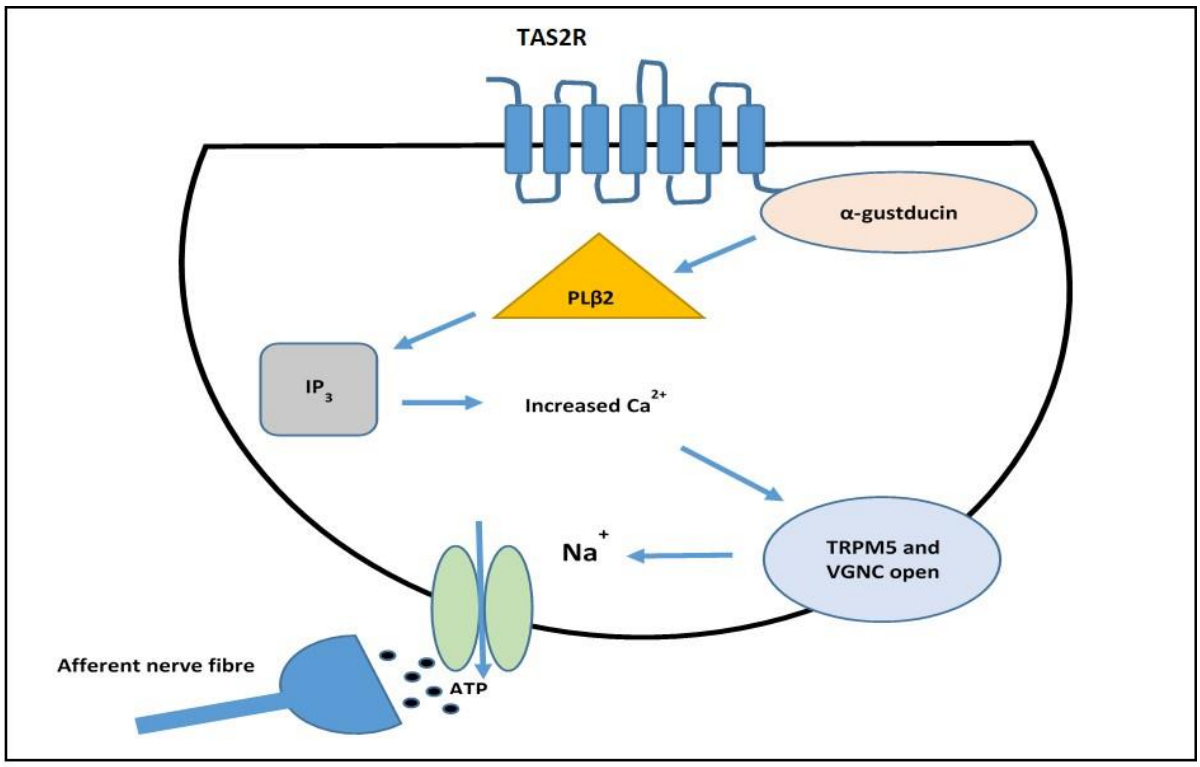

Figure 1 Schematic representation of the human bitter taste pathway. TAS2R; taste 2 receptors, PLβ2; phospholipase C β2, IP_3; inositoltriphosphate, TRPM5; transient receptor potential cation channel M5, VGNC; voltage gated sodium channel.

1.1.1 Genetic diversity and bitterness

Not everyone perceives medicines the same way; nuisances in genetic makeup play a role in the palatability of drugs(Mennella, Mathew and Lowenthal, 2017). TAS2Rs have extensive diversity across human populations around the world(Roudnitzky *et al.*, 2016). Over 150 single-nucleotide polymorphisms have been found in TAS2R coding regions which can result in amino acid substitutions and alter receptor functionality(Kim *et al.*, 2005). Most bitter compounds (including APIs) are perceived as aversive by interaction with a number of TAS2Rs(Roudnitzky *et al.*, 2016). Therefore, a

polymorphism in a specific receptor altering its functionality is unlikely to eliminate the bitter response completely but may result in differences in sensitivity to various agonists. One notable outlier is phenylthiocarbamide (PTC) which is perceived as highly aversive by one bitter-receptor; TAS2R38(*Phenylthiocarbamide (PTC)*, no date). Humans who have a 3 amino acid replacement in their TAS2R38 gene will report PTC as taste-less(Kim *et al.*, 2003). Given this genetic diversity in bitter receptors, it is unlikely that a bitter-blocker will have exactly the same effect in every individual; as is a limitation for traditional taste-masking methods such as sucrose(Mennella *et al.*, 2015) and the artificial sweetener acesulfame-K(Bobowski, Reed and Mennella, 2016) which can be subjective in their bitter-masking efficacy due to genetic diversity of TAS2Rs.

The use of a bitter receptor antagonist will only be effective if the aversive API is hitting this receptor too. The exact role of each subset of bitter receptor, and how they influence taste perception for different medicinal compounds, is not yet fully understood. Databases such as BitterDB(Dagan-Wiener *et al.*, 2019) are beginning to fill these gaps by collating information on bitter molecules and their receptors. As more knowledge is generated, the use of bitter-blocking compounds can be better directed and make the art of taste-masking more precise.

As many API interact with multiple TAS2Rs, a ‘blocker’ acting as a specific bitter receptor antagonist may not mask the aversive taste entirely but is likely to dampen it. This may be sufficient for the API to become palatable enough for improved patient compliance. For highly soluble and aversive API, significant improvement could be achieved using combinations of bitter-

blockers, acting on multiple receptors, or bitter-blockers in conjunction with other taste-masking approaches.

1.2 Bitter-Blocker; an inconsistent term

The screening process for this review highlighted how the term ‘bitter-blocker’ is used inconsistently throughout published literature. It is used synonymously with terms such as ‘taste-modifier’. This review defines bitter-blockers as compounds which modify bitter taste by interacting with the bitter-taste perception pathway in some way, acting at a pharmacological level; interfering with taste receptors or the taste-transduction mechanism. Blocking of bitter taste perception can occur throughout the taste signal cascade**.** A compound can act by directly antagonising bitter taste receptors, preventing the activation of gustducin and inhibiting taste perception. Such compounds are likely to be close structural analogues of bitter compounds enabling them to still bind, allosterically or otherwise to these receptors.

It would be useful if a bitter-blocking molecule was identified that interacts with a multitude of bitter receptors or that interacts with a late stage component of the taste transduction pathway. An ideal site of action for this is the TRPM5 receptor, as shown in figure 1, which facilitates the perception of bitterness to reach the brain. However, TRPM5 receptors also transduce the signals for sweet and umami flavours (Zhang *et al.*, 2003) and a blockade here would abolish these taste sensations leaving only sourness and saltiness to be detected via ion channels. This could result in a more prominent sour or salty taste, albeit less bitter, which could still be aversive to the patient and so little commercial progress has been made to this end.

A number of compounds which have the ability to alter perceived bitterness were compiled by Walsh et al (Walsh *et al.*, 2014), focussing on paediatric medicines. Some of these compounds interact with the bitter-perception pathway as bitter-blocking taste-modifiers but the list also included excipients which were reported to convey bitter suppression due to their sweetener properties, for example neohesperidin dihydrochalcone. This work stated the known limitations of each compound and their regulatory status but did not claim to be an exhaustive list of bitter-blocking agents. In fact, there is no complete review available of all known bitter-blockers nor is there a thorough risk assessment of such compounds for their use in medications. Such an assessment would evaluate their utility as potential excipients by compiling and considering the information known about them in terms of their safety, practical usability and demonstrable efficacy. The compounds can then be assigned a score according to each category to establish their potential. This structured approach would begin to fill the gaps in current knowledge around this taste-masking approach and highlight ways in which bitter-blockers could be applied. Once this knowledge is gathered, medicine regulatory bodies can be consulted to better understand the classification of bitter-blockers in pharmaceutical dosage forms. It is likely that these compounds would still fall under the label ‘excipient’, and not API, even though they act to block bitter receptors; just as is the case for sweeteners which also act on receptors to influence palatability but are still classified as excipients. Furthermore, the world health organisation describes an API as ‘a substance… intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have

direct effect in restoring, correcting or modifying physiological functions in human beings' and bitter-blocking agents do not come under this definition (World Health Organization, 2011).

1.2 Review objectives

The aim of this work is to establish an up to date literature review on bitter-blockers and to evaluate their potential utility as excipients by critically assessing the available information. Improved understanding of the potential benefits and limitations of these compounds would be a useful addition to the formulation toolbox of taste-masking.

The questions this review addresses are; what is the current knowledge of bitter-blockers which act at a molecular level to disrupt bitter perception? How appropriate are these bitter-blockers for use as excipients in medicinal compounds in terms of usability, safety and efficacy? To answer these questions a systematic literature review of bitter-bitter blockers (acting on TAS2Rs) was conducted and a scoring system drawn up to quantitatively assess their practical potential in medicines. This was carried out in accordance with the PRISMA guidelines(Moher *et al.*, 2009).

2 Data search and collection methodology

2.1 Data sources and search strategy

Publications were screened from the following databases; Scopus, PubMed, Embase and Web of Science. All subject areas and years were included in the initial search. The term 'bitter-blocker' is not used consistently in the literature and so search terms included other key words which may have been used synonymously. The search terms selected were; 'taste modifier', 'bitter

blocker', 'bitter antagonist' alongside either 'medicine', 'drug', 'formulation' or 'dosage form'. The search terms were also hyphenated to prevent excluding relevant material.

2.2 Inclusion and exclusion criteria

Duplicate publications were removed before conducting a review of the remaining work according to PRISMA guidelines. Abstracts containing information pertaining to 'taste-modification by bitter blocking' were retained. From here the full texts were screened and excluded if the bitter-blocker was not a compound but a technique, such as hot-melt extrusion, or a genetic modification, for example to bitter receptors. Papers were also excluded if the bitter-blocker did not meet the definition laid out in this review but instead offered sweetening properties or interacted directly with the bitter molecule.

2.3 Criteria of interest for Bitter-Blockers

Utility as a potential excipient can be evaluated by understanding different compound characteristics, namely safety, efficacy (including quality of evidence) and usability.

2.3.1 Safety

Knowing an excipient is safe is vital before use with any medicine to reduce the likelihood of their contribution to adverse events. Adverse events linked to excipients have been reported (Fabiano, Mameli and Zuccotti, 2011) but these tend to occur when excipient levels exceed recommended acceptable daily intake (ADI) (Zajicek *et al.*, 2013). It is now widely understood that many excipients are not inert as was once thought. This means excipients must be deemed safe and supported by robust data. Novel excipients must be subjected to full toxicological evaluation (Food and Drug Administration,

2005). Knowledge of the current regulatory status and any precedence of use of the excipient is also important information.

2.3.1.1 Efficacy

It is key to consider how effective a bitter-blocker has been shown to be in previous research and it is important to understand its mechanism of action. For example, if the compound reduces aftertaste or initial bitterness and to understand in what population it has shown efficacy – bitter blocking abilities have been shown to differ according to age (Mennella *et al.*, 2014). Further understanding of the mechanism of action of a bitter-blocker could help predict how efficacious it is likely to be (Mennella and Beauchamp, 2008) for example, if a specific mechanism is identified, such as the blockade of one or two bitter receptors, this information can be useful to appropriately tailor its use to taste-mask certain bitter substances.

2.3.1.2 Quality of evidence

The models used to demonstrate the efficacy of different bitter-blocking agents vary greatly. The platform used to assess the compounds can be evaluated for reliability and scored according to how rigorous the level of testing was that they received. For example palatability testing can take many forms, the most simple of which are cell-based models (Mohamed-Ahmed *et al.*, 2016) and lipid membrane sensors (Sharma *et al.*, 2015) such as the electronic tongue (E-tongue) (Baldwin *et al.*, 2011). An alternative way to assess the palatability of a given substance is the in-vivo brief access taste aversion (BATA) model which uses the lick response of trained rats to ascertain the aversiveness of a compound compared with bitter controls and

water (Soto *et al.*, 2018). The gold standard test for predicting palatability for human use is human sensory testing (Mohamed-Ahmed *et al.*, 2016). Regardless of the model or methodology used, it is important to highlight any inconsistencies with the research, which may cast doubt over the findings. An example of this is the use of a human panel with a small participant number.

2.3.2 **Usability**

An excipient must be able to be practically used in a formulation intended to be given to patients. As such, it must have good compatibility with potential API (Serajuddin *et al.*, 1999) and should exhibit appropriate stability characteristics. If the excipient needs to be in solution to achieve bitter-blocking action, it should have a reasonable solubility, without the need for a solubilizing aid, in an acceptable vehicle appropriate for dosing (for example not in ethanol) and have acceptable stability in the formulation. Also, the quantities required to produce the desired taste-masking effects would further indicate how appropriate a bitter-blocking excipient would be for use in drug products, especially for use in solid oral dosage form or for children where small volumes of liquid are administered. The nature of its use should be considered; whether it can be incorporated into the dosage form during manufacturing, or if the bitter-blocker must be administered directly prior to taking the medication or if extemporaneous preparation is required. The practical aspects of obtaining the compound must be highlighted, for example if it is readily available or if it requires a number of in-house synthesising steps. It is also important to consider if the compound itself has a taste or smell which could impact its acceptability and use, for example saltiness or sourness.

2.4 Development of a scoring system

In order to best score the bitter-blockers for their potential use as excipients, it is first important to identify the significance of each of the scoring criteria.

2.4.1 Criteria weighting

For an excipient of any sort to be incorporated into a formulation it must be safe, therefore safety is a crucial factor and should be given a weighting reflecting this when scoring. Demonstrable efficacy, including quality of the evidence, is considered equally important as without this the bitter-blocker will not be useful as a component of the formulation toolbox. Usability is the next level down of importance as the use of the bitter-blocker can be tailored according to its characteristics, for example if it does not have long-term stability in solution it could be used for extemporaneous preparation. Such factors may make an excipient less desirable but does not mean they cannot succeed as an excipient given the appropriate conditions. With this in mind when calculating the final score, both safety and efficacy/quality of evidence will carry a weighting of 3 as both are fundamental requirements for use in formulations. Usability will carry a weighting of 2 as this can be tailored.

2.4.2 Scoring each Criteria

Each bitter-blocker will be scored from 0-3 against safety, efficacy (including quality of evidence) and usability (table 1). The score for each criterion will reflect the nature of the information available but also highlight any gaps in information. A score of 3 implies complete information is available on the bitter-blocker’s safety/efficacy/usability and that the evidence suggests excellent characteristics.

Table 1 Scoring criteria for bitter-blockers

	Score of 0	Score of 1	Score of 2	Score of 3
Safety	Evidence of a hazardous nature in low/ efficacious concentrations OR No information available on safety	Incomplete or little information is known on the safety of the compound OR No information found but is a close structural analogue of another compound with 'Generally Regarded as Safe' (GRAS) status	The compound is deemed safe for example has GRAS status OR Has a known ADI that exceeds the efficacious dose OR Is found in the human diet with no concern highlighted on its use although it may be associated with allergies in some patients so requires strict labelling OR Is patented for human use	The compound is deemed safe (for example has GRAS status) AND Not associated with allergies in patients AND one of the following Is patented use for humans OR Has a known ADI that exceeds the efficacious dose, or regulatory bodies have stated there is no limit to the ADI OR Is found in the human diet with no concern highlighted on its use OR The compound has recent precedence for use in human consumables and/or pharmaceuticals
Efficacy and QoE	No demonstrable efficacy shown as a bitter blocker OR	Efficacy shown in a cell-based model expressing a limited number of receptors (which gives no context to its action) or using a sensor technology (which has limitations (Haraguchi *et*	Demonstrates effective transient bitter blocking against one compound which has been demonstrated in a human panel of at least n=8	Demonstrates effective transient bitter blocking against more than one compound with efficacy demonstrated in human sensory panels of sufficient sample size, at least n=8 (unpublished in

	Study demonstrating efficacy has inconclusive or unreliable results	*al.*, 2016) and also gives limited context) OR Transient bitter blocking shown in an animal model (e.g. BATA) against one compound OR Efficacy shown against one bitter compound in a human panel of insufficient number (n<8)	OR Demonstrates effective transient bitter blocking against more than one compound which has been demonstrated in a model that does not involve humans (n>8) or in a human panel of insufficient participant number (n<8)	house data shows n=8 to be sufficient to distinguish between low and high levels of bitterness)
Usability	No known information on stability/ solubility AND/OR Toxic at doses required for bitter-blocking AND/OR Not available to purchase AND/ OR More than three limitations to its use, for example, specific storage is required/ it is only suitable for extemporaneous preparations/ it has a flavour in itself which may	Poor compatibility with API identified or likely to occur AND/OR Solubility either - inappropriate (e.g. only soluble in ethanol/ DMSO) OR - only partially soluble in water OR - poor solubility; too low to convey efficacy if an efficacious concentration has been demonstrated in a human panel or, if no efficacious concentration is known, requires more than 30mL of solvent to dissolve 1g i.e. less soluble than 33.33mg/mL (*Solubility Information Sigma-Aldrich*, no date)	Acceptable solubility; either exceeding that required for efficacy in humans or, if not known, above 33.33mg/mL AND Acceptable stability of at least 3 months (regardless of storage condition required e.g. refrigeration) AND No demonstrable evidence of its ease of use in humans AND Readily available to purchase	Demonstrable ease of use in humans, for example if it is required to be in solution for efficacy, publications report it solubilised in appropriate media and could be administered in sensible quantities (for example 5mL total volume if administered to children (Batchelor and Marriott, 2015) or 10mL if administered to adults (Mohamed-Ahmed *et al.*, 2016)) AND Solubility exceeding that required for efficacy in humans, or if unknown, above 33.33mg/mL and stable for at least 3 months at room temperature AND

	be aversive to some patients/ it is expensive to purchase	AND/OR Stability either - specific time period of stability unknown OR - not stable in solution for at least 3 months (regardless of storage conditions required, e.g. refrigeration) AND/OR Not available to purchase readily/ requires a number of synthesising steps AND/OR This compound has up to three limitations to its use; for example, specific storage is required/ it is only suitable for extemporaneous preparations/ it has a flavour in itself which may be aversive to some patients/ it is expensive to purchase	AND The compound may have up to two additional requirements that limits its use in some way. For example, storage; refrigeration may be necessary, or the compound may only be appropriate for extemporaneous preparations or it may have its own flavour/taste that could be aversive to some patients (e.g. sour)	No aversive taste potential; it is either tasteless or pleasant tasting AND Readily available to purchase. AND The compound has no additional limitations to its use

The score assigned to the bitter-blocker for each criterion will be multiplied by the weight of that category (example in figure 2). This scoring system will be used to assign a mark to each of the bitter-blockers by a number of independent assessors, 3 from academia and 3 from pharmaceutical companies.

Assessing an example taste-modifier; compound A

Known safety information: compound A has GRAS status but has no known ADI nor is it found in the human diet or patented for human use

i) Safety; scores 2 (weighting x3) = 6

Known efficacy information and the QoE: compound A has been shown to reduce the bitter perception of a range of different API using an animal model of palatability (n=12)

i) Efficacy and QoE; scores 2 (weighting x3) =6

Known usability information: compound A needs to be in solution to elicit bitter blocking of target receptors however it is not stable in suitable media for long periods of time and needs to be kept in the fridge, it is also unavailable to purchase

i) Usability; scores 1 (weighting x2) = 2

Total score = 14 (possible highest score is 24)

Figure 2 Worked example of scoring an example bitter-blocker against the three criteria using the different weightings. QoE; quality of evidence

2.4.3 Scoring limitations

The compounds identified had differing amounts of information available about them and this review leveraged information from many sources, depending on the available evidence. This is most apparent when reviewing the level at which the bitter-blocker has been assessed; some have been assessed in paediatric panels whereas others have been assessed in adult panels or in non-human models. In reality, the target patient population's age will affect the safety parameters and efficacy. For example, if the excipient is intended for use in children, but the information regarding safety is only available in adults then further testing would be required for these patients who have underdeveloped organs and a more limited metabolism (Bearer, 1995). This is particularly crucial for neonates who may require a new formulation altogether or a tailored dilution. Safety of excipients is of the

utmost priority across all patient populations but their inclusion in paediatric medicines require further risk assessment focusing on any potential age-related safety concerns (Carter, 2011). Also if a compound has shown bitter blocking ability in adult sensory panels, it will not necessarily confer the same affect in children as demonstrated by Mennella et al (Mennella *et al.*, 2014). However, if a bitter-blocker does work in children, it is likely to work in adults as children are more sensitive to bitter taste. Children and adults have different preferences, for example children prefer salty solutions (Mennella, Pepino and Beauchamp, 2003) so this could also affect their usability.

In this review, two separate tables of results are presented. The scores of bitter-blocking agents which have shown efficacy in a human panel are drawn up separately to those where an alternative method of assessment was used. This is to draw attention to compounds which have been assessed more rigorously but which may fall down due to other reasons of safety or practical use compared with non-proven bitter-blocking compounds which might show potential but about which relatively little is known and evidence is only based on non-human methods of evaluation.

Those assessed using a human panel (of sufficient participant number) will be given the highest score for quality of evidence regardless of the age of the panel, but the patient population investigated will be stated and the safety scores will reflect the age. For example, if assessed in children it is important to know the safety for children. Furthermore, the results from the different human panels were expressed in a number of ways; for example, some gave a percentage inhibition of bitterness, some just quoted a significant reduction in bitterness and importantly the human studies used various concentrations

of bitter controls (for example quinine). To avoid penalising studies which may have used a higher concentration of quinine or to unfairly reward those which did not detail the exact percentage inhibition, a score of 3 was given for efficacy if the human panel reached the criteria for participant number and demonstrated significant bitter blocking - regardless of how this data is presented.

With regards to safety, it is important to note that, as with any novel excipient, bitter-blockers would need to undergo a full battery of safety assessments before being considered for use in a pharmaceutical formulation. This is especially important due to their functional role as receptor blockers.

2.4.4 Reducing bias

In order to reduce bias in this work, the initial scoring system was drawn up before the literature search. The risk of bias associated with the literature screen were minimised by setting inclusion criteria (figure 3) beforehand. After the literature search was conducted and the bitter-blockers identified, each assessor marked and ranked the compounds independently according to their interpretation of the scoring system. The scoring system was then only updated to improve clarity if there were discrepancies in interpretation.

3 Literature screen results

From the literature screen, 21 papers were identified which met the inclusion criteria(Katsuragi *et al.*, 1995; Ming, Ninomiya and Margolskee, 1999; Keast and Breslin, 2002; Nakamura *et al.*, 2002; Mennella, Pepino and Beauchamp, 2003; Ogawa *et al.*, 2004; Ley *et al.*, 2005; Danilova and Hellekant, 2006;

Maehashi *et al.*, 2008; Palmer *et al.*, 2010; Slack *et al.*, 2010; Fletcher *et al.*, 2011; Nicole J. Gaudette and Pickering, 2012; Wilken and Satiroff, 2012; Sharafi, Hayes and Duffy, 2013; Roland *et al.*, 2014; Mennella *et al.*, 2014; Pydi *et al.*, 2015; Patron *et al.*, 2016; Shiraishi *et al.*, 2017; Sotoyama *et al.*, 2017). The rationale for the studies included are laid out in figure 3 according to PRISMA guidelines(Moher *et al.*, 2009).

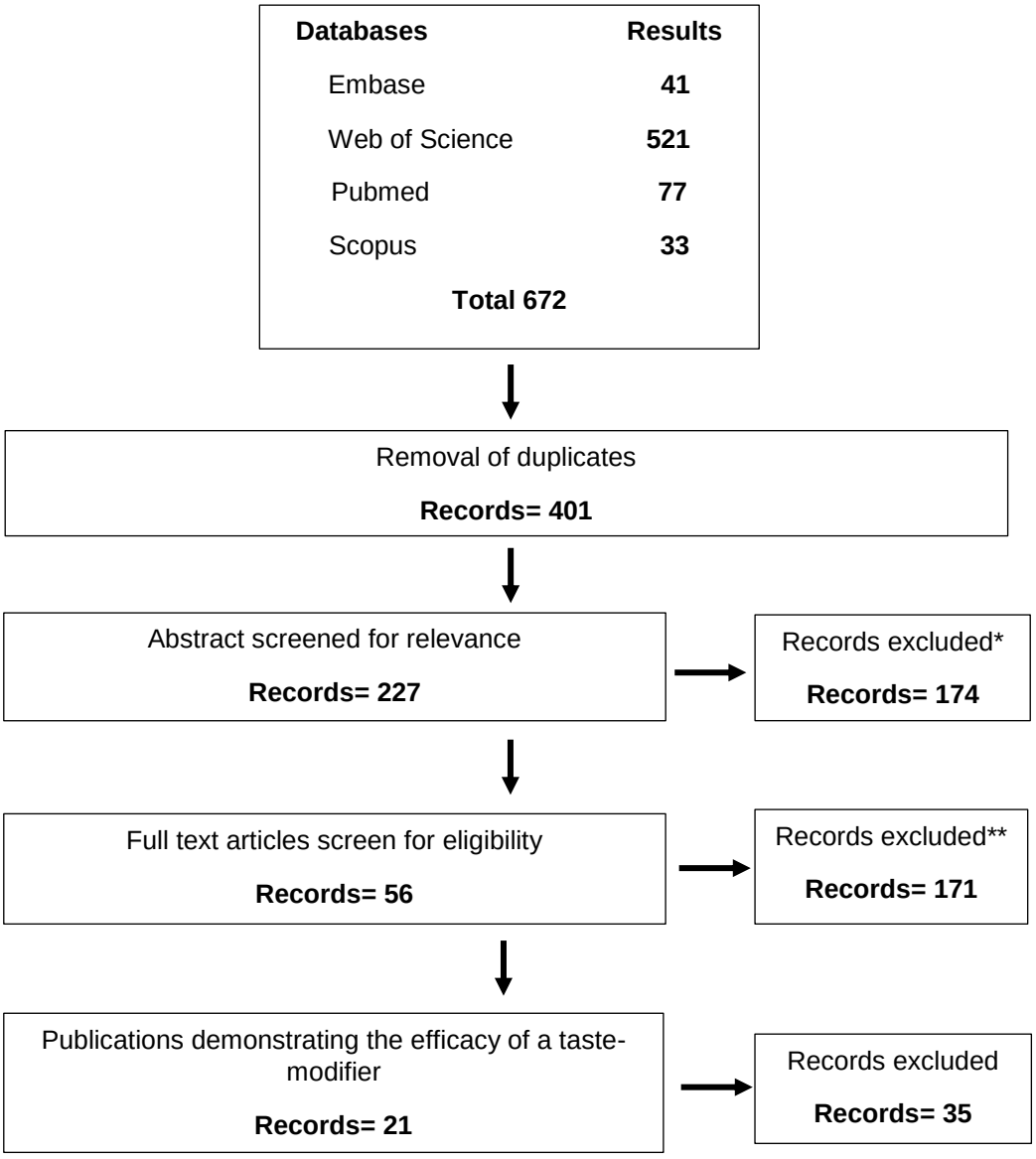

Figure 3 Literature search for bitter-blockers. *Exclusion criteria for abstracts; papers were excluded at this stage if there was no relevant mention of taste-modification by bitter blocking. **Exclusion criteria for full text articles included the bitter-blocking being due to genetic modification of a model, or was describing a technique not a compound that conferred bitter-blocking or inappropriate use of the term bitter-blocker

Two tables of bitter-blockers were compiled, one containing those compounds assessed in human sensory panels (table 2a) and those assessed using other methods (table 2b). Compounds were grouped by class, for example salts.

Table 2a. bitter-blockers extracted from the literature review which were tested in a human panel

Bitter-blocker and level of assessment	Mechanism of Action	Safety information	Demonstration of efficacy	Usability
Acids				
• **Citric acid** **Citric acid if found in citrus fruit** (Sotoyama *et al.*, 2017) **Assessed using a human panel** (Sotoyama *et al.*, 2017)	Calcium imaging has shown citric acid to be an antagonist at TAS2R16 (Sotoyama *et al.*, 2017).	Citric acid is found naturally in human consumables such as citrus fruits and is often added to food, beverages and drug formulations to adjust pH (Sotoyama *et al.*, 2017) There is no defined limit to daily intake (*WHO Food Additives Series 5*, no date) because it is safe and abundant in the human diet It has GRAS status.	In a human sensory panel of 11 adults, a dispersible tablet containing 2.5% (0.3M/ 57mg/mL, pH 1.84) citric acid supressed the bitterness and improved the palatability of olopatidine hydrochloride (Sotoyama *et al.*, 2017)	Citric acid has good solubility in water (592mg/mL) and good stability in solution (> 1 year) (*Citric Acid Assay Kit*, no date). The dry material is moisture sensitive (*Citric acid - PubChem*, no date) Commercially available from Sigma 2.5% citric acid would convey the same sourness as a carton of grapefruit juice (Penniston *et al.*, 2008)
Overall score = 19 Limitations; sourness may be an issue for some patient populations		**Safety score = 3** (found in human diet and GRAS)	**Efficacy/QoE score= 2** (only tested against one bitter compound in a human panel)	**Usability score = 2** (sourness may be an issue for some patients)

• **AMP** **(Adenosine 5' monophosphate); nucleotide found in RNA** **Assessed as NaAMP in human panel** (Keast and Breslin, 2002) **and as AMP in both *in vivo* and *ex vivo* assessment** (Ming, Ninomiya and Margolskee, 1999)	AMP acts on peripheral taste inhibition. The glossopharyngeal nerve innervates taste receptor cells in the tongue and is responsive to bitter stimulus. 0.1mM AMP significantly inhibited the nerve responses to bitter compounds such as quinine and denatonium benzoate. It is thought AMP may alter the receptor G-protein coupling and act as a taste modifier here (Ming, Ninomiya and Margolskee, 1999)	AMP is found in many foods and is found in breast milk (Walsh *et al.*, 2014) It has GRAS status for use in food and drinks and oral pharmaceutical dosage forms (Products, 2004) AMP is patented for use in human consumables and pharmaceuticals (Margolskee and Ming, 2003) AMP has no precedence in pharmaceuticals	Human panel of 14 adults; 20mM NaAMP (7.4mg/mL) in pH 5 deionised water, on average, reduced the bitter perception of the following bitter compounds by 67%; 10mM pseudoephedrine, 4mM ranitidine, 50mM acetaminophen, 0.1mM quinine and 1.2M urea. This study did not give the bitter inhibition results for individual pharmaceuticals (Keast and Breslin, 2002) G-protein activation assay using bovine taste cell membranes; AMP (0.01 - 5mM) dose-dependently inhibited transducin activation by bitter compounds. 2.5 mM AMP inhibits activation of transducin by 5mM denatonium benzoate and 1 mM quinine(Ming, Ninomiya and Margolskee, 1999) Mouse two-bottle preference test; AMP had an inhibitory effect on the bitter perception of concentrations up to 5mM denatonium benzoate and 10 mM quinine. AMP was effective at 0.5 mM for quinine and 1 mM for denatonium benzoate(Ming, Ninomiya and Margolskee, 1999)	Solubility in water of 100 mg/mL (Sigma-Aldrich, 2022) which exceeds efficacious concentration AMP is stable if refrigerated at 4°C; it maintains its initial concentration after 25 weeks of storage. If exposed to room temperature, AMP solution will begin to degrade after a few days (Martínez-García *et al.*, 2002) Commercially available from Sigma Has a savoury taste (Fuke and Shimizu, 1993)

Overall score = 22 Limitations; requires refrigeration and has a slight savoury flavour		**Safety score = 3** (GRAS and in the human diet)	**Efficacy/QoE score= 3**	**Usability score = 2** (requires refrigeration and may have limited use due to its umami flavour (Yamaguchi and Ninomiya, 2000))
Flavanoids				
• **Homoeriodictyol (HED) sodium salt is extracted from the North American Herba Santa shrub (Eriodictyon californicum)** **Homoeriodictyol sodium salt was assessed in human panels** (Ley *et al.*, 2005; Nicole J. Gaudette and Pickering, 2012)	Homoeriodictyol sodium salt does not affect other taste sensations such as sweet or salty. It partially blocks bitter reception for a wide variety of bitter tastants. It is likely to bind allosterically to a site common to all bitter receptors, alternatively it is possible it blocks one bitter receptor subtype which many bitter tastants have affinity for (Ley *et al.*, 2005)	Hydroxyflavanones and their salts have been patented for their use in foods and pharmaceuticals for reducing bitter/metallic tastes (Ley *et al.*, 2014). At present, there is no precedence for pharmaceutical use. Homoeriodictyol sodium salt has GRAS status (European Food Safety Authority, 2010)	In a human sensory panel of 8 people homoeriodictyol sodium salt (0.31mM) reduced the perceived bitterness of 2.58mM caffeine by around 45%. HED decreased the perceived bitterness of a range of bitter tastants with various structures (guaifenesin, paracetamol, quinine, denatonium benzoate, salicin and amarogentin). The concentration of homoeriodictyol necessary to reduce the bitter intensity of each tastants varied from 0.31 to 0.77mM (Ley *et al.*, 2005) In a separate human panel (n=12), 0.31mM homoeriodictyol sodium salt reduced the perceived bitterness of 1.1mM caffeine and 6.2mM (+)-catechin by 15% and 33% respectively (Nicole J. Gaudette and Pickering, 2012)	Homoeriodictyol has a 0.34g/L (1.05mM) water solubility (*FooDB, Homoeriodictyol, sodium salt*, no date) which exceeds the efficacious dose and has good stability up to 40 degrees centigrade (*homoeriodictyol safety data sheet*, no date) however the length of time HED sodium salt is stable for is unknown Homoeriodictyol sodium salt is extracted from Herba Santa. Herba Santa can be obtained from suppliers (Ley *et al.*, 2005). Homoeriodictyol sodium salt itself is not commercially available
Overall score = 20 Limitations; not available to purchase		**Safety score = 3** (patented for human consumption and GRAS)	**Efficacy/QoE score= 3**	**Usability score = 1** (not available to purchase and actual stability unknown)

• **2,4-dihydroxybenzoic acid vanillylamide is a close structural analogue of homoeriodictyol** **2,4-dihydroxybenzoic acid vanillylamide was assessed in a human panel** (Ley *et al.*, 2006)	Unknown but likely to be similar to HED sodium salt and potentially bind allosterically to a site common to all bitter receptors, alternatively it is possible it blocks one bitter receptor subtype which many bitter tastants have affinity for (Ley *et al.*, 2005)	No safety information found although a close structural analogue of homoeriodictyol which is safe	In a human sensory panel (n≥10), 2,4-Dihydroxybenzoic Acid N- (4-Hydroxy-3-methoxybenzyl)-amide (also known as 2,4-Dihydroxybenzoic acid vanillylamide) showed dose-dependent activity as an inhibitor of the bitter taste of 2.6mM caffeine solution. At 0.017mM the compound inhibited caffeine's bitterness by around 12% and at 1.7M it inhibited caffeine's bitterness by around 40%. 0.017mM 2,4-dihydroxybenzoic acid vanillylamide also suppressed the bitterness of 0.015mM quinine by around 25%	No solubility or stability data found Not found to be available commercially. Can be synthesised (Ley *et al.*, 2006)
Overall score = 12 Limitations; not available to purchase and lack of information available		**Safety score = 1**	**Efficacy/QoE score= 3**	**Usability score = 0** (not available to purchase and no stability/ solubility data available)
• **Jaceosidin and sakuranetin are isolated from rice leaves** (Kodama *et al.*, 1992) **Assessed in a small human panel with inconclusive results** (Fletcher *et al.*, 2011)	In vitro assays show the flavanones jaceosidin and sakuranetin to be antagonists at TAS2R31 (Fletcher *et al.*, 2011)	Sakuranetin is reportedly harmful (*Sakuranetin - PubChem*, no date) as is jaceosidin due to its effect on cell apoptosis (*Jaceosidin - Cayman Chemical*, no date)	A panel of 4 tasters reviewed the effect on palatability of 1% sakuranetin dissolved in ethanol on the palatability of acesulfame K – results were documented as 'inconsistent' and due to lack of aqueous solubility no full results could be	Sakuranetin has very poor aqueous solubility (109.2mg/L) (*sakuranetin - TGSC*, no date) and jaceosidin is only soluble in DMSO and ethanol (*Jaceosidin - Cayman Chemical*, no date)

			drawn (Fletcher *et al.*, 2011)	These compounds may be harmful at doses required for bitter-blocking
Overall score = 0 Limitations; unsafe and only soluble in ethanol/DMSO		**Safety score = 0** (unsafe substance)	**Efficacy/QoE score= 0** (inconclusive results)	**Usability score = 0**
• **GIV3727 (4-(2,2,3-trimethylcyclopentyl) butanoic acid)** **Assessed using HEK293 cells expressing various receptor subtypes and human sensory panel** (Slack *et al.*, 2010)	GIV3727 it thought to be an insurmountable antagonist at the orthosteric binding site of TAS2R31.This mechanism of action may result in GIV3727 having a slow release profile which could render it unacceptable for use in drug products unless this proves to be a short lived effect. Molecular modelling suggests Lys^{265} in helix 7 of both hTAS2R31 and hTAS2R43 is important for GIV3727's action (Slack *et al.*, 2010).	It is currently used as a flavouring and is patented for use to rectify off-tastes (Ungureanu *et al.*, 2008). GIV3727 has no assigned ADI as it is deemed safe at levels used as a flavouring agent (*4-(2,2,3-Trimethylcyclopentyl)butanoic acid - PubChem*, no date) and it has GRAS status (*4-(2,2,3-TRIMETHYLCYCLOPENTYL)BUTANOIC ACID | FEMA*, no date) GIV3727 has no precedence in pharmaceuticals	In human sensory panel of 50 people, 150μM (0.03mg/mL) GIV3727 added to 2mM acesulfame K or to 2mM saccharin significantly reduced the perceived bitterness compared to control whilst having no effect on perceived sweetness of these sweeteners or of sucrose (Slack *et al.*, 2010) In vitro, GIV3727 inhibits activation of six subtypes of TAS2Rs (TAS2R4, 7, 31, 40, 43 and 49) (Slack *et al.*, 2010)	GIV3727 is very expensive even in low quantities (around £20,000 per gram) GIV3727 is stable in methanol/DMSO/ ethanol for over 2 years (*4-(2,2,3-Trimethylcyclopentyl)butanoic Acid - Cayman Chemical*, no date). If this is evaporated off, GIV3727 can be resupsended in PBS; the supplier's information states that GIV3727 is soluble in PBS, pH 7.2 up to 0.25mg/mL, these solutions can only be kept for a short period of time (no longer than a working day) due to oxidation. Therefore, GIV3727 is not stable in suitable media GIV3727 in methanol must be stored at -20 degrees centigrade

				GIV3727 is commercially available from cayman chemical
Overall score = 20		**Safety score = 3** (patented and GRAS)	**Efficacy/QoE score= 3**	**Usability score = 1** (expensive to purchase, required specific storage and not stable in an appropriate media; could have use for extemporaneous preparations)
Lipoproteins • **Lipoprotein mixture, composed of phosphatidic acid (PA) and beta-lactoglobulin (LG)** **Assessed using a human panel** (Katsuragi *et al.*, 1995)	It is thought that PA-LG acts primarily on bitter taste-receptors directly. When PA-LG is given alone, any subsequent administration of a bitter compound is perceived as more palatable than the control (Katsuragi *et al.*, 1995).	PA originates from soybeans and LG originates from milk and are safe. PA-LG complexes are held by hydrophobic interactions and hydrogen-binding and hence can be hydrolysed in the digestive system easily (Katsuragi *et al.*, 1995). Other combination fatty acids (FA) are patented for their use in food and drink. Linoleic acid and heptanoic acid in combination are used reduce the bitter off-taste of artificial sweeteners in beverages (Aglione *et al.*, 2014). At present, there is no precedence for pharmaceutical use.	In a human sensory panel, (n=8-10) 0.85% PA (12.6mM) + 2.15% LG (0.584mM) in 5mL water selectively and reversibly inhibited bitter perception of 12 compounds, in particular basic and hydrophobic substances. PA-LG reduced the bitter perception of 5mM promethazine and 10mM propranolol to almost zero and greatly reduced that of 50mM caffeine and 0.5mM quinine. PA-LG complex did not affect perception to salty or sweet stimulus (Katsuragi *et al.*, 1995)	This phospholipid-protein complex can be made by suspending PA and LG in water and homogenizing. The homogenate could then be freeze dried and the powder at 3% can be dispersed in 5mL deionised water (pH 5-7) (Katsuragi *et al.*, 1995). Both PA and LG, in the powder form, have stability of over 1 year but in solution they have poor stability PA and LG are both available from Sigma

		Potential allergens are associated with PA-LG so may limit its utility (Savage *et al.*, 2007)		
Overall score = 17 Limitations; not suitable for those with egg or milk allergies and used for extemporaneous preparation		**Safety score = 2** (found in human diet and FAs have been patented for human use however it is associated with allergies in some patients)	**Efficacy/QoE score= 3**	**Usability score = 1** (not reported to fully dissolve in solution but is dispersed so may be used for extemporaneous preparation)
• **Phosphatidic acid (PA) alone** **Assessed using a human panel** (Nakamura *et al.*, 2002)**.**	Phosphatidic acid adsorbs to the bitter compound but mostly acts directly on bitter receptors (Nakamura *et al.*, 2002)	As above. Caution for use in milk allergy sufferers	14.8 mM PA supressed the bitter perception of 0.1 mM quinine in a human panel of 11 people by around 80% (Nakamura *et al.*, 2002)	PA is not soluble in water but is dispersed and it does not have good stability in solution
Overall score = 14 Limitations; not suitable for those with milk allergies and used for extemporaneous preparation		**Safety score = 2** (as above)	**Efficacy/QoE score= 2** (efficacy shown against one bitter compound)	**Usability score = 1**
• **Riboflavin-Binding Protein (RBP)** **Riboflavin-binding protein is isolated**	RBP has been shown to bind to quinine by hydrophobic interactions to supress its bitterness but there is	Found in the human diet (it is in egg whites at 0.09%)(Maehashi *et al.*, 2008)	In a human sensory panel of 4 participants, 0.2mM RBP decreased the bitterness of 0.125mM quinine by almost 100%	RBP has poor solubility in water of 1mg/mL (0.03 mM) RBP powder is commercially

from chicken egg (*Riboflavin Binding Protein from chicken egg white lyophilized powder* | *Sigma-Aldrich*, no date) **RBP was assessed in a human panel** (Maehashi *et al.*, 2008)	evidence to suggest RBP directly antagonises a number of bitter receptors (it is not known which ones) due to RBP's bitter supressing action of structurally different tastants (Maehashi *et al.*, 2008).	No current use in pharmaceuticals Unsuitable for those with egg allergies	(Maehashi *et al.*, 2008)	available from Sigma RBP has been shown to inhibit sweetness from some proteins which may limit its use (Maehashi *et al.*, 2006).
Overall score = 11 Limitations; poor usability		**Safety score = 2** (found in human diet but associated with egg allergy)	**Efficacy/QoE score= 1** (insufficient sample size and only one bitter substance tested)	**Usability score = 1** (solubility is insufficient for efficacy and can inhibit sweetness)
Sodium salts • **Sodium gluconate** **Assessed in paediatric sensory panels** (Mennella, Pepino and Beauchamp, 2003; Mennella *et al.*, 2014)	Sodium ions are thought to act on specific bitter receptors directly. The exact mechanism is unknown, sodium may shield the receptor proteins, modulate ion channels or act on second messenger systems (Keast and Breslin, 2002). It is not a universal bitter receptor blocker or modulator as its influence on compounds differs (Kroeze and Bartoshuk, 1985; Breslin and Beauchamp, 1995)	Sodium gluconate is generally regarded as safe for use in pharmaceuticals and foods (FDA, 2018). There are some concerns about exposing patients to excess sodium but 2mL of 0.3M sodium gluconate provides approximately 14mg sodium, the daily limit for children has been recommended to be 1,500mg per day and 2,300mg for adults (Health.gov, 2015) so this quantity is unlikely to be an issue with regulatory bodies Sodium containing compounds have been patented for bitterness inhibition in pharmaceuticals	In a paediatric sensory panel of 41 children, ages 7-10, 2mL 0.3 M sodium gluconate improved the perceived palatability of 0.5 M urea in 70% of the children. There was no difference in the palatability of urea + salt compared to salt alone. 0.3 M sodium gluconate improved the perceived bitterness of 0.08M caffeine in 68% of the children but this solution was perceived as more bitter than the salt alone. Children also ranked sodium gluconate as equally preferable to water (Mennella, Pepino and Beauchamp, 2003) In another paediatric panel of 154 children 0.3M sodium gluconate reduced bitter perception of 0.119mM quinine	The solubility of sodium gluconate in water is approximately 600mg/mL (2.75M) at 25°C (Pedrosa and Serrano, 2000) Sodium gluconate is reportedly very stable, especially in water (Prescott *et al.*, 1953) and has been demonstrated to be stable for at least 3 months as part of a medicinal cream (Jungbunzlauer, 2022) Sodium gluconate is commercially available from sigma The salty flavour may be off-putting for some patient populations such as adults – children do not find the saltiness

		(Hikaru and Akiko, 2003)	(Mennella *et al.*, 2014)	aversive (Mennella, Pepino and Beauchamp, 2003)
Overall score = 22 Limitations; saltiness may be aversive		**Safety score = 3**	**Efficacy/QoE score= 3**	**Usability score = 2** (salty flavour may be aversive to some patients)
• **Sodium chloride + L-Arg** **Assessed in human sensory trial** (Ogawa *et al.*, 2004)	See above	Both components have GRAS status (FDA, no date; FEMA, no date). 5mL of 30mM sodium chloride provides approximately 3.5mg sodium Sodium containing compounds have been patented for bitterness inhibition in pharmaceuticals (Hikaru and Akiko, 2003).	Sodium chloride (30 mM) in combination with 2.87mM L-Arg has also been shown to be effective in human sensory trials, the number of participants was 6 per group, against a number of bitter tastants including quinine at 0.1mM. All samples were in 5mL (Ogawa *et al.*, 2004). However, NaCl is perceived as saltier and more aversive than sodium gluconate and so less preferable (Breslin and Beauchamp, 1995)	Good solubility in water (PubChem, no date c) and individual components have good stability (Science Direct Topics, no date)(PubChem, no date c) Components are commercially available from sigma Sodium chloride has a salty flavour (Smith and van der Klaauw, 1995)
Overall score = 19 Limitations; saltiness may be aversive		**Safety score = 3**	**Efficacy/QoE score= 2** (insufficient sample size)	**Usability score = 2** (salty flavour may be aversive to some patients)
• **Sodium acetate** **Assessed in human sensory trials** (Keast	See above	GRAS status (FDA, 2018).	100mM sodium acetate reduced the bitter perception of a range of bitter	Good solubility in water (PubChem, no date b), 246.1g/L (3M)(Chemical

and Breslin, 2002; Sharafi, Hayes and Duffy, 2013)		10mL of 100mM sodium acetate provides approximately 23mg sodium Sodium containing compounds have been patented for bitterness inhibition in pharmaceuticals (Hikaru and Akiko, 2003)	pharmaceuticals, including 0.1mM quinine and 1.2M urea, by 55% on average in a human sensory panel of 14 participants, the solutions were in 10mL (Keast and Breslin, 2002) 1.33M sodium acetate also reduced the perceived bitterness of a range of green vegetables by 42% in a human panel of 37 people (Sharafi, Hayes and Duffy, 2013)	Book, no date) and good stability for up to 1 year in solution (Cayman Chemical, 2022) Commercially available from sigma Sodium acetate has a mild salty flavour (Henney, Taylor and Boon, 2010)
Overall score = 22 Limitations; saltiness may be aversive		**Safety score = 3**	**Efficacy/QoE score= 3**	**Usability score = 2** (salty flavour may be aversive to some patients)

Table 2b. bitter-blockers extracted from the literature review which were tested using a non-human method

Bitter-blocker and level of assessment	Mechanism of Action	Safety information	Demonstration of efficacy	Usability
Acids				
• **Chlorogenic acid** **Chlorogenic acid is an ester of caffeic acid and quinic acid. It is found in many fruits, vegetables and**	The inhibition of taste sensor outputs is thought to occur mainly via chlorogenic acid acting on the surface of taste sensor membrane and competing with the API	Chlorogenic acid is abundant in the human diet and is GRAS. It is found in coffee at approximately 0.2-0.6mg/mL (0.56-1.69mM) (Mikiko and Mutsuko, 2008). The concentration shown to be	Taste sensor outputs of bitter basic drugs tested at 0.5mM were significantly reduced by the addition of 0.1, 0.5, 1.0 mM chlorogenic acid dose-dependently. 0.5mM chlorogenic acid decreased the	Chlorogenic acid as a crystalline solid is stable for at least two years but it is recommended that the solution is not kept for more than one day due to poor stability (Cayman

coffee (Walsh *et al.*, 2014) **Assessed using an artificial-lipid membrane taste sensor; SA402B** (Shiraishi *et al.*, 2017)	(Shiraishi *et al.*, 2017)	effective was well within this range Caffeic acid and its salts are currently patented as bitterness inhibitors in food – masking the bitter aftertaste of artificial sweeteners (Walsh *et al.*, 2014). At present, there is no precedence for pharmaceutical use	sensor output for these drugs by up to 46.0 ± 3.6% The sensor outputs for bitter acidic drugs tested at 0.8mM were reduced but less effectively by addition of 0.3, 0.8, 1.4mM chlorogenic acid. 0.8mM of chlorogenic acid (1:1 with drug) inhibited bitterness by up to 12.2 ± 1.3% (Shiraishi *et al.*, 2017)	Chemical, no date) The solubility of chlorogenic acid in phosphate-buffered saline (pH 7.2) is 25mg/mL. Commercially available from Sigma
Overall score = 14 Limitations; not stable in solution for long period of time		**Safety score = 3** (GRAS and patented for human use and present in high quantities in the human diet)	**Efficacy/QoE score = 1** (not shown in a human panel)	**Usability score = 1** (not stable in solution for long periods of time and low solubility but could have use for extemporaneous preparations)
• **Abscisic acid** **Abscisic acid is a plant hormone** (Pydi *et al.*, 2015) **Assessed using HEK293T cells expressing TAS2Rs** (Pydi *et al.*, 2015)	Pharmacological characterisation confirms that abscisic acid acts as an antagonist at T2R4 (Pydi *et al.*, 2015)	Abscisic acid is found in fruits and vegetables such as blueberries, with a concentration of around 30 µg/g (Pydi *et al.*, 2015) which equates to 180.8 µM. It has also been reported that abscisic acid is endogenously produced by insulin secreting cells in humans (Bruzzone *et al.*, 2007) Abscisic acid has GRAS status	Abscisic acid antagonises 1 mM quinine at T2R4 with an IC50 value of 34.4 ± 1.1 µM. However, this study found that known T2R4 'agonists' were not able to activate the receptor so a reliable conclusion cannot be thoroughly drawn as to its efficacy (Pydi *et al.*, 2015)	Abscisic acid needs to be in solution to confer efficacy but is soluble in ethanol and DMSO and requires storage at -20 degrees centigrade (PubChem, no date a) Commercially available from Sigma

		No ADI exists at present (Zocchi *et al.*, 2017)		
Overall score = 11 Limitations; study which demonstrated efficacy came to unreliable conclusions and is insoluble in biocompatible media		**Safety score = 3** (found in human diet and GRAS)	**Efficacy/QoE score = 0** (unreliable evidence from study, with known T2R4 agonists not able to activate the receptor)	**Usability score = 1** (Needs to be in solution to have effect but soluble in inappropriate media and requires specific storage)
Flavanoids				
• **4′-fluoro-6-methoxyflavanone, 6,3′-dimethoxyflavanone, and 6-methoxyflavanone** **Assessed using HEK293 cells expressing TAS2R39 and hTAS2R14** (Roland *et al.*, 2014)	It is likely the flavanones 4′-fluoro-6-methoxyflavanone, 6,3′-dimethoxyflavanone, and 6-methoxyflavanone are antagonists of TAS2R39 (Roland *et al.*, 2014)	The safety of 4′-fluoro-6-methoxyflavanone is unknown No precedence of use found	In vitro assays show the flavanones; 4′-fluoro-6-methoxyflavanone, 6,3′-dimethoxyflavanone, and 6-methoxyflavanone (in order of decreasing potency) to inhibit the activation of TAS2R39 by 1.7mM denatonium, 4′-fluoro-6-methoxyflavanone eliminated the response completely. The three flavanones also inhibited the activation of hTAS2R14 but to a lesser extent (Roland *et al.*, 2014)	Solubility/ stability data for 4′-fluoro-6-methoxyflavanone, 6,3′-dimethoxyflavanone, and 6-methoxyflavanone is not readily available Not commercially available
Overall score = 3 Limitations; not enough information available on usability or safety		**Safety score = 0** (not enough information known)	**Efficacy/QoE score= 1** (cell based model of only two bitter receptor subtypes)	**Usability score = 0** (not enough information known)

• **Substituted 3-(pyrazol-4-yl) imidazolidine-2,4-diones** • **3- (1- ((3,5-dimethylisoxazol-4-yl) methyl)-1*H*-pyrazol-4-yl)-1- (3-hydroxybenzyl)imidazolidine-2,4-dione** • **3- (1- ((3,5-dimethylisoxazol-4-yl)methyl)-1*H*-pyrazol-4-yl)-1- (3-hydroxybenzyl)-5,5-dimethylimidazolidine-2,4-dione** **Assessed in HEK293 cells expressing hTAS2R8** (Patron *et al.*, 2016) **Overall score = 11** Limitations; insoluble in suitable media and limited evidence of efficacy	These compounds selectively antagonise hTAS2R8 (IC_{50}'s = 0.035 and 0.073μM) (Patron *et al.*, 2016)	Both compounds are GRAS for use as an excipient A full toxicological report found that the no observable averse effect level (NOAEL) for each compound is orders of magnitude above the expected human exposure (Karanewsky *et al.*, 2016) **Safety score = 2**	Both substituted 3-(pyrazol-4-yl) imidazolidine-2,4-diones have been shown to significantly attenuate the bitter taste of a variety of bitter tastants including caffeine (1mM) in cell models expressing TAS2R8 (Patron *et al.*, 2016) **Efficacy/QoE score= 1** (single receptor expressing cell line)	Both insoluble in water. Require ethanol to solubilise (Patron *et al.*, 2016) Commercial availability cannot be found **Usability score = 1** (insoluble in suitable media and not available to purchase)
• **Triphenylphosphine oxide (TPPO)** **Assessed in Hek293 cells expressing TRPM5** (Palmer *et al.*, 2010)	TPPO selectively inhibits the TRPM5 receptor. TRPM5 is activated by intracellular calcium release after taste cell activation by sweet, bitter and umami tastants (Palmer, 2007) and so by blocking here these taste	TPPO is reported as very toxic at high levels (PubChem, no date d). A reference dose of 0.02mg/kg-day has been extrapolated with safety margins from dog toxicology studies (US Environmental Protection Agency, 2007). This would mean a 70kg person can be	TPPO inhibited human TRPM5 heterologously expressed in HEK293 cells (IC_{50} = 12μM) (Palmer *et al.*, 2010)	TPPO is almost insoluble in deionized water. It is soluble in ethanol, formic acid, acetic acid, and dichloromethane (Hu *et al.*, 2009) Unsafe to use as doses

	signals cannot be transduced.	exposed to a maximum of approximately 5.03µM per day, which is well below the IC_{50}.		required for bitter blocking. It is commercially available from sigma
Overall score = 3		**Safety score = 0** (efficacious concentration from study could be toxic in humans)	**Efficacy/QoE score= 1** (efficacy shown in model of only TRPM5 and this will affect other taste sensations)	**Usability score = 0** (insoluble in suitable media and unsafe at required doses)

4 Scoring system results

Using the scoring system laid out in table 1 the bitter-blockers were assessed and ranked accordingly, again separated by method of assessment (table 3a & 3b).

Table 3a Bitter-blocking agents tested using human panels. Overall score according to 2 x usability, 3 x efficacy/quality of evidence and 3 x safety

Compound	Overall score
AMP	22
Sodium acetate	22
Sodium gluconate	22
GIV3727	20
Homoeriodictyol sodium salt	20
Citric acid	19
Sodium chloride + L-arginine	19
Phosphatidic acid + beta-lactoglobulin	17
Phosphatidic acid	14
2,4-dihydroxybenzoic acid vanillylamide	12
Riboflavin-binding protein	11
Jaceosidin and sakuranetin	0

Table 3b Bitter-blocking agents tested using non-human methods. Overall score according to 2 x usability, 3 x efficacy/quality of evidence and 3 x safety

Compound	Overall score
Chlorogenic acid	14
Substituted 3- (pyrazol-4-yl) imidazolidine-2,4-diones	11
Abscisic acid	11
Triphenylphosphine oxide (TPPO)	3
4'-fluoro-6-methoxyflavanone, 6,3'-dimethoxyflavanone, *and 6-methoxyflavanone*	3

5 Other reported compounds

Many cited bitter-blockers in the literature reference sweeteners or sweet proteins. Examples include thaumatin (Chinedu *et al.*, 2014), aspartame (Sharafi, Hayes and Duffy, 2013), monellin (Cagan, 1973) and neotame (Zheng *et al.*, 2018). Some sweeteners are ligands for bitter receptors, acting as agonists and resulting in bitter aftertastes in the patient. For example saccharin and acesulfame K activate hTAS2R43 and hTAS2R44 at millimolar concentrations (Kuhn *et al.*, 2004). It is known that particular combinations of sweeteners can be added together to offset the bitterness of the other. For example, both saccharin and cyclamate have bitter 'off-tastes'. Saccharin activates the bitter receptors TAS2R31 and TAS2R43 at millimolar concentrations (0.17 and 0.08mM respectively)(Kuhn *et al.*, 2004) whereas the half-maximal effective concentration (EC50) for the sweet receptor target (the TAS1R2/TAS1R3 heterodimer) is approximately 0.2mM saccharin. This means that saccharin is eliciting a bitter taste-response before reaching signal saturation of the sweet receptor(Behrens, Blank and Meyerhof, 2017). Cyclamate can modify the bitter-taste response to saccharin, primarily by acting on TAS2R43, a receptor partially responsible for saccharin's bitter off-taste. However, the concentrations of cyclamate required to significantly impact upon saccharin induced TAS2R43 bitterness are relatively high (half-maximal inhibitory concentration of 19.0 ± 4.6 mM)(Behrens, Blank and Meyerhof, 2017). Furthermore, cyclamate itself activates certain bitter receptors, (TAS2R1 and TAS2R38) at 30mM (Meyerhof *et al.*, 2010), a concentration far higher than the 2.2mM EC50 of the sweet receptor target (Winnig *et al.*, 2007) but a concentration that may be required to fully block

saccharin's bitterness. Such sweeteners are not included in this review as bitter-blocking agents as their primary taste-masking action is on sweet receptors.

Miraculin, is not a sweetener as such but a glycoprotein extracted from the miracle berry that is known to make sour taste appear sweet [19]. Interestingly, it was shown to improve the perception of food and drink reported as metallic in a small group of patients with altered sense of taste undergoing chemotherapy [39]. It has not been assessed against bitter compounds in sensory trials however, and it's primary effect is as a sweet receptor agonist(Paladino *et al.*, 2008)(Sanematsu *et al.*, 2016)

Other taste-masking agents cited in the literature as bitter blocking taste modifiers include cyclodextrins, ion-exchange complexes (Zheng *et al.*, 2018), and fatty-acids (Homma *et al.*, 2012). Additives which coat the mouth and prevent interaction with the taste receptors such as lipophilic vehicles (Sohi, Sultana and Khar, 2004) and surfactant compounds (DeSimone, Heck and Bartoshuk, 1980) were also excluded from this review.

5.1 Compounds which influence other taste perceptions

Other commonly reported 'bitter blockers' include 3β-Hydroxydihydrocostunolide and 3β-hydroxypelenolide which can be extracted from wormwood and inhibit TAS2R46. However, they are agonists at a number of other bitter receptors (Brockhoff *et al.*, 2011) and therefore are unsuitable for addition to pharmaceutical compounds. GIV3727 was included in this review even though it has been reported to be an agonist at TAS2R14 (Pydi *et al.*, 2014) because it has shown to be promising in human sensory

trials. Other compounds have been shown to inhibit bitter perception but effect other taste sensations, for example $ZnSO_4$ reduced the perceived bitterness of quinine in a human sensory experiment but also reduced the panellists' perception of sweetness (Keast and Breslin, 2005). Zinc salt solutions also have a prominent astringency that makes them unappealing as excipients for medicines (Keast, 2003). Other salts that have been reported as bitter-blocking include $MgSO_4$, which is also a bitter stimulus at higher concentrations(Keast and Breslin, 2002) and has bitter inhibiting effects at lower concentrations (Gaudette and Pickering, 2013) but has not shown consistent results.

Both γ-aminobutryic acid (GABA) and *N*α,*N*α-bis (carboxymethyl)-l-lysine (BCML) have been shown to act as bitter blockers to 1mM quinine, with an IC50 of 3.2±0.3 μM and 59±18 nM respectively (Pydi *et al.*, 2014). Aside from the fact GABA is a neurotransmitter and so is not an ideal excipient to add to medicines, amino acid derivatives have their own umami taste which seems to be key to their bitter suppression. Peptides which are tasteless, such as Gly-Gly, did not have any effect on bitter perception in an in vitro model of TAS2R16 (Kim *et al.*, 2015). Using umami flavour as a taste-masking excipient does not provide an ideal solution to bitter blocking as this taste preference is subjective and may not increase the acceptance of medicines in many patient populations.

5.2 Compounds with other limitations

Probenecid has been shown in vitro to inhibit the activation of TAS2R16, 38 and 43 and this correlates in humans with 10mM probenecid rinse significantly reducing the bitter perception of 10mM salicin, a TAS2R16 ligand, in a human

panel of 15 people (Greene *et al.*, 2011). Its utility as a bitter-blocker has been shown by using it as a 10mM pre-treatment rinse and not as an excipient. Furthermore, Probenecid is an FDA approved treatment for gout and, like most medicinal compounds, is associated with side effects (Boger and Strickland, 1955) making it an undesirable excipient choice. Other rinse approaches have shown success; pre-treatment with Chlorhexidine antiseptic can alter bitter perception of tastants given directly after (Gent, Frank and Hettinger, 2002). However, not only is this rinse approach unsuitable but Chlorhexidine is bitter in itself.

Other modifiers that have been reported include flavan-3-ol-spiro-C-glycosides reaction products (Zhang, Xia and Peterson, 2014) and 1-carboxymethyl-5-hydroxy-2-hydroxymethylpyridinium inner salt (Soldo and Hofmann, 2005). These have shown some bitter blocking effect in small human panels. However, these are produced by long chemical reactions and not available to purchase and therefore unideal excipient candidates.

Studies trying to elucidate the mechanisms behind taste perception have highlighted how cascade blockers can be used to alter perception of taste sensations including bitterness. For example, U73122, a phospholipase C blocker and thapsigargin, a Ca^{++}- ATPase blocker, have been shown to have efficacy in preventing bitter taste transmission by investigating nerve responses in the rat (DeSimone *et al.*, 2012). Both compounds are only soluble in unsuitable media (Sigma-Aldrich, no date a, no date b) and their mechanism of action is non-specific, rendering them unsuitable for use.

Some compounds attribute molecular action to their bitter-suppression but not on the bitter receptor pathway. For example Δ^9- tetrahydrocannabinol has

been shown to enhance quinine palatability in rats by acting on CB1 receptors (Jarrett, Scantlebury and Parker, 2007). This mechanism of action is undesirable for use as a taste-masking excipient as it is likely to lead to off target effects (Mackie, 2008). TRPM8 agonists have shown promise with bitter-masking through the cooling effect they impart (González-Muñiz *et al.*, 2019); menthol has been shown to successfully improve the acceptability of bitter compounds in the BATA model (Andrews *et al.*, 2017) and in human sensory trials (work done in house, unpublished). The limitation with menthol is the strong smell which may be aversive to patients but other agonists which do not have the same scent need to be further investigated for their potential as taste-masking excipients.

Other bitter-blocking compounds were not discussed in this review because there is too little published data available to make any judgements on their usefulness. For example, MR15, 24A and MZ70 have been quoted to mask bitter melon's unpleasant taste using in vitro methods (Donya *et al.*, 2007) but not there is not enough information in the public domain to evaluate them.

6 Conclusions

Palatability plays an important role in patient adherence to a medicinal regime. Bitter-blockers are a category of bitter-masking compounds which directly target the taste-pathway at a molecular level. This review highlights a number of molecules which have demonstrated, to various degrees, bitter taste-modification. The scores given to each compound based on parameters such as safety and usability put the available information in perspective. This review found that AMP and some sodium salts may be productive avenues to

explore in future research to improve the palatability of bitter compounds. GIV3727 and homoeriodictyol sodium salt also scored highly but these have limited commercial viability with lack of availability and other usability issues being the major barrier for these compounds.

In order for bitter-blockers to be used more widely in pharmaceutical products it is key to understand their safety within a formulation and learn more about their use as a functional excipient. It is also important to explore factors such as length of efficacy; any effect on bitter suppression must be transient (ideally seconds, perhaps single minutes) as to not disrupt taste-perception longer than necessary. For widespread use, it may be necessary to generate new toxicological data on these compounds, it is unlikely that the promising bitter-blockers highlighted in this review would have a safety issue due to their GRAS status and the low levels required for efficacy. These compounds could offer an invaluable option to improve the palatability of medicines and help to increase patient compliance.

Funding Information

This work was supported by the CDT in Advanced Therapeutics and Nanomedicines (EP/L01646X), which is funded by the Engineering and Physical Sciences Research Council (EPSRC).

References

4-(2,2,3-Trimethylcyclopentyl)butanoic Acid - Cayman Chemical (no date). Available at: https://www.caymanchem.com/product/19606 (Accessed: 16 October 2019).

4-(2,2,3-Trimethylcyclopentyl)butanoic acid - PubChem (no date). Available at: https://pubchem.ncbi.nlm.nih.gov/compound/25099817#section=Computed-Properties (Accessed: 15 February 2019).

4-(2,2,3-TRIMETHYLCYCLOPENTYL)BUTANOIC ACID | FEMA (no date). Available at: https://www.femaflavor.org/flavor-library/4-223-trimethylcyclopentylbutanoic-acid (Accessed: 15 February 2019).

Abou-Donia, M. B. *et al.* (2008) 'Splenda Alters Gut Microflora and Increases Intestinal P-Glycoprotein and Cytochrome P-450 in Male Rats', *Journal of Toxicology and Environmental Health, Part A*, 71(21), pp. 1415–1429. doi: 10.1080/15287390802328630.

Abou-Hamdan, A. *et al.* (2000) 'High-Pressure Studies as a Novel Approach in Determining Inclusion Mechanisms: Thermodynamics and Kinetics of the Host−Guest Interactions for α-Cyclodextrin Complexes'. American Chemical Society. doi: 10.1021/JA993139M.

Adhikari, B. *et al.* (2007) 'Effect of surface tension and viscosity on the surface stickiness of carbohydrate and protein solutions', *Journal of Food Engineering*. Elsevier, 79(4), pp. 1136–1143. doi: 10.1016/J.JFOODENG.2006.04.002.

Adkin, D. *et al.* (1995) 'The effects of pharmaceutical excipients on small intestinal transit.', *British Journal of Clinical Pharmacology*. Wiley/Blackwell (10.1111), 39(4), pp. 381–387. doi: 10.1111/j.1365-2125.1995.tb04466.x.

Adkin, D. A., Gowland, P., *et al.* (1995) 'Echo-Planar Magnetic Resonance Imaging to Assess Water Volume in the Distal Small Bowel', *Pharmaceutical*

Research: An Official Journal of the American Association of Pharmaceutical Scientists. Pharm Res, 12(8), pp. 1134–1139. doi: 10.1023/A:1016251624220.

Adkin, D. A., Davis, S. S., *et al.* (1995) 'The Effect of Different Concentrations of Mannitol in Solution on Small Intestinal Transit: Implications for Drug Absorption', *Pharmaceutical Research*. Kluwer Academic Publishers-Plenum Publishers, 12(3), pp. 393–396. doi: 10.1023/A:1016256619309.

Afonso-Pereira, F. *et al.* (2016) 'Sex differences in excipient effects: Enhancement in ranitidine bioavailability in the presence of polyethylene glycol in male, but not female, rats', *International Journal of Pharmaceutics*. Elsevier B.V., 506(1–2), pp. 237–241. doi: 10.1016/j.ijpharm.2016.04.022.

Aglione, A. *et al.* (2014) 'Modulation of bitterness and mouthfeel via synergistic mixtures of long chain fatty acids'. Available at: https://patents.google.com/patent/EP3082460A1/en (Accessed: 9 May 2018).

Ahmad, R. and Dalziel, J. E. (2020) 'G Protein-Coupled Receptors in Taste Physiology and Pharmacology', *Frontiers in Pharmacology*. Frontiers Media S.A., 11, p. 1771. doi: 10.3389/FPHAR.2020.587664/BIBTEX.

Al-kasmi, B. *et al.* (2017) 'Mechanical microencapsulation: The best technique in taste masking for the manufacturing scale - Effect of polymer encapsulation on drug targeting', *Journal of Controlled Release*. Elsevier, 260, pp. 134–141. doi: 10.1016/J.JCONREL.2017.06.002.

Al-Kasmi, B. *et al.* (2018) 'Structural and in vitro in vivo evaluation for taste masking', *Expert Opinion on Drug Delivery*, 15(11), pp. 1105–1116. doi: 10.1080/17425247.2018.1535590.

Alam, M. A., Al-Jenoobi, F. I. and Al-mohizea, A. M. (2012) ‘Everted gut sac model as a tool in pharmaceutical research: limitations and applications’, *Journal of Pharmacy and Pharmacology*. John Wiley & Sons, Ltd, 64(3), pp. 326–336. doi: 10.1111/j.2042-7158.2011.01391.x.

American Academy of Pediatrics (2000) *Periodic Survey #44. Patient Compliance With Prescription Regimens*. Available at: http://www.aap.org/research/periodicsurvey/ps44bexs.htm (Accessed: 22 February 2022).

Andrews, D. *et al.* (2017) ‘A novel approach to assess and improve palatability of an inhaled asset using the Rat Brief Access Taste Aversion Assay and an In Silico model of salivary flow’, *F1000Research*, 6. doi: 10.7490/F1000RESEARCH.1115096.1.

Andrews, D. *et al.* (2021) ‘Bitter-blockers as a taste masking strategy: A systematic review towards their utility in pharmaceuticals’, *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier, 158, pp. 35–51. doi: 10.1016/J.EJPB.2020.10.017.

de Angelis, I. and Turco, L. (2011) ‘Caco-2 cells as a model for intestinal absorption’, *Current Protocols in Toxicology*. John Wiley and Sons Inc., (SUPPL.47). doi: 10.1002/0471140856.tx2006s47.

Arey, L. B., Tremaine, M. J. and Monzingo, F. L. (1935) ‘The numerical and topographical relations of taste buds to human circumv allate papillae throughout the life span’, *The Anatomical Record*, 64(1), pp. 9–25. doi: 10.1002/AR.1090640103.

Arima, H. *et al.* (2001) ‘Contribution of P-glycoprotein to the Enhancing Effects

of Dimethyl-β-cyclodextrin on Oral Bioavailability of Tacrolimus', *Journal of Pharmacology and Experimental Therapeutics*, 297(2), pp. 547–555.

Arima, H. *et al.* (2004) 'Contribution of Cholesterol and Phospholipids to Inhibitory Effect of Dimethyl-β-Cyclodextrin on Efflux Function of P-Glycoprotein and Multidrug Resistance-Associated Protein 2 in Vinblastine-Resistant Caco-2 Cell Monolayers', *Pharmaceutical Research 2004 21:4*. Springer, 21(4), pp. 625–634. doi: 10.1023/B:PHAM.0000022409.27896.D4.

Arima, H., Higashi, T. and Motoyama, K. (2012) 'Improvement of the bitter taste of drugs by complexation with cyclodextrins: Applications, evaluations and mechanisms', *Therapeutic Delivery*. Future Science Ltd London, UK , 3(5), pp. 633–644. doi: 10.4155/TDE.12.28/ASSET/IMAGES/LARGE/FIGURE15.JPEG.

Arthur, S. and Burgess, A. (2017) 'How to identify and manage "problem" excipients in medicines for children', *The Pharmaceutical Journal*.

Arvidson, K. (1979) 'Location and variation in number of taste buds in human fungiform papillae', *European Journal of Oral Sciences*. John Wiley & Sons, Ltd, 87(6), pp. 435–442. doi: 10.1111/J.1600-0722.1979.TB00705.X.

Ashiru, D. A. I., Patel, R. and Basit, A. W. (2007) 'Simple and universal HPLC-UV method to determine cimetidine, ranitidine, famotidine and nizatidine in urine: Application to the analysis of ranitidine and its metabolites in human volunteers', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. J Chromatogr B Analyt Technol Biomed Life Sci, 860(2), pp. 235–240. doi: 10.1016/j.jchromb.2007.10.029.

Baldwin, E. A. *et al.* (2011) 'Electronic Noses and Tongues: Applications for

the Food and Pharmaceutical Industries', *Sensors*, 11(5), pp. 4744–4766. doi: 10.3390/s110504744.

Bartoshuk, L. M. *et al.* (1982) 'Effects of temperature on the perceived sweetness of sucrose', *Physiology and Behavior*. Physiol Behav, 28(5), pp. 905–910. doi: 10.1016/0031-9384(82)90212-8.

Basit, A. W. *et al.* (2001) 'The effect of polyethylene glycol 400 on gastrointestinal transit: implications for the formulation of poorly-water soluble drugs.', *Pharmaceutical research*, 18(8), pp. 1146–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11587486 (Accessed: 17 May 2018).

Batchelor, H. K., Fotaki, N. and Klein, S. (2014) 'Paediatric oral biopharmaceutics: Key considerations and current challenges', *Advanced Drug Delivery Reviews*. Elsevier, pp. 102–126. doi: 10.1016/j.addr.2013.10.006.

Batchelor, H. K. and Marriott, J. F. (2015) 'Formulations for children: problems and solutions.', *British journal of clinical pharmacology*. Wiley-Blackwell, 79(3), pp. 405–18. doi: 10.1111/BCP.12268.

Bearer, C. F. (1995) 'How are children different from adults?', *Environmental Health Perspectives*, 103(suppl 6), pp. 7–12. doi: 10.1289/ehp.95103s67.

Behrens, M., Blank, K. and Meyerhof, W. (2017) 'Blends of Non-caloric Sweeteners Saccharin and Cyclamate Show Reduced Off-Taste due to TAS2R Bitter Receptor Inhibition', *Cell Chemical Biology*, 24(10), pp. 1199-1204.e2. doi: 10.1016/j.chembiol.2017.08.004.

BeMiller, J. (2003) 'Dextrins', in Caballero, B. (ed.) *Encyclopedia of Food*

Sciences and Nutrition. 2nd edn. Available at: https://www.sciencedirect.com/referencework/9780122270550/encyclopedia-of-food-sciences-and-nutrition (Accessed: 19 November 2021).

Bergeron, R. J. *et al.* (1977) 'Disposition requirements for binding in aqueous solution of polar substrates in the cyclohexaamylose cavity', *Journal of the American Chemical Society*. American Chemical Society, 99(15), pp. 5146–5151. doi: 10.1021/ja00457a040.

Blazek-Welsh, A. I. and Rhodes, D. G. (2001) 'Maltodextrin-based proniosomes', *AAPS PharmSci*. Springer New York, 3(1), pp. 1–8. doi: 10.1208/ps030101.

Bobowski, N., Reed, D. R. and Mennella, J. A. (2016) 'Variation in the TAS2R31 bitter taste receptor gene relates to liking for the nonnutritive sweetener Acesulfame-K among children and adults', *Scientific Reports*. Nature Publishing Group, 6(1), pp. 1–7. doi: 10.1038/srep39135.

Boger, W. P. and Strickland, S. C. (1955) 'Probenecid (Benemid), Its Uses and Side-Effects in 2,502 Patients', *A.M.A. Archives of Internal Medicine*. American Medical Association, 95(1), p. 83. doi: 10.1001/archinte.1955.00250070099012.

Breslin, P. A. S. and Beauchamp, G. K. (1995) 'Suppression of bitterness by sodium: Variation among bitter taste stimuli', *Chemical Senses*, 20(6), pp. 609–623. doi: 10.1093/chemse/20.6.609.

Breslin, P. and Spector, A. (2008) 'Mammalian taste perception', *Current Biology*, 18(4). Available at: https://www.cell.com/current-biology/pdf/S0960-9822(07)02370-6.pdf (Accessed: 9 March 2022).

Breza, J. M., Curtis, K. S. and Contreras, R. J. (2006) ‘Temperature modulates taste responsiveness and stimulates gustatory neurons in the rat geniculate ganglion’, *Journal of Neurophysiology*. J Neurophysiol, 95(2), pp. 674–685. doi: 10.1152/jn.00793.2005.

Brockhoff, A. *et al.* (2011) ‘Receptor Agonism and Antagonism of Dietary Bitter Compounds’, *Journal of Neuroscience*, 31(41), pp. 14775–14782. doi: 10.1523/JNEUROSCI.2923-11.2011.

Brusick, D. *et al.* (2009) ‘Expert Panel report on a study of Splenda in male rats’, *Regulatory Toxicology and Pharmacology*. Academic Press Inc., 55(1), pp. 6–12. doi: 10.1016/j.yrtph.2009.06.013.

Bruzzone, S. *et al.* (2007) ‘Abscisic acid is an endogenous cytokine in human granulocytes with cyclic ADP-ribose as second messenger’, *Proceedings of the National Academy of Sciences*, 104(14), pp. 5759–5764. doi: 10.1073/pnas.0609379104.

Bryson, S. P. (2014) ‘Patient-centred, administration friendly medicines for children - an evaluation of children’s preferences and how they impact medication adherence’, *International journal of pharmaceutics*. Int J Pharm, 469(2), pp. 257–259. doi: 10.1016/J.IJPHARM.2014.04.069.

Buggins, T. R., Dickinson, P. A. and Taylor, G. (2007) ‘The effects of pharmaceutical excipients on drug disposition’, *Advanced drug delivery reviews*. Adv Drug Deliv Rev, 59(15), pp. 1482–1503. doi: 10.1016/J.ADDR.2007.08.017.

Cabras, T. *et al.* (2012) ‘Responsiveness to 6-n-Propylthiouracil (PROP) Is Associated with Salivary Levels of Two Specific Basic Proline-Rich Proteins in

Humans', *PLoS ONE*. Edited by W. Meyerhof. Public Library of Science, 7(2), p. e30962. doi: 10.1371/journal.pone.0030962.

Cagan, R. H. (1973) 'Chemostimulatory protein: a new type of taste stimulus.', *Science (New York, N.Y.)*, 181(4094), pp. 32–5. doi: 10.2307/1735921.

Carter, W. D. (2011) 'Risk Assessment for Excipients for Enhanced Patient Safety', *Pharmaceutical Technology*, pp. S29–S30, S32–S33. Available at: http://connection.ebscohost.com/c/articles/60002631/risk-assessment-excipients-enhanced-patient-safety (Accessed: 2 July 2018).

Cayman Chemical (2022) *Sodium Acetate Stock Solution (3 M, pH 5.2)*. Available at: https://www.caymanchem.com/product/600228 (Accessed: 3 December 2018).

Cayman Chemical (no date) *Product Information; Chlorogenic Acid*. Available at: https://www.caymanchem.com/pdfs/70930.pdf (Accessed: 5 July 2018).

Chay, S. K. *et al.* (2018) 'Evaluation of the taste-masking effects of (2-hydroxypropyl)-β-cyclodextrin on ranitidine hydrochloride; a combined biosensor, spectroscopic and molecular modelling assessment', *RSC Advances*. The Royal Society of Chemistry, 8(7), pp. 3564–3573. doi: 10.1039/C7RA11015D.

Chemical Book (2019) *Sodium acetate*. Available at: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB1230044.htm (Accessed: 16 March 2022).

Chemical Book (no date) *Sodium acetate*. Available at: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB1230044.ht

m (Accessed: 18 March 2019).

Chen, A. W., Resurreccion, A. V. A. and Paguio, L. P. (1996) ‘Age Appropriate Hedonic Scales To Measure Food Preferences In Young Children’, *Journal of Sensory Studies*. John Wiley & Sons, Ltd, 11(2), pp. 141–163. doi: 10.1111/J.1745-459X.1996.TB00038.X.

Chen, M. L. *et al.* (2007) ‘A modern view of excipient effects on bioequivalence: Case study of sorbitol’, *Pharmaceutical Research*, 24(1), pp. 73–80. doi: 10.1007/s11095-006-9120-4.

Chinedu, S. N. *et al.* (2014) ‘Analyses of the leaf, fruit and seed of Thaumatococcus daniefii (Benth.): exploring potential uses.’, *Pakistan journal of biological sciences : PJBS*, 17(6), pp. 849–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26035959 (Accessed: 21 November 2018).

Chun, L., Zhang, W. H. and Liu, J. F. (2012) ‘Structure and ligand recognition of class C GPCRs’, *Acta Pharmacologica Sinica*. Nature Publishing Group, 33(3), p. 312. doi: 10.1038/APS.2011.186.

Cid-Samamed, A. *et al.* (2022) ‘Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications’, *Food Chemistry*. Elsevier, 384, p. 132467. doi: 10.1016/J.FOODCHEM.2022.132467.

Citric acid - PubChem (no date). Available at: https://pubchem.ncbi.nlm.nih.gov/compound/citric_acid (Accessed: 23 November 2018).

Citric Acid Assay Kit (no date). Available at: https://www.megazyme.com/Citric-Acid-Assay-Kit (Accessed: 20 October 2018).

Clapham, D. *et al.* (2012) 'Assessing taste without using humans: Rat brief access aversion model and electronic tongue', *International Journal of Pharmaceutics*, 435(2), pp. 137–139. doi: 10.1016/j.ijpharm.2012.05.056.

Clapham, D. *et al.* (2021) 'Proposed Tool to Compare and Assess the Applicability of Taste Assessment Techniques for Pharmaceuticals', *Journal of Pharmaceutical Sciences*. Elsevier. doi: 10.1016/J.XPHS.2021.09.002.

ClinicalTrials.gov (2017) *A Phase IV, Open Label, 4-period Cross-over Study to Investigate a Drug Interaction Between Lamivudine and Sorbitol Oral Solutions in Healthy Volunteers*. Available at: https://clinicaltrials.gov/ct2/show/study/NCT02634073 (Accessed: 5 February 2020).

ClinicalTrials.gov (2018) *Effects of Sucralose on Drug Absorption and Metabolism (The SweetMeds Study)*. Available at: https://clinicaltrials.gov/ct2/show/results/NCT03407079 (Accessed: 26 January 2022).

Cocorocchio, M. *et al.* (2016) 'Bitter tastant responses in the amoeba Dictyostelium correlate with rat and human taste assays', *ALTEX*, 33(3), pp. 225–236. doi: 10.14573/altex.1509011.

Coupland, J. N. and Hayes, J. E. (2014) 'Physical Approaches to Masking Bitter Taste: Lessons from Food and Pharmaceuticals', *Pharmaceutical research*. NIH Public Access, 31(11), p. 2921. doi: 10.1007/S11095-014-349

1480-6.

Cram, A. *et al.* (2009) ‘Challenges of developing palatable oral paediatric formulations’, *International Journal of Pharmaceutics*. Elsevier, 365(1–2), pp. 1–3. doi: 10.1016/J.IJPHARM.2008.09.015.

Cravotto, G. *et al.* (2006) ‘Cyclodextrins as Food Additives and in Food Processing’, *Current Nutrition & Food Science*, 2(4), pp. 343–350. doi: 10.2174/157340106778699485.

Dagan-Wiener, A. *et al.* (2019) ‘Bitterdb: Taste ligands and receptors database in 2019’, *Nucleic Acids Research*. Oxford University Press, 47(D1), pp. D1179–D1185. doi: 10.1093/nar/gky974.

Danilova, V. and Hellekant, G. (2006) ‘Elucidating coding of taste qualities with the taste modifier miraculin in the common marmoset’, *Brain Research Bulletin*, 68(5), pp. 315–321. doi: 10.1016/j.brainresbull.2005.09.008.

Dass, C. R. and Jessup, W. (2000) ‘Apolipoprotein A-I, Cyclodextrins and Liposomes as Potential Drugs for the Reversal of Atherosclerosis. A Review’, *Journal of Pharmacy and Pharmacology*, 52(7), pp. 731–761. doi: 10.1211/0022357001774606.

Davies, E. H. and Tuleu, C. (2008) ‘Medicines for Children: A Matter of Taste’, *The Journal of Pediatrics*. Mosby, 153(5), pp. 599-604.e2. doi: 10.1016/J.JPEDS.2008.06.030.

Davis, H. J. and Phipps, R. J. (2017) ‘Harnessing non-covalent interactions to exert control over regioselectivity and site-selectivity in catalytic reactions’, *Chemical Science*. Royal Society of Chemistry, 8(2), pp. 864–877. doi:

10.1039/C6SC04157D.

Dawson, L. A. (2019) ‘What factors affect adherence to medicines?’, *Archives of Disease in Childhood - Education and Practice*. Royal College of Paediatrics and Child Health, 104(1), pp. 49–52. doi: 10.1136/ARCHDISCHILD-2017-312820.

Depoortere, I. (2014) ‘Taste receptors of the gut: emerging roles in health and disease’, *Gut*, 63(1), pp. 179–190. doi: 10.1136/gutjnl-2013-305112.

DeSimone, J. A. *et al.* (2012) ‘Changes in taste receptor cell [Ca $^{2+}$] $_{i}$ modulate chorda tympani responses to bitter, sweet, and umami taste stimuli’, *Journal of Neurophysiology*, 108(12), pp. 3221–3232. doi: 10.1152/jn.00129.2012.

DeSimone, J. A., Heck, G. L. and Bartoshuk, L. M. (1980) ‘Surface active taste modifiers: a comparison of the physical and psychophysical properties of gymnemic acid and sodium lauryl sulfate’, *Chemical Senses*. Oxford University Press, 5(4), pp. 317–330. doi: 10.1093/chemse/5.4.317.

Devantier, H. R. *et al.* (2008) ‘Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion assay’, *Behavioural Pharmacology*, 19(7), pp. 673–682. doi: 10.1097/FBP.0b013e3283123cd6.

Donya, A. *et al.* (2007) ‘Effects of Processing Methods on the Proximate Composition and Momordicosides K and L Content of Bitter Melon Vegetable’. American Chemical Society. doi: 10.1021/JF070428I.

DPP project (2021) *Developing Paediatric Primaquine*. Available at:

https://dpp-project.org/ (Accessed: 20 March 2022).

Drewnowski, A. *et al.* (1997) 'Nontasters, Tasters, and Supertasters of 6-n-Propylthiouracil (PROP) and Hedonic Response to Sweet', *Physiology & Behavior*. Elsevier, 62(3), pp. 649–655. doi: 10.1016/S0031-9384(97)00193-5.

Drewnowski, A. *et al.* (2012) 'Sweetness and Food Preference', *The Journal of Nutrition*. Oxford University Press (OUP), 142(6), pp. 1142S-1148S. doi: 10.3945/jn.111.149575.

Drozdzik, M. *et al.* (2014) 'Protein abundance of clinically relevant multidrug transporters along the entire length of the human intestine', *Molecular Pharmaceutics*. American Chemical Society, 11(10), pp. 3547–3555. doi: 10.1021/mp500330y.

Dürr, D. *et al.* (2000) 'St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4', *Clinical pharmacology and therapeutics*. Clin Pharmacol Ther, 68(6), pp. 598–604. doi: 10.1067/MCP.2000.112240.

Dwaine Reuber, M. (1978) 'Carcinogenicity of saccharin.', *Environmental Health Perspectives*. National Institute of Environmental Health Sciences, 25, p. 173. doi: 10.1289/EHP.7825173.

EDA (2015) *Improving the design of animal experiments: Introducing the Experimental Design Assistant (EDA)* | *NC3Rs*. Available at: https://www.nc3rs.org.uk/news/improving-design-animal-experiments-introducing-experimental-design-assistant-eda (Accessed: 14 March 2022).

EFSA (2013) 'Conclusion on the peer review of the pesticide risk assessment of the active substance maltodextrin', *EFSA Journal*. Wiley-Blackwell

Publishing Ltd, 11(1). doi: 10.2903/J.EFSA.2013.3007.

EMA (2001) ‘Note for guidance on clinical investigation of medicinal products in the paediatric populations CPMP/ICH/2711/99’. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-1.pdf (Accessed: 2 March 2022).

EMA (2006) *Reflection paper: formulations of choice for the paediatric population EMEA/CHMP/PEG/194810/2005*. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003782.pdf.

EMA (2013) *Guideline on pharmaceutical development of medicines for paediatric use. EMA/CHMP/QWP/805880/2012*. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/07/WC500147002.pdf (Accessed: 5 June 2020).

EMA (2014) ‘Background review for cyclodextrins used as excipients’. Available at: www.ema.europa.eu/contact (Accessed: 10 November 2021).

EMA (2017) *Cyclodextrins used as excipients. EMA/CHMP/333892/2013.*

EMA (2018) *Nonclinical Safety Testing in Support of Development of Paediatric pharmaceuticals. EMA/CHMP/ICH/616110/2018*. Available at: http://www.ich.org/products/guidelines/safety/safety-single/article/nonclinical-safety-testing-in-support-of-development-of-paediatric-medicines.html (Accessed: 30 August 2018).

EMC (2017) *Ranitidine 150mg/10ml Oral Solution - Summary of Product*

Characteristics (SmPC). Available at: https://www.medicines.org.uk/emc/product/3371/smpc (Accessed: 10 March 2022).

EMC (2021) *Revatio 10 mg/ml powder for oral suspension - Summary of Product Characteristics (SmPC)*. Available at: https://www.medicines.org.uk/emc/medicine/27153/SPC/Revatio+10+mg+ml+powder+for+oral+suspension/ (Accessed: 10 March 2022).

Eurofins (2021) *Method Transfer*. Available at: https://www.eurofins.com/biopharma-services/product-testing/services/biopharma-product-testing-services/method-development/method-transfer/ (Accessed: 5 January 2022).

European Food Safety Authority (2010) 'Flavouring Group Evaluation 32 - EFSA', *EFSA Journal*, 8(9), p. 1065. doi: 10.2903/j.efsa.2010.1065.

Fabiano, V., Mameli, C. and Zuccotti, G. V. (2011) 'Paediatric pharmacology: Remember the excipients', *Pharmacological Research*, 63(5), pp. 362–365. doi: 10.1016/j.phrs.2011.01.006.

Fakhoury, M. *et al.* (2005) 'Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age', *Drug Metabolism and Disposition*. American Society for Pharmacology and Experimental Therapeutics, 33(11), pp. 1603–1607. doi: 10.1124/dmd.105.005611.

FDA (2004) *Agency Response Letter GRAS Notice No. GRN 000144. Office of Food Additive Safety, Center 464 for Food Safety and Applied Nutrition, Food and Drug Administration.* Available at: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasS354

afeGRAS/GRASListings/ucm153976.htm.

FDA (2006) *Guidance for Industry Nonclinical Safety Evaluation of Pediatric Drug Products*. Available at: http://www.fda.gov/cder/guidance/index.htm (Accessed: 8 March 2022).

FDA (2016) *Food for human consumption. Direct food substances affirmed as generally recoginized as safe*. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=184.1444 (Accessed: 24 February 2022).

FDA (2018) *Food for human consumption - FDA; Code of Federal Regulations*. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.6757 (Accessed: 10 July 2018).

FDA (2020) *FDA Requests Removal of All Ranitidine Products (Zantac) from the Market | FDA*. Available at: https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidine-products-zantac-market (Accessed: 20 March 2022).

FDA (2022) *Inactive Ingredient Search for Approved Drug Products*. Available at: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm (Accessed: 23 February 2022).

FDA (no date) *Food Additive Status List*. Center for Food Safety and Applied Nutrition. Available at: https://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm091048.htm (Accessed: 5 December 2018).

FEMA (no date) *L-arginine*. Available at: https://www.femaflavor.org/flavor-library/l-arginine (Accessed: 5 December 2018).

Fifere, A. *et al.* (2012) 'Theoretical study on β-cyclodextrin inclusion complexes with propiconazole and protonated propiconazole.', *Beilstein journal of organic chemistry*. Beilstein-Institut, 8, pp. 2191–201. doi: 10.3762/bjoc.8.247.

Filho, E. R. T. *et al.* (2020) 'Dynamic aspects of salt reduction in tomato sauce by use of flavor enhancers and a bitter blocker':, *https://doi.org/10.1177/1082013220913361*. SAGE PublicationsSage UK: London, England, 26(6), pp. 549–559. doi: 10.1177/1082013220913361.

Finger, T. E. (2005) 'Cell Types and Lineages in Taste Buds', *Chemical Senses*. Narnia, 30(Supplement 1), pp. i54–i55. doi: 10.1093/chemse/bjh110.

Fletcher, J. N. *et al.* (2011) 'In Vitro Evaluation of Flavonoids from *Eriodictyon californicum* for Antagonist Activity against the Bitterness Receptor hTAS2R31', *Journal of Agricultural and Food Chemistry*, 59(24), pp. 13117–13121. doi: 10.1021/jf204359q.

Food and Drug Administration (2005) 'Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients', pp. 20852–1448. Available at: http://www.fda.gov/cder/guidance/index.htm (Accessed: 30 June 2018).

FooDB, Homoeriodictyol, sodium salt (no date). Available at: http://foodb.ca/compounds/FDB009586 (Accessed: 26 July 2018).

Forestell, C. A. (2017) 'Flavor Perception and Preference Development in

Human Infants', *Annals of Nutrition and Metabolism*. Karger Publishers, 70(Suppl. 3), pp. 17–25. doi: 10.1159/000478759.

Fotsing, J. R. *et al.* (2020) 'Discovery and Development of S6821 and S7958 as Potent TAS2R8 Antagonists', *Journal of Medicinal Chemistry*. American Chemical Society, 63(9), pp. 4957–4977. doi: 10.1021/ACS.JMEDCHEM.0C00388.

Frutos, M. G. S. *et al.* (2012) 'Feed efficiency, food choice, and food reward behaviors in young and old Fischer rats', *Neurobiology of Aging*. Elsevier, 33(1), pp. 206.e41-206.e53. doi: 10.1016/J.NEUROBIOLAGING.2010.09.006.

Fuke, S. and Shimizu, T. (1993) 'Sensory and preference aspects of umami', *Trends in Food Science & Technology*. Elsevier, 4(8), pp. 246–251. doi: 10.1016/0924-2244(93)90139-2.

Furrer, P. (2013) 'The central role of excipients in drug formulation', *European Pharmaceutical Review*, 18(2), pp. 67–70. Available at: https://www.europeanpharmaceuticalreview.com/article/18434/the-central-role-of-excipients-in-drug-formulation-2/ (Accessed: 24 August 2021).

Gaillard, D. and Stratford, J. M. (2016) 'Measurement of Behavioral Taste Responses in Mice: Two-Bottle Preference, Lickometer, and Conditioned Taste-Aversion Tests', *Current Protocols in Mouse Biology*. John Wiley & Sons, Ltd, 6(4), pp. 380–407. doi: 10.1002/cpmo.18.

Gao, X. *et al.* (2008) 'Nonparametric multiple comparison procedures for unbalanced one-way factorial designs', *Journal of Statistical Planning and Inference*. North-Holland, 138(8), pp. 2574–2591. doi: 10.1016/J.JSPI.2007.10.015.

Gaudette, Nicole J and Pickering, G. J. (2012) ‘Optimizing the orosensory properties of model functional beverages: the influence of novel sweeteners, odorants, bitter blockers, and their mixtures on (+)-catechin.’, *Journal of food science*, 77(6), pp. S226-32. doi: 10.1111/j.1750-3841.2012.02707.x.

Gaudette, Nicole J. and Pickering, G. J. (2012) ‘The efficacy of bitter blockers on health-relevant bitterants’, *Journal of Functional Foods*, 4(1), pp. 177–184. doi: 10.1016/j.jff.2011.10.003.

Gaudette, N. J. and Pickering, G. J. (2013) ‘Modifying Bitterness in Functional Food Systems’, *Critical Reviews in Food Science and Nutrition*. Taylor & Francis Group , 53(5), pp. 464–481. doi: 10.1080/10408398.2010.542511.

Gent, J. F., Frank, M. E. and Hettinger, T. P. (2002) ‘Taste confusions following chlorhexidine treatment’, *Chemical Senses*. Oxford University Press, 27(1), pp. 73–80. doi: 10.1093/chemse/27.1.73.

Gillette, M. (1990) ‘Sensory Evaluation: Analytical and Affective Testing’, *Perfumer & Flavorist*, 15.

Gilsenan, M. B. (2011) ‘Additives in Dairy Foods | Safety’, *Encyclopedia of Dairy Sciences: Second Edition*. Academic Press, pp. 55–60. doi: 10.1016/B978-0-12-374407-4.00005-4.

Ginsberg, G., Hattis, D. and Sonawane, B. (2004) ‘Incorporating pharmacokinetic differences between children and adults in assessing children’s risks to environmental toxicants’, *Toxicology and Applied Pharmacology*. Academic Press Inc., 198(2), pp. 164–183. doi: 10.1016/J.TAAP.2003.10.010.

GlaxoSmithKline (2004) *ZANTAC prescribing information*. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/20251scf015_zantac_lbl.pdf (Accessed: 20 January 2022).

González-Muñiz, R. *et al.* (2019) 'Recent Progress in TRPM8 Modulation: An Update', *International Journal of Molecular Sciences*, 20(11), p. 2618. doi: 10.3390/ijms20112618.

Gore-Langton, J. K. *et al.* (2015) 'Measures of the aversive effects of drugs: A comparison of conditioned taste and place aversions', *Pharmacology Biochemistry and Behavior*. Elsevier Inc., 134, pp. 99–105. doi: 10.1016/j.pbb.2015.05.002.

Greene, T. A. *et al.* (2011) 'Probenecid Inhibits the Human Bitter Taste Receptor TAS2R16 and Suppresses Bitter Perception of Salicin', *PLoS ONE*. Edited by H. Matsunami, 6(5), p. e20123. doi: 10.1371/journal.pone.0020123.

Grembecka, M. (2015) 'Natural sweeteners in a human diet', *Roczniki Panstwowego Zakladu Higieny*. Rocz Panstw Zakl Hig, 66(3), pp. 195–202. Available at: https://pubmed.ncbi.nlm.nih.gov/26400114/ (Accessed: 22 February 2022).

Grembecka, M. (2018) 'Sugar Alcohols as Sugar Substitutes in Food Industry', *Reference Series in Phytochemistry*. Springer, Cham, pp. 547–573. doi: 10.1007/978-3-319-27027-2_23.

Gupta, H. *et al.* (2010) 'E-Tongue: A Tool for Taste Evaluation', *Recent Patents on Drug Delivery & Formulation*, 4(1), pp. 82–89. doi: 10.2174/187221110789957309.

Hampton, T. (2008) ‘Sugar substitutes linked to weight gain’, *JAMA*. JAMA, 299(18), pp. 2137–2138. doi: 10.1001/JAMA.299.18.2137.

Handbook of Pharmaceutical Excipients (2006) *Handbook of Pharmaceutical Excipients*, *Fifth edition*. Edited by R. Rowe, P. Sheskey, and S. Owen. Pharmaceutical Press.

Hanghøj, S. and Boisen, K. A. (2014) ‘Self-reported barriers to medication adherence among chronically ill adolescents: a systematic review’, *The Journal of adolescent health : official publication of the Society for Adolescent Medicine*. J Adolesc Health, 54(2), pp. 121–138. doi: 10.1016/J.JADOHEALTH.2013.08.009.

Haraguchi, T. *et al.* (2016) ‘Usefulness and limitations of taste sensors in the evaluation of palatability and taste-masking in oral dosage forms’, *Asian Journal of Pharmaceutical Sciences*. Elsevier, 11(4), pp. 479–485. doi: 10.1016/J.AJPS.2016.03.001.

Health.gov (2015) *Executive Summary - 2015-2020 Dietary Guidelines - health.gov*. Available at: https://health.gov/dietaryguidelines/2015/guidelines/executive-summary/ (Accessed: 16 February 2019).

Henney, J., Taylor, C. and Boon, C. (2010) *Strategies to Reduce Sodium Intake in the United States*. Washington, D.C.: National Academies Press. doi: 10.17226/12818.

Hikaru, F. and Akiko, K. (2003) ‘Pharmaceutical formulations comprising sodium laurylsulfate as bitterness masking agent’. American Pharmaceutical Association. Available at:

https://patents.google.com/patent/WO2004075900A2/en (Accessed: 16 February 2019).

Hofman, D. L., van Buul, V. J. and Brouns, F. J. P. H. (2015) ‘Nutrition, Health, and Regulatory Aspects of Digestible Maltodextrins’, *https://doi.org/10.1080/10408398.2014.940415*. Taylor & Francis, 56(12), pp. 2091–2100. doi: 10.1080/10408398.2014.940415.

Hollister, E. B. *et al.* (2015) ‘Structure and function of the healthy pre-adolescent pediatric gut microbiome’, *Microbiome*. BioMed Central, 3, p. 36. doi: 10.1186/S40168-015-0101-X.

Home office (2013) *Animal testing and research: guidance for the regulated community*. Available at: https://www.gov.uk/guidance/research-and-testing-using-animals (Accessed: 25 February 2022).

Home Office (2014) *Code of Practice for the housing and care of animals used in scientific procedures*, *Animals (Scientific Procedures) Act 1986*. Available at: https://www.gov.uk/government/publications/code-of-practice-for-the-housing-and-care-of-animals-bred-supplied-or-used-for-scientific-purposes (Accessed: 7 January 2022).

Homma, R. *et al.* (2012) ‘Identification of Bitterness-Masking Compounds from Cheese’, *Journal of Agricultural and Food Chemistry*, 60(18), pp. 4492–4499. doi: 10.1021/jf300563n.

homoeriodictyol safety data sheet (no date). Available at: https://www.greyhoundchrom.com/Content/files/Downloads/128623/1283.pdf (Accessed: 14 August 2018).

Hu, F.-H. *et al.* (2009) ‘Solubilities of Triphenylphosphine Oxide in Selected Solvents’, *Journal of Chemical & Engineering Data*, 54(4), pp. 1382–1384. doi: 10.1021/je800842z.

Al Humaid, J. (2018) ‘Sweetener content and cariogenic potential of pediatric oral medications: A literature.’, *International journal of health sciences*. Qassim University, 12(3), pp. 75–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29896075 (Accessed: 11 October 2019).

International Pharmaceutical Excipients Council (2008) *Qualification of Excipients for Use in Pharmaceuticals*. Available at: file:///C:/Users/DANIEL~1.AND/AppData/Local/Temp/ExcipientQualificationGuide.pdf (Accessed: 16 October 2019).

Inui-Yamamoto, C. *et al.* (2017) ‘Taste preference changes throughout different life stages in male rats’, *PLOS ONE*. Public Library of Science, 12(7), p. e0181650. doi: 10.1371/JOURNAL.PONE.0181650.

Islam, M. S. and Sakaguchi, E. (2006) ‘Sorbitol-based osmotic diarrhea: Possible causes and mechanism of prevention investigated in rats’, *World Journal of Gastroenterology : WJG*. Baishideng Publishing Group Inc, 12(47), p. 7635. doi: 10.3748/WJG.V12.I47.7635.

Ives, R., Tuleu, C. and Bachmanov, A. (2018) ‘Rat Brief Access Taste Aversion Test Predicts Human Perception of Bitter Medicines: Comparison of Data from Two Laboratories. Presented at 10th EuPFI Conference, London – EuPFI’. Available at: http://www.eupfi.org/past-conferences/10th-eupfi-conference/ (Accessed: 13 January 2022).

Jaceosidin - Cayman Chemical (no date). Available at:

https://www.caymanchem.com/product/19758 (Accessed: 28 November 2018).

Jaggupilli, A. *et al.* (2019) ‘Advanced Glycation End-Products Can Activate or Block Bitter Taste Receptors’, *Nutrients 2019, Vol. 11, Page 1317*. Multidisciplinary Digital Publishing Institute, 11(6), p. 1317. doi: 10.3390/NU11061317.

Jarrett, M. M., Scantlebury, J. and Parker, L. A. (2007) ‘Effect of Δ9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test’, *Physiology & Behavior*, 90(2–3), pp. 425–430. doi: 10.1016/j.physbeh.2006.10.003.

Johnson, T. N. and Thomson, M. (2008) ‘Intestinal metabolism and transport of drugs in children: The effects of age and disease’, *Journal of Pediatric Gastroenterology and Nutrition*, pp. 3–10. doi: 10.1097/MPG.0b013e31816a8cca.

Juluri, A. *et al.* (2016) ‘Taste Masking of Griseofulvin and Caffeine Anhydrous Using Kleptose Linecaps DE17 by Hot Melt Extrusion’, *AAPS PharmSciTech*. Springer New York LLC, 17(1), pp. 99–105. doi: 10.1208/S12249-015-0374-1/FIGURES/8.

Jungbunzlauer (2022) *Facial Moisturizer with Sodium Lactate, Sodium Gluconate and Citrofol*. Available at: https://www.personalcaremagazine.com/formulation-details/1423/facial-moisturizer-with-sodium-lactate-sodium-gluconate-and-citrofol-ai?companyid=2672 (Accessed: 16 October 2019).

Kalra, A., Bhat, P. and Kaur, I. P. (2021) ‘Deciphering molecular mechanics in

the taste masking ability of Maltodextrin: Developing pediatric formulation of Oseltamivir for viral pandemia', *Carbohydrate Polymers*. Elsevier, 260, p. 117703. doi: 10.1016/J.CARBPOL.2021.117703.

Karanewsky, D. *et al.* (2011) 'Compounds that inhibit (block) bitter taste in composition and use thereof'. United States Patent.

Karanewsky, D. S. *et al.* (2016) 'Toxicological evaluation of two novel bitter modifying flavour compounds: 3-(1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)imidazolidine-2,4-dione and 3-(1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-5,5-dimethylimidazolidine-2,4-dione.', *Toxicology reports*. Elsevier, 3, pp. 310–327. doi: 10.1016/j.toxrep.2016.02.007.

Katdare, A. and Chaubal, M. V (2006) 'Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems'. Available at: www.informa.com (Accessed: 14 March 2022).

Katrajkar, K. *et al.* (2019) 'Shedding light on interaction of so called inactive ingredients (excipients) with permeability-glycoprotein', *Journal of Drug Delivery Science and Technology*. Editions de Sante, pp. 531–552. doi: 10.1016/j.jddst.2019.05.022.

Katsuragi, Y. *et al.* (1995) 'Selective Inhibition of Bitter Taste of Various Drugs by Lipoprotein', *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists*. doi: 10.1023/A:1016295122461.

Kaushal, N. *et al.* (2016) 'Study of regulatory requirements for the conduct of bioequivalence studies in US, Europe, Canada, India, ASEAN and SADC countries: Impact on generic drug substitution', *Journal of Applied*

Pharmaceutical Science, 6(04), pp. 206–222. doi: 10.7324/JAPS.2016.60430.

Kaushik, D. and Dureja, H. (2014) 'Recent patents and patented technology platforms for pharmaceutical taste masking.', *Recent patents on drug delivery & formulation*, 8(1), pp. 37–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24499438 (Accessed: 7 March 2019).

Kearns, G. L. *et al.* (2003) 'Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children', *New England Journal of Medicine*. Edited by A. J. J. Wood. Massachusetts Medical Society , 349(12), pp. 1157–1167. doi: 10.1056/NEJMra035092.

Keast, R. S. J. (2003) 'The Effect of Zinc on Human Taste Perception', *Journal of Food Science*. Wiley/Blackwell (10.1111), 68(5), pp. 1871–1877. doi: 10.1111/j.1365-2621.2003.tb12345.x.

Keast, R. S. J. and Breslin, P. A. S. (2002) 'Modifying the bitterness of selected oral pharmaceuticals with cation and anion series of salts', *Pharmaceutical Research*. Springer, 19(7), pp. 1019–1026. doi: 10.1023/A:1016474607993.

Keast, R. S. J. and Breslin, P. A. S. (2005) 'Bitterness Suppression with Zinc Sulfate and Na-Cyclamate: A Model of Combined Peripheral and Central Neural Approaches to Flavor Modification', *Pharmaceutical Research*, 22(11), pp. 1970–1977. doi: 10.1007/s11095-005-6136-0.

Keeley, A. *et al.* (2017) 'Are Rats Supertasters?' Presented at EuPFI 9th Annual Conference 'Formulating Better Medicines for Children'.

Keeley, A. *et al.* (2019) 'In Vitro Dissolution Model Can Predict the in Vivo

Taste Masking Performance of Coated Multiparticulates', *Molecular Pharmaceutics*. American Chemical Society, 16(5), pp. 2095–2105. doi: 10.1021/ACS.MOLPHARMACEUT.9B00060/ASSET/IMAGES/ACS.MOLPHARMACEUT.9B00060.SOCIAL.JPEG_V03.

Khalil, F. and Läer, S. (2014) 'Physiologically based pharmacokinetic models in the prediction of oral drug exposure over the entire pediatric age range-sotalol as a model drug', *The AAPS journal*. AAPS J, 16(2), pp. 226–239. doi: 10.1208/S12248-013-9555-6.

Khan, D. *et al.* (2022) 'Paediatric specific dosage forms: Patient and formulation considerations', *International Journal of Pharmaceutics*. Elsevier, 616, p. 121501. doi: 10.1016/J.IJPHARM.2022.121501.

Kim, M. J. *et al.* (2015) 'Umami–bitter interactions: The suppression of bitterness by umami peptides via human bitter taste receptor', *Biochemical and Biophysical Research Communications*, 456(2), pp. 586–590. doi: 10.1016/j.bbrc.2014.11.114.

Kim, U. *et al.* (2005) 'Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci', *Human Mutation*. Hum Mutat, 26(3), pp. 199–204. doi: 10.1002/humu.20203.

Kim, U. kyung *et al.* (2003) 'Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide', *Science*. Science, 299(5610), pp. 1221–1225. doi: 10.1126/science.1080190.

Klinkesorn, U. *et al.* (2004) 'Stability and rheology of corn oil-in-water emulsions containing maltodextrin', *Food Research International*. Elsevier, 37(9), pp. 851–859. doi: 10.1016/J.FOODRES.2004.05.001.

Koch, K. M. *et al.* (1993) ‘Effect of sodium acid pyrophosphate on ranitidine bioavailability and gastrointestinal transit time.’, *Pharmaceutical research*, 10(7), pp. 1027–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8397394 (Accessed: 17 May 2018).

Kodama, O. *et al.* (1992) ‘Sakuranetin, a flavanone phytoalexin from ultraviolet-irradiated rice leaves’, *Phytochemistry*. Pergamon, 31(11), pp. 3807–3809. doi: 10.1016/S0031-9422(00)97532-0.

Koldovsky, O., Johnson, S. M. and Koldovsky, O. (1988) ‘In vitro intestinal processing of prostaglandin F2 alpha in rats: developmental aspects’, *Journal of pediatric gastroenterology and nutrition*. J Pediatr Gastroenterol Nutr, 7(5), pp. 755–761. doi: 10.1097/00005176-198809000-00022.

Korteja¨rvi, H. *et al.* (2005) *Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Ranitidine Hydrochloride*, *J Pharm Sci*. doi: 10.1002/jps.20392.

Kroeze, J. H. A. and Bartoshuk, L. M. (1985) ‘Bitterness suppression as revealed by split-tongue taste stimulation in humans’, *Physiology & Behavior*. Elsevier, 35(5), pp. 779–783. doi: 10.1016/0031-9384(85)90412-3.

Kuhn, C. *et al.* (2004) ‘Bitter taste receptors for saccharin and acesulfame K’, *Journal of Neuroscience*. Society for Neuroscience, 24(45), pp. 10260–10265. doi: 10.1523/JNEUROSCI.1225-04.2004.

Kytariolos, J. *et al.* (2013) ‘Stability and physicochemical characterization of novel milk-based oral formulations’, *International Journal of Pharmaceutics*, 444(1–2), pp. 128–138. doi: 10.1016/j.ijpharm.2013.01.022.

Labib, G. (2015) ‘Novel levocetirizine HCl tablets with enhanced palatability: synergistic effect of combining taste modifiers and effervescence technique’, *Drug Design, Development and Therapy*, 9, p. 5135. doi: 10.2147/DDDT.S92245.

Lajoinie, A. *et al.* (2014) ‘Solid oral forms availability in children: a cost saving investigation’, *British Journal of Clinical Pharmacology*. Wiley-Blackwell, 78(5), p. 1080. doi: 10.1111/BCP.12442.

Lander, A. and Newman, J. (2013) ‘Paediatric anatomy’, *Surgery (Oxford)*. Elsevier, 31(3), pp. 101–105. doi: 10.1016/J.MPSUR.2013.01.002.

Latha, R. S. and Lakshmi, P. K. (2012) ‘Electronic tongue: An analytical gustatory tool.’, *Journal of advanced pharmaceutical technology & research*. Wolters Kluwer -- Medknow Publications, 3(1), pp. 3–8. doi: 10.4103/2231-4040.93556.

Lawless, H. and Heymann, H. (2010) ‘Principles of Good Practice’, in *Sensory Evaulation of Food*. New York, NY: Springer New York, p. 58.

Lemon, C. H. (2015) ‘Perceptual and Neural Responses to Sweet Taste in Humans and Rodents’, *Chemosensory Perception*. Springer New York LLC, 8(2), pp. 46–52. doi: 10.1007/s12078-015-9177-8.

Lenhart, A. and Chey, W. D. (2017) ‘A Systematic Review of the Effects of Polyols on Gastrointestinal Health and Irritable Bowel Syndrome.’, *Advances in nutrition (Bethesda, Md.)*. American Society for Nutrition, 8(4), pp. 587–596. doi: 10.3945/an.117.015560.

Ley, J. P. *et al.* (2005) ‘Evaluation of Bitter Masking Flavanones from Herba

Santa (*Eriodictyon californicum* (H. & A.) Torr., Hydrophyllaceae)', *Journal of Agricultural and Food Chemistry*, 53(15), pp. 6061–6066. doi: 10.1021/jf0505170.

Ley, J. P. *et al.* (2006) 'New Bitter-Masking Compounds: Hydroxylated Benzoic Acid Amides of Aromatic Amines as Structural Analogues of Homoeriodictyol', *Journal of Agricultural and Food Chemistry*, 54(22), pp. 8574–8579. doi: 10.1021/jf0617061.

Ley, J. P. *et al.* (2014) 'Use Of Hydroxyflavanones For Masking Bitter Taste'. Available at: https://patentimages.storage.googleapis.com/0a/df/60/a88712de9dff03/US8685436.pdf (Accessed: 26 July 2018).

Lin, L. *et al.* (2015) 'SLC transporters as therapeutic targets: emerging opportunities', *Nature Reviews Drug Discovery 2015 14:8*. Nature Publishing Group, 14(8), pp. 543–560. doi: 10.1038/nrd4626.

Liu, B. *et al.* (2011) 'Molecular Mechanism of Species-Dependent Sweet Taste toward Artificial Sweeteners', *Journal of Neuroscience*. Society for Neuroscience, 31(30), pp. 11070–11076. doi: 10.1523/JNEUROSCI.0791-11.2011.

Liu, F. *et al.* (2014) 'Patient-Centered Pharmaceutical Design to Improve Acceptability of Medicines: Similarities and Differences in Paediatric and Geriatric Populations', *Drugs*. Springer, 74(16), p. 1871. doi: 10.1007/S40265-014-0297-2.

Locher, K. P. (2009) 'Structure and mechanism of ATP-binding cassette transporters', *Philosophical Transactions of the Royal Society B: Biological*

Sciences. Royal Society, pp. 239–245. doi: 10.1098/rstb.2008.0125.

Loftsson, T., Hreinsdóttir, D. and Másson, M. (2007) 'The complexation efficiency', *Journal of Inclusion Phenomena and Macrocyclic Chemistry*. Springer, 57(1–4), pp. 545–552. doi: 10.1007/S10847-006-9247-2/FIGURES/2.

Lopez, F. L. *et al.* (2015) 'Formulation approaches to pediatric oral drug delivery: benefits and limitations of current platforms.', *Expert opinion on drug delivery*. Taylor & Francis, 12(11), pp. 1727–40. doi: 10.1517/17425247.2015.1060218.

Lu, B., Breza, J. M. and Contreras, R. J. (2016) 'Temperature Influences Chorda Tympani Nerve Responses to Sweet, Salty, Sour, Umami, and Bitter Stimuli in Mice', *Chemical Senses*. Oxford Academic, 41(9), pp. 727–736. doi: 10.1093/CHEMSE/BJW082.

Lu, H. and Rosenbaum, S. (2014) 'Developmental Pharmacokinetics in Pediatric Populations', *The Journal of Pediatric Pharmacology and Therapeutics : JPPT*. Pediatric Pharmacology Advocacy Group, 19(4), p. 262. doi: 10.5863/1551-6776-19.4.262.

Lu, P. *et al.* (2017) 'Extraoral bitter taste receptors in health and disease.', *The Journal of general physiology*. The Rockefeller University Press, 149(2), pp. 181–197. doi: 10.1085/jgp.201611637.

Lvovskaya, S. and Smith, D. P. (2013) 'A spoonful of bitter helps the sugar response go down', *Neuron*. NIH Public Access, pp. 612–614. doi: 10.1016/j.neuron.2013.07.038.

Mackie, K. (2008) ‘Cannabinoid Receptors: Where They are and What They do’, *Journal of Neuroendocrinology*. Wiley/Blackwell (10.1111), 20(s1), pp. 10–14. doi: 10.1111/j.1365-2826.2008.01671.x.

Maehashi, K. *et al.* (2006) ‘Riboflavin-Binding Protein Exhibits Selective Sweet Suppression toward Protein Sweeteners’, *Chemical Senses*. Oxford University Press, 32(2), pp. 183–190. doi: 10.1093/chemse/bjl048.

Maehashi, K. *et al.* (2008) ‘Riboflavin-Binding Protein Is a Novel Bitter Inhibitor’, *Chemical Senses*. Oxford University Press, 33(1), pp. 57–63. doi: 10.1093/chemse/bjm062.

Mahalapbutr, P. *et al.* (2019) ‘Atomistic mechanisms underlying the activation of the G protein-coupled sweet receptor heterodimer by sugar alcohol recognition’, *Scientific Reports 2019 9:1*. Nature Publishing Group, 9(1), pp. 1–11. doi: 10.1038/s41598-019-46668-w.

Mai, Y. *et al.* (2019) ‘Sex-Dependence in the Effect of Pharmaceutical Excipients: Polyoxyethylated Solubilising Excipients Increase Oral Drug Bioavailability in Male but not Female Rats.’, *Pharmaceutics*. Multidisciplinary Digital Publishing Institute (MDPI), 11(5). doi: 10.3390/pharmaceutics11050228.

Malode, S. and Gudsoorkar, V. (2014) ‘Taste Masking : Overview of Taste Assessment Approaches in the development of oral pharmaceutical formulations’, *Indo American Journal of Pharmaceutical Research*, 4(01), pp. 204–211. doi: 10.1044/1980-iajpr.01322.

Manor, P. C. and Saenger, W. (1974) ‘Topography of cyclodextrin inclusion complexes. III. Crystal and molecular structure of cyclohexaamylose

hexahydrate, the water dimer inclusion complex', *Journal of the American Chemical Society*. American Chemical Society, 96(11), pp. 3630–3639. doi: 10.1021/ja00818a042.

Margolskee, R. and Ming, D. (2003) 'Inhibitors of the bitter taste response'. Available at: https://patentimages.storage.googleapis.com/f1/fd/02/f75894eee2c319/US6540978.pdf (Accessed: 26 July 2018).

Martel, F. *et al.* (1998) 'Postnatal development of organic cation transport in the rat liver', *Pharmacological Research*. Academic Press, 37(2), pp. 131–136. doi: 10.1006/phrs.1997.0283.

Martínez-García, M. A. *et al.* (2002) 'Analysis of the Stability of Stored Adenosine 5′-monophosphate used for Bronchoprovocation', *Pulmonary Pharmacology & Therapeutics*, 15(2), pp. 157–160. doi: 10.1006/pupt.2001.0334.

Masamoto, M. *et al.* (2020) 'Effects of bitter receptor antagonists on behavioral lick responses of mice', *Neuroscience Letters*. Elsevier, 730, p. 135041. doi: 10.1016/J.NEULET.2020.135041.

Matsson, P. *et al.* (2009) 'Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs', *Pharmaceutical Research*. Pharm Res, 26(8), pp. 1816–1831. doi: 10.1007/s11095-009-9896-0.

Matsuoka, Y. *et al.* (1999) 'Developmental expression of P-glycoprotein (multidrug resistance gene product) in the rat brain', *Journal of Neurobiology*. J Neurobiol, 39(3), pp. 383–392. doi: 10.1002/(SICI)1097-

4695(19990605)39:3<383::AID-NEU5>3.0.CO;2-4.

McEwan, J. A. and Lyon, D. H. (2003) ‘Sensory Rating and Scoring Methods’, in *Encyclopedia of Food Sciences and Nutrition*. Academic Press, pp. 5148–5152. doi: 10.1016/B0-12-227055-X/01064-6.

McGregor, R. (2007) ‘Bitter blockers in foods and pharmaceuticals’, in *Modifying Flavour in Food*. Elsevier, pp. 221–231. doi: 10.1533/9781845693367.221.

McKinley, M. J. *et al.* (2006) ‘Differential effects of aging on fluid intake in response to hypovolemia, hypertonicity, and hormonal stimuli in Munich Wistar rats’, *Proceedings of the National Academy of Sciences of the United States of America*, 103(9), pp. 3450–3455. doi: 10.1073/PNAS.0511099103/ASSET/3BC37D4C-7E1A-4F47-A513-BFC230884DBE/ASSETS/GRAPHIC/ZPQ0060611730005.JPEG.

Mclaughlin, S. K., Mckinnon, P. J. and Margolskee, R. F. (1992) ‘Gustducin is a taste-cell-specific G protein closely related to the transducins’.

McOmber, M. E., Ou, C. N. and Shulman, R. J. (2010) ‘Effects of Timing, Sex, and Age on Site-Specific Gastrointestinal Permeability Testing in Children and Adults’, *Journal of pediatric gastroenterology and nutrition*. NIH Public Access, 50(3), p. 269. doi: 10.1097/MPG.0B013E3181AA3AA9.

Mehrotra, N. *et al.* (2006) ‘The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy’, *International Journal of Impotence Research 2007 19:3*. Nature Publishing Group, 19(3), pp. 253–264. doi: 10.1038/sj.ijir.3901522.

Mennella, J. A. *et al.* (2013) 'The Bad Taste of Medicines: Overview of Basic Research on Bitter Taste', *Clinical Therapeutics*. Elsevier, 35(8), pp. 1225–1246. doi: 10.1016/j.clinthera.2013.06.007.

Mennella, J. A. *et al.* (2014) 'Age-Related Differences in Bitter Taste and Efficacy of Bitter Blockers', *PLoS ONE*. Edited by M. Behrens, 9(7), p. e103107. doi: 10.1371/journal.pone.0103107.

Mennella, J. A. *et al.* (2015) '"A spoonful of sugar helps the medicine go down": bitter masking by sucrose among children and adults', *Chemical senses*. Chem Senses, 40(1), pp. 17–25. doi: 10.1093/chemse/bju053.

Mennella, J. A. and Beauchamp, G. K. (2008) 'Optimizing oral medications for children.', *Clinical therapeutics*. NIH Public Access, 30(11), pp. 2120–32. doi: 10.1016/j.clinthera.2008.11.018.

Mennella, J. A. and Bobowski, N. K. (2015) 'The sweetness and bitterness of childhood: Insights from basic research on taste preferences', *Physiology and Behavior*. Elsevier Inc., 152(0 0), pp. 502–507. doi: 10.1016/j.physbeh.2015.05.015.

Mennella, J. A., Mathew, P. S. and Lowenthal, E. D. (2017) 'Use of Adult Sensory Panel to Study Individual Differences in the Palatability of a Pediatric HIV Treatment Drug', *Clinical Therapeutics*. Excerpta Medica Inc., 39(10), pp. 2038–2048. doi: 10.1016/j.clinthera.2017.08.012.

Mennella, J. A., Pepino, M. Y. and Beauchamp, G. K. (2003) 'Modification of bitter taste in children.', *Developmental psychobiology*. NIH Public Access, 43(2), pp. 120–7. doi: 10.1002/dev.10127.

Metry, M. and Polli, J. E. (2022) ‘Evaluation of Excipient Risk in BCS Class I and III Biowaivers’, *AAPS Journal*. Springer Science and Business Media Deutschland GmbH, 24(1), pp. 1–11. doi: 10.1208/S12248-021-00670-1/TABLES/1.

Meyerhof, W. *et al.* (2010) ‘The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors’, *Chem. Senses*, 35, pp. 157–170. doi: 10.1093/chemse/bjp092.

Mikiko, I. and Mutsuko, T. (2008) ‘Effect of extraction conditions and chlorogenic acid content on the taste of coffee’, *Japan Cookery Science Journal*, 41(4), pp. 257–261. doi: 10.11402/cookeryscience1995.41.4_257.

Milne, C. (2005) ‘The pediatric studies initiative.’, in Mathieu, M. (ed.) *New Drug Development: A Regulatory Overview.* 8th ed. Waltham, Mass: PAREXEL Internation, pp. 339–362.

Ming, D., Ninomiya, Y. and Margolskee, R. F. (1999) ‘Blocking taste receptor activation of gustducin inhibits gustatory responses to bitter compounds’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 96(17), pp. 9903–9908. doi: 10.1073/pnas.96.17.9903.

Miyoshi, M. A., Abe, K. and Emori, Y. (2001) ‘IP(3) receptor type 3 and PLCbeta2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells.’, *Chemical senses*, 26(3), pp. 259–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11287386 (Accessed: 24 April 2019).

Mohamed-Ahmed, A. H. A. *et al.* (2016) ‘Non-human tools for the evaluation of bitter taste in the design and development of medicines: a systematic

review', *Drug Discovery Today*. Elsevier Current Trends, 21(7), pp. 1170–1180. doi: 10.1016/J.DRUDIS.2016.05.014.

Moher, D. *et al.* (2009) 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement', *PLoS Medicine*. Public Library of Science, 6(7), p. e1000097. doi: 10.1371/journal.pmed.1000097.

Mojet, J., Christ-Hazelhof, E. and Heidema, J. (2001) 'Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes?', *Chemical senses*. Chem Senses, 26(7), pp. 845–860. doi: 10.1093/CHEMSE/26.7.845.

Monk, K. J. *et al.* (2014) 'Licking Microstructure Reveals Rapid Attenuation of Neophobia', *Chemical Senses*. Oxford University Press, 39(3), p. 203. doi: 10.1093/CHEMSE/BJT069.

Mortensen, A. *et al.* (2016) 'Re-evaluation of β-cyclodextrin (E 459) as a food additive', *EFSA Journal*. Wiley, 14(12). doi: 10.2903/J.EFSA.2016.4628.

Mossavar-Rahmani, Y. *et al.* (2019) 'Artificially sweetened beverages and stroke, coronary heart disease, and all-cause mortality in the women's health initiative', *Stroke*. Lippincott Williams and Wilkins, 50(3), pp. 555–562. doi: 10.1161/STROKEAHA.118.023100.

Münster, M. *et al.* (2017) 'Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations', *International journal of pharmaceutics*. Int J Pharm, 529(1–2), pp. 310–318. doi: 10.1016/J.IJPHARM.2017.06.084.

Mura, E. *et al.* (2018) 'Innate and acquired tolerance to bitter stimuli in mice'. doi: 10.1371/journal.pone.0210032.

Murakami, T. (2017) ‘Absorption sites of orally administered drugs in the small intestine’, *http://dx.doi.org/10.1080/17460441.2017.1378176*. Taylor & Francis, 12(12), pp. 1219–1232. doi: 10.1080/17460441.2017.1378176.

Nader, A. M. *et al.* (2016) ‘Effect of Gastric Fluid Volume on the In Vitro Dissolution and In Vivo Absorption of BCS Class II Drugs: a Case Study with Nifedipine’, *The AAPS journal*. AAPS J, 18(4), pp. 981–988. doi: 10.1208/S12248-016-9918-X.

Nair, A. B. and Jacob, S. (2016) ‘A simple practice guide for dose conversion between animals and human’, *Journal of Basic and Clinical Pharmacy*. Wolters Kluwer -- Medknow Publications, 7(2), p. 27. doi: 10.4103/0976-0105.177703.

Nakamura, T. *et al.* (2002) ‘The effect of various substances on the suppression of the bitterness of quinine-human gustatory sensation, binding, and taste sensor studies.’, *Chemical & pharmaceutical bulletin*, 50(12), pp. 1589–93. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12499596 (Accessed: 21 November 2018).

Narukawa, M. *et al.* (2017) ‘Participation of the peripheral taste system in aging-dependent changes in taste sensitivity’, *Neuroscience*. Pergamon, 358, pp. 249–260. doi: 10.1016/J.NEUROSCIENCE.2017.06.054.

National Cancer Institute (2016) *Artificial Sweeteners and Cancer - National Cancer Institute*. Available at: https://www.cancer.gov/about-cancer/causes-prevention/risk/diet/artificial-sweeteners-fact-sheet (Accessed: 24 February 2022).

Neath, K. N. *et al.* (2010) ‘Increased liking for a solution is not necessary for

the attenuation of neophobia in rats', *Behavioral neuroscience*. NIH Public Access, 124(3), p. 398. doi: 10.1037/A0019505.

Niazi, S. K. (2004) 'Handbook of Pharmaceutical Manufacturing Formulations: Liquid Products'.

Nicolas, J. M. *et al.* (2017) 'Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions', *Biopharmaceutics & Drug Disposition*. Wiley-Blackwell, 38(3), p. 209. doi: 10.1002/BDD.2052.

Nieuwlaat, R. *et al.* (2014) 'Interventions for enhancing medication adherence', *The Cochrane database of systematic reviews*. Cochrane Database Syst Rev, 2014(11). doi: 10.1002/14651858.CD000011.PUB4.

Nigam, S. K. and Bhatnagar, V. (2013) 'How Much Do We Know About Drug Handling by SLC and ABC Drug Transporters in Children?', *Clinical Pharmacology & Therapeutics*. John Wiley & Sons, Ltd, 94(1), pp. 27–29. doi: 10.1038/CLPT.2013.82.

Noorjahan, A., Amrita, B. and Kavita, S. (2014) '*In vivo* evaluation of taste masking for developed chewable and orodispersible tablets in humans and rats', *Pharmaceutical Development and Technology*, 19(3), pp. 290–295. doi: 10.3109/10837450.2013.778870.

Nordenmalm, S. *et al.* (2019) 'Children's views on taking medicines and participating in clinical trials', *Archives of Disease in Childhood*. BMJ Publishing Group Ltd, 104(9), pp. 900–905. doi: 10.1136/ARCHDISCHILD-2018-316511.

Nunn, T. and Williams, J. (2005) ‘Formulation of medicines for children’, *British Journal of Clinical Pharmacology*. Wiley-Blackwell, 59(6), p. 674. doi: 10.1111/J.1365-2125.2005.02410.X.

O’Mahony, M. and Rousseau, B. (2003) ‘Discrimination testing: a few ideas, old and new’, *Food Quality and Preference*. Elsevier, 14(2), pp. 157–164. doi: 10.1016/S0950-3293(02)00109-X.

Ogawa, T. *et al.* (2004) ‘The combination effect of L-arginine and NaCl on bitterness suppression of amino acid solutions’, *Chemical and Pharmaceutical Bulletin*. Chem Pharm Bull (Tokyo), 52(2), pp. 172–177. doi: 10.1248/cpb.52.172.

Ohkubo, S. and Nakahata, N. (2007) ‘The Role of Lipd Rafts in Trimeric G Protein-Mediated Signal Transduction’, *ChemInform*. Wiley, 38(19). doi: 10.1002/CHIN.200719265.

Ojiro, I. *et al.* (2021) ‘Trp-Trp acts as a multifunctional blocker for human bitter taste receptors, hTAS2R14, hTAS2R16, hTAS2R43, and hTAS2R46’, *Bioscience, Biotechnology, and Biochemistry*. Oxford Academic, 85(6), pp. 1526–1529. doi: 10.1093/BBB/ZBAB061.

Okada, S. (2015) ‘The taste system of small fish species’, *Bioscience, Biotechnology, and Biochemistry*. Japan Society for Bioscience Biotechnology and Agrochemistry, 79(7), pp. 1039–1043. doi: 10.1080/09168451.2015.1023251.

Al Omari, M. M. *et al.* (2006) ‘Sildenafil/cyclodextrin complexation: Stability constants, thermodynamics, and guest–host interactions probed by 1H NMR and molecular modeling studies’, *Journal of Pharmaceutical and Biomedical*

Analysis. Elsevier, 41(3), pp. 857–865. doi: 10.1016/J.JPBA.2006.01.055.

Ono, N. *et al.* (2011) 'Reduction of Bitterness of Antihistaminic Drugs by Complexation with β-Cyclodextrins', *Journal of Pharmaceutical Sciences*, 100(5), pp. 1935–1943. doi: 10.1002/jps.22417.

Østergaard, G. and Knudsen, I. (1998) 'The applicability of the ADI (Acceptable Daily Intake) for food additives to infants and children', *Food additives and contaminants*. Food Addit Contam, 15 Suppl(SUPPL.), pp. 63–74. doi: 10.1080/02652039809374617.

Ouranidis, A. *et al.* (2021) 'Sildenafil 4.0—Integrated Synthetic Chemistry, Formulation and Analytical Strategies Effecting Immense Therapeutic and Societal Impact in the Fourth Industrial Era', *Pharmaceuticals*. Multidisciplinary Digital Publishing Institute (MDPI), 14(4). doi: 10.3390/PH14040365.

Paladino, A. *et al.* (2008) 'Molecular modelling of miraculin: Structural analyses and functional hypotheses', *Biochemical and Biophysical Research Communications*. Academic Press, 367(1), pp. 26–32. doi: 10.1016/J.BBRC.2007.12.102.

Palmer, R. K. (2007) 'The Pharmacology and Signaling of Bitter, Sweet, and Umami Taste Sensing', *Molecular Interventions*, 7(2), pp. 87–98. doi: 10.1124/mi.7.2.9.

Palmer, R. K. *et al.* (2010) 'Triphenylphosphine Oxide Is a Potent and Selective Inhibitor of the Transient Receptor Potential Melastatin-5 Ion Channel', *ASSAY and Drug Development Technologies*, 8(6), pp. 703–713. doi: 10.1089/adt.2010.0334.

Palmer, R. K. *et al.* (2013) ‘A High Throughput In Vivo Assay for Taste Quality and Palatability’, *PLoS ONE*. Edited by J. I. Glendinning. Public Library of Science, 8(8), p. e72391. doi: 10.1371/journal.pone.0072391.

Panakanti, R. and Narang, A. S. (2012) ‘Impact of excipient interactions on drug bioavailability from solid dosage forms’, *Pharmaceutical research*. Pharm Res, 29(10), pp. 2639–2659. doi: 10.1007/S11095-012-0767-8.

Parikh, A., Agarwal, S. and Raut, K. (2014) ‘A review on applications of maltodextrin in pharmaceutical industry’, *International Journal of Pharmacy and Biological Sciences*, 4(4), pp. 67–74. Available at: https://www.semanticscholar.org/paper/A-REVIEW-ON-APPLICATIONS-OF-MALTODEXTRIN-IN-Parikh-Agarwal/c74b58a29ccff435f2908fc428eb7dfb274ce432 (Accessed: 3 March 2022).

Patron, A. *et al.* (2016) ‘Bittertaste modifiers including substituted 1-benzyl-3-(1-(isoxazol-4-ylmethyl)- 1H-pyrazol-4-YL)imidazolidine-2, 4-dones and compositions thereof’. Available at: https://patentimages.storage.googleapis.com/86/de/c9/229194288bce7b/US20160376263A1.pdf (Accessed: 2 August 2018).

Pavlov, G. M. *et al.* (2010) ‘Hydrodynamic properties of cyclodextrin molecules in dilute solutions.’, *European biophysics journal : EBJ*. Springer, 39(3), pp. 371–9. doi: 10.1007/s00249-008-0394-9.

Pawar, S. and Kumar, A. (2002) ‘Issues in the formulation of drugs for oral use in children: role of excipients’, *Paediatric drugs*. Paediatr Drugs, 4(6), pp. 371–379. doi: 10.2165/00128072-200204060-00004.

Pearlman, M., Obert, J. and Casey, L. (2017) ‘The Association Between Artificial Sweeteners and Obesity’, *Current Gastroenterology Reports*. Current Medicine Group LLC 1, 19(12), pp. 1–8. doi: 10.1007/S11894-017-0602-9/TABLES/1.

Pediatric Drug Delivery Systems - Drinking Straw XStraw (no date). Available at: https://www.pharmaceuticalonline.com/doc/pediatric-drug-delivery-systems-drinking-straw-xstraw-0001.

Pedrosa, A. and Serrano, M. (2000) ‘Solubilities of Sodium Gluconate in Water and in Aqueous Solutions of Ethanol and Methanol’, *J. Chem. Eng. Data*. American Chemical Society, 45(3), pp. 461–463. doi: 10.1021/JE990305X.

Pein, M. *et al.* (2014) ‘Taste-masking assessment of solid oral dosage forms–A critical review’, *International Journal of Pharmaceutics*. Elsevier, 465(1–2), pp. 239–254. doi: 10.1016/J.IJPHARM.2014.01.036.

Penniston, K. L. *et al.* (2008) ‘Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products.’, *Journal of endourology*. NIH Public Access, 22(3), pp. 567–70. doi: 10.1089/end.2007.0304.

Pereira, A. G. *et al.* (2021) ‘Main Applications of Cyclodextrins in the Food Industry as the Compounds of Choice to Form Host–Guest Complexes’, *International Journal of Molecular Sciences*. Multidisciplinary Digital Publishing Institute (MDPI), 22(3), pp. 1–23. doi: 10.3390/IJMS22031339.

Peryam, D. R. and Girardot, N. F. (1952) ‘Advanced taste test method’, *Food Engineering*, 24(194), pp. 58–61.

Phenylthiocarbamide (PTC) (no date). Available at: http://bitterdb.agri.huji.ac.il/compound.php?mode_organism=default&id=119 (Accessed: 24 August 2020).

Podrażka, M. *et al.* (2017) 'Electronic Tongue-A Tool for All Tastes?', *Biosensors*. Multidisciplinary Digital Publishing Institute (MDPI), 8(1). doi: 10.3390/bios8010003.

Popescu, C. *et al.* (2012) 'NSAIDs Taste Masking by New Maltodextrins (KLEPTOSE® Linecaps)'. Available at: https://www.roquette.com/-/media/contenus-gbu/pharma/scientific-posters/roquette-pharma-case-study-nsaids-taste-masking-by-new-maltodextrins-kleptose-linecaps.pdf.

Popper, R. and Kroll, J. J. (2011) 'Consumer testing of food products using children', in *Developing Children's Food Products*. Woodhead Publishing, pp. 163–187. doi: 10.1533/9780857091130.3.163.

Prasad, B. *et al.* (2016) 'Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics', *Clinical pharmacology and therapeutics*. Clin Pharmacol Ther, 100(4), pp. 362–370. doi: 10.1002/CPT.409.

Preis, M. *et al.* (2014) 'A comparative study on solubilizing and taste-masking capacities of hydroxypropyl-β-cyclodextrin and maltodextrins with high amylose content', *Sensors and Actuators B: Chemical*. Elsevier, 193, pp. 442–450. doi: 10.1016/J.SNB.2013.12.005.

Preis, M., Pein, M. and Breitkreutz, J. (2021) 'Development of a Taste-Masked Orodispersible Film Containing Dimenhydrinate', *Pharmaceutics*, 4, pp. 551–562. doi: 10.3390/pharmaceutics4040551.

Prescott, F. J. *et al.* (1953) ‘Gluconic Acid and Its Derivatives’, *Industrial & Engineering Chemistry*. American Chemical Society (ACS), 45(2), pp. 338–342. doi: 10.1021/ie50518a030.

Products, N. (2004) *Linguagens AMP Product to Obtain GRAS Status*, *Natural Products*. Available at: https://www.naturalproductsinsider.com/regulatory/linguagens-amp-product-obtain-gras-status (Accessed: 30 November 2018).

PubChem (no date a) *Abscisic acid*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/abscisic_acid#section=Top (Accessed: 23 November 2018).

PubChem (no date b) *Sodium acetate*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Sodium_acetate#section=Melting-Point (Accessed: 28 November 2018).

PubChem (no date c) *Sodium chloride*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/sodium_chloride#section=Melting-Point (Accessed: 28 November 2018).

PubChem (no date d) *Triphenylphosphine oxide*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/triphenylphosphine_oxide#section=GHS-Classification (Accessed: 23 November 2018).

Putseys, J. A., Lamberts, L. and Delcour, J. A. (2010) ‘Amylose-inclusion complexes: Formation, identity and physico-chemical properties’, *Journal of Cereal Science*. Academic Press, 51(3), pp. 238–247. doi: 10.1016/J.JCS.2010.01.011.

Pydi, S. P. *et al.* (2014) ‘Amino acid derivatives as bitter taste receptor (T2R) blockers.’, *The Journal of biological chemistry*, 289(36), pp. 25054–66. doi: 10.1074/jbc.M114.576975.

Pydi, S. P. *et al.* (2015) ‘Abscisic Acid Acts as a Blocker of the Bitter Taste G Protein-Coupled Receptor T2R4.’, *Biochemistry*, 54(16), pp. 2622–31. doi: 10.1021/acs.biochem.5b00265.

Radeloff, M. A. and Beck, R. H. F. (2013) ‘Polyols – more than sweeteners’, *Sugar Industry*. Verlag Dr. Albert Bartens KG, pp. 226–234. doi: 10.36961/SI14095.

Raffaini, G. and Ganazzoli, F. (2020) ‘Understanding Surface Interaction and Inclusion Complexes between Piroxicam and Native or Crosslinked β-Cyclodextrins: The Role of Drug Concentration’, *Molecules*. Multidisciplinary Digital Publishing Institute (MDPI), 25(12). doi: 10.3390/MOLECULES25122848.

Ranbaxy Limited (2020) ‘Clarithromycin oral suspension patient information leaflet’. Available at: https://www.medicines.org.uk/emc/files/pil.4360.pdf (Accessed: 24 February 2022).

Rashed, A. N. *et al.* (2021) ‘Feasibility of developing children’s Pill School within a UK hospital’, *Archives of disease in childhood*. Arch Dis Child, 106(7), pp. 705–708. doi: 10.1136/ARCHDISCHILD-2020-319154.

Reed-Knight, B., Lewis, J. D. and Blount, R. L. (2011) ‘Association of disease, adolescent, and family factors with medication adherence in pediatric inflammatory bowel disease’, *Journal of pediatric psychology*. J Pediatr Psychol, 36(3), pp. 308–317. doi: 10.1093/JPEPSY/JSQ076.

Riboflavin Binding Protein from chicken egg white lyophilized powder | *Sigma-Aldrich* (no date). Available at: https://www.sigmaaldrich.com/catalog/product/sigma/r5258?lang=en&region=GB (Accessed: 16 February 2019).

Riera, C. E. *et al.* (2007) 'Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. American Physiological Society, 293(2), pp. R626–R634. doi: 10.1152/ajpregu.00286.2007.

Van Riet-Nales, D. A. *et al.* (2013) 'Acceptability of different oral formulations in infants and preschool children', *Archives of Disease in Childhood*. BMJ Publishing Group Ltd, 98(9), pp. 725–731. doi: 10.1136/ARCHDISCHILD-2012-303303.

Robino, A. *et al.* (2014) 'A population-based approach to study the impact of PROP perception on food liking in populations along the Silk Road', *PloS one*. PLoS One, 9(3). doi: 10.1371/JOURNAL.PONE.0091716.

Roland, W. S. U. *et al.* (2014) '6-Methoxyflavanones as Bitter Taste Receptor Blockers for hTAS2R39', *PLoS ONE*. Edited by J. Reisert, 9(4), p. e94451. doi: 10.1371/journal.pone.0094451.

Rouaz, K. *et al.* (2021) 'Excipients in the Paediatric Population: A Review', *Pharmaceutics*, 13(3), p. 387. doi: 10.3390/pharmaceutics13030387.

Roudnitzky, N. *et al.* (2015) 'Receptor Polymorphism and Genomic Structure Interact to Shape Bitter Taste Perception', *PLoS Genetics*. Public Library of Science, 11(9). doi: 10.1371/journal.pgen.1005530.

Roudnitzky, N. *et al.* (2016) ‘Copy number variation in TAS2R bitter taste receptor genes: Structure, origin, and population genetics’, *Chemical Senses*. Oxford University Press, 41(8), pp. 649–659. doi: 10.1093/chemse/bjw067.

Royal College of Paediatrics and Child Health (2021) *Medicine safety alert - ranitidine (withdrawn)* . Available at: https://www.rcpch.ac.uk/news-events/news/medicine-safety-alert-ranitidine-withdrawn (Accessed: 20 March 2022).

Rudnitskaya, A. *et al.* (2013) ‘Assessment of bitter taste of pharmaceuticals with multisensor system employing 3 way PLS regression’, *Analytica Chimica Acta*, 770, pp. 45–52. doi: 10.1016/j.aca.2013.02.006.

Ruiz-Ojeda, F. J. *et al.* (2019) ‘Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials.’, *Advances in nutrition (Bethesda, Md.)*. American Society for Nutrition, 10(suppl_1), pp. S31–S48. doi: 10.1093/advances/nmy037.

Ruiz, F. *et al.* (2019) ‘Sex differences in medicine acceptability: A new factor to be considered in medicine formulation’, *Pharmaceutics*. MDPI AG, 11(8). doi: 10.3390/pharmaceutics11080368.

Rupesh, S. and Nayak, U. (2006) ‘Genetic sensitivity to the bitter taste of 6-n propylthiouracil: A new risk determinant for dental caries in children’, *Journal of Indian Society of Pedodontics and Preventive Dentistry*. Medknow Publications, 24(2), p. 63. doi: 10.4103/0970-4388.26018.

Sakuranetin - PubChem (no date). Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Sakuranetin#section=Safety-and-Hazards (Accessed: 28 November 2018).

sakuranetin - TGSC (no date). Available at: http://www.thegoodscentscompany.com/data/rw1588731.html (Accessed: 16 October 2019).

Salminen, E. K. *et al.* (1989) 'Xylitol vs glucose: effect on the rate of gastric emptying and motilin, insulin, and gastric inhibitory polypeptide release', *The American Journal of Clinical Nutrition*, 49(6), pp. 1228–1232. doi: 10.1093/ajcn/49.6.1228.

Sanematsu, K. *et al.* (2016) 'Intracellular acidification is required for full activation of the sweet taste receptor by miraculin', *Scientific Reports*. Nature Publishing Group, 6(1), pp. 1–7. doi: 10.1038/srep22807.

Santer, M. *et al.* (2014) 'Treatment non-adherence in pediatric long-term medical conditions: systematic review and synthesis of qualitative studies of caregivers' views', *BMC pediatrics*. BMC Pediatr, 14(1). doi: 10.1186/1471-2431-14-63.

Saokham, P. *et al.* (2018) 'Solubility of Cyclodextrins and Drug/Cyclodextrin Complexes', *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*. Multidisciplinary Digital Publishing Institute (MDPI), 23(5). doi: 10.3390/MOLECULES23051161.

Sarah French, A. *et al.* (2015) 'Dual mechanism for bitter avoidance in drosophila', *Journal of Neuroscience*. Society for Neuroscience, 35(9), pp. 3990–4004. doi: 10.1523/JNEUROSCI.1312-14.2015.

Savage, J. H. *et al.* (2007) 'The natural history of egg allergy', *Journal of Allergy and Clinical Immunology*, 120(6), pp. 1413–1417. doi: 10.1016/j.jaci.2007.09.040.

Schiffman, S. S. and Abou-Donia, M. B. (2012) ‘Sucralose revisited: Rebuttal of two papers about Splenda safety’, *Regulatory Toxicology and Pharmacology*. Academic Press Inc., 63(3), pp. 505–508. doi: 10.1016/J.YRTPH.2012.05.002.

Schulze, J. D. R. *et al.* (2006) ‘Impact of formulation excipients on human intestinal transit’, *Journal of Pharmacy and Pharmacology*, 58(6), pp. 821–825. doi: 10.1211/jpp.58.6.0012.

Schwartz, D. D. and Axelrad, M. E. (2015) ‘Healthcare Partnerships for Pediatric Adherence’. Cham: Springer International Publishing (SpringerBriefs in Public Health). doi: 10.1007/978-3-319-13668-4.

Schwiebert, E. *et al.* (2021) ‘Inhibition of Bitter Taste from Oral Tenofovir Alafenamide’, *Molecular Pharmacology*. American Society for Pharmacology and Experimental Therapeutics, 99(5), pp. 319–327. doi: 10.1124/MOLPHARM.120.000071.

Science Direct Topics (no date) *L-Arginine*. Available at: https://www.sciencedirect.com/topics/chemistry/l-arginine (Accessed: 3 December 2018).

Sclafani, A. (1986) ‘Carbohydrate Taste, Appetite, and Obesity: An Overview I’, *Neuroscience & Biobehavioral Reviews*, pp. 131–153.

Sclafani, A. (2004) ‘The sixth taste?’, *Appetite*. Academic Press, 43(1), pp. 1–3. doi: 10.1016/J.APPET.2004.03.007.

Sengupta, P. (2013) ‘The laboratory rat: Relating its age with human’s’, *International Journal of Preventive Medicine*. Wolters Kluwer -- Medknow

Publications, pp. 624–630.

Senopsys (2018) *The Role of Sweeteners in Taste Masking*. Available at: https://www.senopsys.com/taste-masking-blog/role-sweeteners-taste-masking/ (Accessed: 23 February 2022).

Serajuddin, A. T. M. *et al.* (1999) 'Selection of solid dosage form composition through drug–excipient compatibility testing', *Journal of Pharmaceutical Sciences*. John Wiley & Sons, Ltd, 88(7), pp. 696–704. doi: 10.1021/js980434g.

du Sert, N. P. *et al.* (2020) 'Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0', *PLOS Biology*. Public Library of Science, 18(7), p. e3000411. doi: 10.1371/JOURNAL.PBIO.3000411.

Sharafi, M., Hayes, J. E. and Duffy, V. B. (2013) 'Masking Vegetable Bitterness to Improve Palatability Depends on Vegetable Type and Taste Phenotype.', *Chemosensory perception*. NIH Public Access, 6(1), pp. 8–19. doi: 10.1007/s12078-012-9137-5.

Sharma, G. *et al.* (2015) 'Development of Lipid Membrane Based Taste Sensors for Electronic Tongue', *Procedia Computer Science*. Elsevier, 70, pp. 146–152. doi: 10.1016/J.PROCS.2015.10.062.

Sharom, F. J. (2011) 'The P-glycoprotein multidrug transporter', *Essays In Biochemistry*, 50(1), pp. 161–178. doi: 10.1042/bse0500161.

Shigemura, N. *et al.* (2013) 'Angiotensin II Modulates Salty and Sweet Taste Sensitivities', *Journal of Neuroscience*, 33(15), pp. 6267–6277. doi: 10.1523/JNEUROSCI.5599-12.2013.

Shil, A. and Chichger, H. (2021) ‘Artificial Sweeteners Negatively Regulate Pathogenic Characteristics of Two Model Gut Bacteria, E. coli and E. faecalis’, *International Journal of Molecular Sciences 2021, Vol. 22, Page 5228*. Multidisciplinary Digital Publishing Institute, 22(10), p. 5228. doi: 10.3390/IJMS22105228.

Shin, Y. K. *et al.* (2012) ‘Age-Related Changes in Mouse Taste Bud Morphology, Hormone Expression, and Taste Responsivity’, *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*. Oxford University Press, 67A(4), p. 336. doi: 10.1093/GERONA/GLR192.

Shiraishi, S. *et al.* (2017) ‘Taste-Masking Effect of Chlorogenic Acid (CGA) on Bitter Drugs Evaluated by Taste Sensor and Surface Plasmon Resonance on the Basis of CGA–Drug Interactions’, *CHEMICAL & PHARMACEUTICAL BULLETIN*. The Pharmaceutical Society of Japan, 65(2), pp. 127–133. doi: 10.1248/cpb.c16-00621.

Sigma-Aldrich (2022) *Adenosine 5'-monophosphate sodium salt from yeast, ≥99%* | *5'-Adenylic acid* | *Sigma-Aldrich*. Available at: https://www.sigmaaldrich.com/catalog/product/sigma/a1752?lang=en&region=GB (Accessed: 6 January 2020).

Sigma-Aldrich (no date a) *Thapsigargin ≥98% (HPLC), solid film*. Available at: https://www.sigmaaldrich.com/catalog/product/sigma/t9033?lang=en&region=GB (Accessed: 21 November 2018).

Sigma-Aldrich (no date b) *U-73122 hydrate powder*. Available at: https://www.sigmaaldrich.com/catalog/product/sigma/u6756?lang=en&region=GB (Accessed: 21 November 2018).

Silvius, J. R. (2003) ‘Role of cholesterol in lipid raft formation: Lessons from lipid model systems’, *Biochimica et Biophysica Acta - Biomembranes*. Elsevier, 1610(2), pp. 174–183. doi: 10.1016/S0005-2736(03)00016-6.

Sjöstedt, N. *et al.* (2017) ‘Interaction of Food Additives with Intestinal Efflux Transporters’, *Molecular Pharmaceutics*. American Chemical Society, 14(11), pp. 3824–3833. doi: 10.1021/acs.molpharmaceut.7b00563.

Slack, J. P. *et al.* (2010) ‘Modulation of Bitter Taste Perception by a Small Molecule hTAS2R Antagonist’, *Current Biology*. Cell Press, 20(12), pp. 1104–1109. doi: 10.1016/J.CUB.2010.04.043.

Smith, D. V. and van der Klaauw, N. J. (1995) ‘The Perception of Saltiness is Eliminated by NaCl Adaptation: Implications for Gustatory Transduction and Coding’, *Chemical Senses*. Narnia, 20(5), pp. 545–557. doi: 10.1093/chemse/20.5.545.

Smith, J. C. (2001) ‘The history of the “Davis Rig”.’, *Appetite*, 36(1), pp. 93–98. doi: 10.1006/APPE.2000.0372.

Soetewey Antoine (2020) *Outliers detection in R*. Available at: https://statsandr.com/blog/outliers-detection-in-r/ (Accessed: 14 June 2022).

Sohi, H., Sultana, Y. and Khar, R. K. (2004) ‘Taste Masking Technologies in Oral Pharmaceuticals: Recent Developments and Approaches’, *Drug Development and Industrial Pharmacy*. Taylor & Francis, 30(5), pp. 429–448. doi: 10.1081/DDC-120037477.

Soldo, T. and Hofmann, T. (2005) ‘Application of Hydrophilic Interaction Liquid Chromatography/Comparative Taste Dilution Analysis for Identification of a

Bitter Inhibitor by a Combinatorial Approach Based on Maillard Reaction Chemistry'. American Chemical Society. doi: 10.1021/JF051701O.

Solubility Information Sigma-Aldrich (no date). Available at: https://www.sigmaaldrich.com/united-kingdom/technical-services/solubility.html (Accessed: 16 October 2019).

Soto, J. *et al.* (2015) 'Development of a model for robust and exploratory analysis of the rodent brief-access taste aversion data', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier B.V., 91, pp. 47–51. doi: 10.1016/j.ejpb.2015.01.016.

Soto, J. (2016) *Assessing the feasbility of using an animal model for in vivo taste assessment of pharmaceutical compounds and formulations*. UCL School of Pharmacy.

Soto, J. *et al.* (2018) 'Rats can predict aversiveness of Active Pharmaceutical Ingredients', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier B.V., 133, pp. 77–84. doi: 10.1016/j.ejpb.2018.09.027.

Sotoyama, M. *et al.* (2017) 'Citric Acid Suppresses the Bitter Taste of Olopatadine Hydrochloride Orally Disintegrating Tablets', *Biological & Pharmaceutical Bulletin*, 40(4), pp. 451–457. doi: 10.1248/bpb.b16-00828.

Spector, A. C. and Kopka, S. L. (2002) 'Rats fail to discriminate quinine from denatonium: Implications for the neural coding of bitter-tasting compounds', *Journal of Neuroscience*. Society for Neuroscience, 22(5), pp. 1937–1941. doi: 10.1523/jneurosci.22-05-01937.2002.

Stone, H., Bleibaum, R. and Thomas, H. (2012) 'Descriptive Analysis', in

Sensory Evaluation Practices. 4th edn. Elsevier, pp. 233–289. doi: 10.1016/B978-0-12-382086-0.00006-6.

Suez, J. *et al.* (2014) 'Artificial sweeteners induce glucose intolerance by altering the gut microbiota', *Nature*. Nature, 514(7521), pp. 181–186. doi: 10.1038/NATURE13793.

Suez, J. *et al.* (2015) 'Non-caloric artificial sweeteners and the microbiome: findings and challenges', *https://doi.org/10.1080/19490976.2015.1017700*. Taylor & Francis, 6(2), pp. 149–155. doi: 10.1080/19490976.2015.1017700.

Swithers, S. E. and Davidson, T. L. (2008) 'A role for sweet taste: calorie predictive relations in energy regulation by rats', *Behavioral neuroscience*. Behav Neurosci, 122(1), pp. 161–173. doi: 10.1037/0735-7044.122.1.161.

Sylvetsky, A. C. *et al.* (2016) 'Hormonal responses to non-nutritive sweeteners in water and diet soda', *Nutrition & metabolism*. Nutr Metab (Lond), 13(1). doi: 10.1186/S12986-016-0129-3.

Szejtli, J. and Szente, L. (2005) 'Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins', *European Journal of Pharmaceutics and Biopharmaceutics*. Elsevier, 61(3), pp. 115–125. doi: 10.1016/J.EJPB.2005.05.006.

Szente, L. and Szejtli, J. (2004) 'Cyclodextrins as food ingredients', *Trends in Food Science & Technology*. Elsevier, 15(3–4), pp. 137–142. doi: 10.1016/J.TIFS.2003.09.019.

Tan, V. W. K. *et al.* (2019) 'Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA)', *Food Research*

International. Elsevier, 121, pp. 39–47. doi: 10.1016/J.FOODRES.2019.03.019.

Tandel, K. R. (2011) 'Sugar substitutes: Health controversy over perceived benefits', *Journal of Pharmacology & Pharmacotherapeutics*. Wolters Kluwer -- Medknow Publications, 2(4), p. 236. doi: 10.4103/0976-500X.85936.

Temussi, P. A. (2009) 'Sweet, bitter and umami receptors: a complex relationship', *Trends in Biochemical Sciences*. Elsevier Current Trends, 34(6), pp. 296–302. doi: 10.1016/J.TIBS.2009.02.005.

The United States Pharmacopeial Convention (2016a) *Transfer of Analytical Procedures (USP 1224)*. Available at: http://www.ropack.com/wp-content/uploads/c1224.pdf.

The United States Pharmacopeial Convention (2016b) *Validation of Compendial Procedures*. Available at: https://latam-edu.usp.org/wp-content/uploads/2021/08/1225.pdf.

Tiwari, R. V. *et al.* (2017) 'Rat Palatability Study for Taste Assessment of Caffeine Citrate Formulation Prepared via Hot-Melt Extrusion Technology', *AAPS PharmSciTech*, 18(2), pp. 341–348. doi: 10.1208/s12249-015-0447-1.

Toda, J. *et al.* (1985) 'Interaction of cyclodextrins with taste substances', *Proceedings of the 4th International Flavour Conference*, pp. 19–34. Available at: https://agris.fao.org/agris-search/search.do?recordID=US8248478 (Accessed: 9 March 2022).

Tordoff, M. G. (2007) 'Taste solution preferences of C57BL/6J and 129X1/SvJ mice: Influence of age, sex, and diet', *Chemical Senses*, 32(7), pp. 655–671.

doi: 10.1093/CHEMSE/BJM034.

Tucker, T. G. H. A. *et al.* (2012) ‘Absolute immunoquantification of the expression of ABC transporters P-glycoprotein, breast cancer resistance protein and multidrug resistance-associated protein 2 in human liver and duodenum’, *Biochemical Pharmacology*. Biochem Pharmacol, 83(2), pp. 279–285. doi: 10.1016/j.bcp.2011.10.017.

Ungureanu, I. M. *et al.* (2008) ‘Off-note blocking sensory organic compounds’.

Unitaid (2020) *Innovative Delivery Systems for Technology Landscape*. Available at: https://unitaid.org/assets/Innovative-delivery-systems-for-paediatric-medicines-technology-landscape.pdf (Accessed: 20 March 2022).

US Environmental Protection Agency (2007) *Provisional Peer-Reviewed Toxicity Values for Triphenylphosphine Oxide*. Washington DC EPA/690/R-07/038F. Available at: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=339078 (Accessed: 23 November 2018).

Del Valle, E. M. M. (2004) ‘Cyclodextrins and their uses: a review’, *Process Biochemistry*. Elsevier, 39(9), pp. 1033–1046. doi: 10.1016/S0032-9592(03)00258-9.

Venables, R. *et al.* (2015) ‘Determination of formulation factors that affect oral medicines acceptability in a domiciliary paediatric population’, *International journal of pharmaceutics*. Int J Pharm, 480(1–2), pp. 55–62. doi: 10.1016/J.IJPHARM.2015.01.023.

Volpe, D. A. (2008) ‘Variability in caco-2 and MDCK cell-based intestinal

permeability assays', *Journal of Pharmaceutical Sciences*. John Wiley and Sons Inc., pp. 712–725. doi: 10.1002/jps.21010.

Walsh, J. *et al.* (2014) 'Playing hide and seek with poorly tasting paediatric medicines: Do not forget the excipients', *Advanced Drug Delivery Reviews*, 73, pp. 14–33. doi: 10.1016/j.addr.2014.02.012.

Wankar, Jitendra *et al.* (2020) 'Recent Advances in Host–Guest Self-Assembled Cyclodextrin Carriers: Implications for Responsive Drug Delivery and Biomedical Engineering', *Advanced Functional Materials*. John Wiley & Sons, Ltd, 30(44), p. 1909049. doi: 10.1002/ADFM.201909049.

Wee, M., Tan, V. and Forde, C. (2018) 'A comparison of psychophysical dose-response behaviour across 16 sweeteners', *Nutrients*. MDPI AG, 10(11). doi: 10.3390/NU10111632.

Weiffenbach, J. M., Baum, B. J. and Burghauser, R. (1982) 'Taste thresholds: quality specific variation with human aging', *Journal of gerontology*. J Gerontol, 37(3), pp. 373–377. doi: 10.1093/GERONJ/37.3.372.

Whittaker, A. *et al.* (2009) 'Toxic additives in medication for preterm infants.', *Archives of disease in childhood. Fetal and neonatal edition*. BMJ Publishing Group, 94(4), pp. F236-40. doi: 10.1136/adc.2008.146035.

WHO (1998) *Quality control methods for medicinal plant materials World Health Organization Geneva*.

WHO (2007) *Development of Paediatric Medicines: Points to Consider in Formulation. WHO Expert Committee*.

WHO Food Additives Series 5 (no date). Available at:

http://www.inchem.org/documents/jecfa/jecmono/v05je24.htm (Accessed: 15 February 2019).

Wilken, M. K. and Satiroff, B. A. (2012) ‘Pilot Study of Miracle Fruit to Improve Food Palatability for Patients Receiving Chemotherapy’, *Clinical Journal of Oncology Nursing*, 16(5), pp. E173–E177. doi: 10.1188/12.CJON.E173-E177.

Willia, M. F. *et al.* (1992) ‘Influence of gastrointestinal site of drug delivery on the absorption characteristics of ranitidine’, *Pharmaceutical research*. Pharm Res, 9(9), pp. 1190–1194. doi: 10.1023/A:1015860007380.

Williams, H. D. *et al.* (2013) ‘Strategies to Address Low Drug Solubility in Discovery and Development’, *The American Society for Pharmacology and Experimental Therapeutics* , 65(1), pp. 315–499. doi: 10.1124/pr.112.005660.

Winnig, M. *et al.* (2007) ‘The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor’, *BMC Structural Biology*. BioMed Central, 7(1), p. 66. doi: 10.1186/1472-6807-7-66.

Wise, P. M., Damani, S. and Breslin, P. A. S. (2019) ‘Sodium, but not potassium, blocks bitterness in simple model chicken broths’, *Journal of food science and technology*. J Food Sci Technol, 56(6), pp. 3151–3156. doi: 10.1007/S13197-019-03770-1.

Woertz, K. *et al.* (2011a) ‘A comparative study on two electronic tongues for pharmaceutical formulation development’, *Journal of Pharmaceutical and Biomedical Analysis*. J Pharm Biomed Anal, 55(2), pp. 272–281. doi: 10.1016/j.jpba.2011.02.002.

Woertz, K. *et al.* (2011b) ‘Taste sensing systems (electronic tongues) for

pharmaceutical applications', *International Journal of Pharmaceutics*. Elsevier, 417(1–2), pp. 256–271. doi: 10.1016/J.IJPHARM.2010.11.028.

Wollmer, E. *et al.* (2022) 'Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines', *Advanced Drug Delivery Reviews*. Elsevier, 181, p. 114084. doi: 10.1016/J.ADDR.2021.114084.

Wooding, S. *et al.* (2006) 'Independent evolution of bitter-taste sensitivity in humans and chimpanzees', *Nature*. Nature Publishing Group, 440(7086), pp. 930–934. doi: 10.1038/nature04655.

World Health Organization (2011) *Definition of active pharmaceutical ingredient.* QAS/11.426/Rev.1. Available at: https://www.who.int/medicines/areas/quality_safety/quality_assurance/DefinitionAPI-QAS11-426Rev1-08082011.pdf (Accessed: 26 June 2019).

Xu, Q. *et al.* (2019) 'Hen protein-derived peptides as the blockers of human bitter taste receptors T2R4, T2R7 and T2R14', *Food Chemistry*. Elsevier, 283, pp. 621–627. doi: 10.1016/J.FOODCHEM.2019.01.059.

Xu, Y. *et al.* (2021) 'An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers', *Advanced Drug Delivery Reviews*. Elsevier, 175, p. 113795. doi: 10.1016/J.ADDR.2021.05.005.

Yamaguchi, S. and Ninomiya, K. (2000) 'Umami and Food Palatability', *The Journal of Nutrition*. Oxford University Press, 130(4), pp. 921S-926S. doi: 10.1093/jn/130.4.921S.

Yamane, M. *et al.* (2021) ‘The Provisional No-Effect Threshold of Sugar Alcohols on Oral Drug Absorption Estimated by Physiologically Based Biopharmaceutics Model’, *Journal of Pharmaceutical Sciences*. Elsevier B.V., 110(1), pp. 467–477. doi: 10.1016/j.xphs.2020.05.013.

Yu, Z. *et al.* (2022) ‘Identification of Oncorhynchus mykiss nebulin-derived peptides as bitter taste receptor TAS2R14 blockers by in silico screening and molecular docking’, *Food Chemistry*. Elsevier, 368, p. 130839. doi: 10.1016/J.FOODCHEM.2021.130839.

Zajicek, A. *et al.* (2013) ‘A Report from the Pediatric Formulations Task Force: Perspectives on the State of Child-Friendly Oral Dosage Forms’, *The AAPS Journal*. Springer US, 15(4), pp. 1072–1081. doi: 10.1208/s12248-013-9511-5.

Zakharova, L. *et al.* (2016) ‘Supramolecular strategy of the encapsulation of low-molecular-weight food ingredients’, *Encapsulations*. Academic Press, pp. 295–362. doi: 10.1016/B978-0-12-804307-3.00008-9.

Zhang, C. *et al.* (2019) ‘Glycated Beef Protein Hydrolysates as Sources of Bitter Taste Modifiers’, *Nutrients 2019, Vol. 11, Page 2166*. Multidisciplinary Digital Publishing Institute, 11(9), p. 2166. doi: 10.3390/NU11092166.

Zhang, L., Xia, Y. and Peterson, D. G. (2014) ‘Identification of Bitter Modulating Maillard-Catechin Reaction Products’, *Journal of Agricultural and Food Chemistry*. American Chemical Society, 62(33), pp. 8470–8477. doi: 10.1021/jf502040e.

Zhang, W. *et al.* (2016) ‘The Effects of Pharmaceutical Excipients on Gastrointestinal Tract Metabolic Enzymes and Transporters—an Update’,

AAPS Journal. Springer New York LLC, 18(4), pp. 830–843. doi: 10.1208/s12248-016-9928-8.

Zhang, Y. *et al.* (2003) 'Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways.', *Cell*. Elsevier, 112(3), pp. 293–301. doi: 10.1016/S0092-8674(03)00071-0.

Zheng, X. *et al.* (2018) 'Developments in Taste-Masking Techniques for Traditional Chinese Medicines', *Pharmaceutics*, 10(3), p. 157. doi: 10.3390/pharmaceutics10030157.

Zhou, D. *et al.* (2020) 'Interaction of Organic Anion Transporter 3-Mediated Uptake of Steviol Acyl Glucuronide, a Major Metabolite of Rebaudioside A, with Selected Drugs', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 68(6), pp. 1579–1587. doi: 10.1021/ACS.JAFC.9B05808/ASSET/IMAGES/ACS.JAFC.9B05808.SOCIAL.JPEG_V03.

Zidovetzki, R. and Levitan, I. (2007) 'Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies', *Biochimica et biophysica acta*. NIH Public Access, 1768(6), p. 1311. doi: 10.1016/J.BBAMEM.2007.03.026.

Zocchi, E. *et al.* (2017) 'Abscisic Acid: A Novel Nutraceutical for Glycemic Control.', *Frontiers in nutrition*. Frontiers Media SA, 4, p. 24. doi: 10.3389/fnut.2017.00024.