Formulation and Assessment of Taste Masked Combination Therapies for the Treatment of Paediatric Tuberculosis

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Thesis submitted in accordance with the requirements of UCL School of Pharmacy for the degree of Doctor of Philosophy

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DECLARATION

I, Alison Keating, confirm that the work presented in this thesis is my own. This work was conducted at University College London School of Pharmacy from May 2014 to September 2017 under the supervision of Professor Duncan Craig, Dr. Catherine Tuleu and Dr. Claire Forbes. Wherever collaborative work is described every effort is made to indicate this clearly. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

_________________________________  ______________
Alison Keating  Date
For Mam and Dad
Imíonn an tuirse,

ach fanann an tairbhe
Abstract

Tuberculosis is a major global health problem, which ranks alongside HIV as a leading cause of death worldwide. Adherence to tuberculosis treatment regimens is quite low, particularly in paediatric patients, and the aversive taste of these medicines is often cited as a major reason for this. In the first part of this thesis, the taste of these four drugs was assessed using both a human taste panel and the rodent Brief Access Taste Aversion (BATA) model. Human EC_{50} (i.e. the concentration of drug which elicits 50% of the maximum taste response) values were determined for each drug and the BATA model was identified as being useful for the assessment of formulations containing isoniazid, rifampicin and ethambutol.

The ability of an in vitro technique, the Insent TS-5000Z electronic tongue, to detect and assess the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was investigated. The best correlation between human responses and electronic tongue responses was observed for ethambutol dihydrochloride.

The latter half of this thesis focused on the use of hot melt extrusion (HME) as a processing technique to develop taste masked polymeric formulations of isoniazid and rifampicin and a fixed dose combination containing both isoniazid and rifampicin.

A fixed dose combination (FDC) formulation containing 20% w/w isoniazid and 30% w/w rifampicin was produced using Eudragit E-PO as a carrier. The extrudate was milled and incorporated into a dispersible tablet. The weight uniformity, thickness, hardness, disintegration time and content uniformity of the tablets were investigated and found to conform to the specifications for solid dosage forms. The dispersible tablet was found to effectively mask the taste of the drugs when dispersed in water with the drug release remaining below the human EC_{50} value for each drug.
Impact Statement

This thesis focuses on the development of a taste-masked fixed dose combination for the treatment of paediatric tuberculosis. In the initial part of this work the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride (the four first line drugs used for the treatment of paediatric tuberculosis) was assessed using a human taste panel. This allowed for EC50 values, i.e. the concentration of drug which elicits half the maximum taste response, to be determined. This is the first time the bitterness levels of these drugs have been quantified in a human panel. The data obtained from this study will be extremely useful to future researchers working on paediatric formulations of these drugs. Patient adherence to tuberculosis treatment regimens is quite low, and the poor palatability of currently available formulations is cited as a key factor in this. A greater understanding of the taste of these drugs will allow for more palatable formulations to be developed which in turn will lead to greater patient adherence and improved treatment outcomes.

Two non-human taste assessment techniques were also investigated i.e. the rodent brief access taste aversion (BATA) model and the electronic tongue. The comparison of the results from the BATA experiments to the human panel have been helpful in the ongoing validation of this model and suggest that the BATA model will be useful for taste assessment of formulations containing isoniazid, rifampicin and ethambutol dihydrochloride. The ability of the electronic tongue to both detect and assess the taste of these drugs was investigated. Ethambutol dihydrochloride was the only drug to exhibit a linear response which correlated well with human taste responses indicating that this is the only drug of the four assessed which can be effectively assessed using the electronic tongue. This data will also help to contribute to the validation of the electronic tongue and help researchers determine when this technique should be used.

In the latter parts of this thesis hot melt extrusion was demonstrated to be useful for the production of taste-masked formulations of isoniazid and a fixed dose combination containing isoniazid and rifampicin. These results help to validate the usage of hot melt extrusion as a taste-masking technique. This may encourage future researchers both in academia and industry to use this technique for taste masking of other drugs which may lead to development of more effectively taste-masked formulations which should help to improve patient adherence to drug regimens, particularly in the paediatric population.
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ABBREVIATIONS

µA – Microampere
ADI – Acceptable Daily Intake
ANOVA – Analysis of Variance
API – Active Pharmaceutical Ingredient
ATP – Adenosine triphosphate
βCD – β-cyclodextrin
BATA – Brief Access Taste Aversion
BCS – Biopharmaceutics Classification System
BP – British Pharmacopoeia
BPCA – Best Pharmaceuticals for Children Act
CALMH1 – Calcium homeostasis modulator 1
ChemFET – Chemically modified field effect transistor
CPA – Change in membrane potential after adsorption
CTA – Conditioned Taste Aversion
DNA – Deoxyribonucleic acid
DOT – Directly Observed Therapy
DSC – Differential Scanning Calorimetry
EMA – European Medicines Association
EPO – Eudragit E-PO
ETH – Ethambutol
FDA – Food and Drug Administration
FDAAA – FDA Amendment Act
FDAMA – FDA Modernisation Act
FDASIA – FDA Safety and Innovation Act
Abbreviations

FDC – Fixed Dose Combination
GDF – Global Drug Facility
GI – Gastrointestinal
GPCR – G-protein coupled receptor
GRAS – Generally Recognised as Safe
HPLC – High Performance Liquid Chromatography
HPMC – Hydroxypropylmethylcellulose
IGRA – Interferon Gamma Release Assay
InhA – 2-trans- enoyl-acyl carrier protein reductase
IP₃ – Inositol-1,4,5-triphosphate
IP₃R3 – Inositol-1,4,5-triphosphate receptor type 3
IZD – Isoniazid
M – Molar
MDR-TB – Multidrug Resistant Tuberculosis
mM – Millimolar
MTDSC – Modulated Temperature Differential Scanning Calorimetry
mV – Millivolt
N – Newton
NADH – Nicotinamide Adenine Dinucleotide
NCE – New Chemical Entity
NMR – Nuclear Magnetic Resonance Spectroscopy
PCA – Principal Component Analysis
PDCO – Paediatric Committee
PIP – Paediatric Investigation Plan
pKₐ – Negative base-10 logarithm of the acid dissociation constant
Abbreviations

PLC-β2 – Phospholipase C-β2
PREA – Paediatric Research Equity Act
PROP – 6-n-Propylthiouracil
PSP – Paediatric Study Plan
PTC – Phenylthiocarbamide
PUMA – Paediatric Use Marketing Authorisation
PXRD – Powder X-Ray Diffraction
PYZ – Pyrazinamide
PZase – Pyrazinamidase
REC – Research Ethics Committee
RIF – Rifampicin
RNA – Ribonucleic acid
RR-TB – Rifampicin Resistant Tuberculosis
S.D. – Standard Deviation
S.E.M. – Standard Error of the Mean
SLP – Soluplus
SPC – Supplementary Protection Certificate
SSF – Simulated Salivary Fluid
SSNMR – Solid State Nuclear Magnetic Resonance Spectroscopy
STEP – Safety and Toxicity of Excipients in Paediatrics
T1R – Type 1 taste receptor
T2R – Type 2 taste receptor
TAS2R – Taste 2 receptor gene
TB – Tuberculosis
Td – Degradation temperature
Abbreviations

$T_g$ – Glass transition temperature

TGA – Thermogravimetric Analysis

$T_m$ – Melting temperature

TRC – Taste Receptor Cell

TRPM5 – Transient receptor potential cation channel subfamily M member 5

TST – Tuberculin Skin Test

UCL – University College London

USP – United States Pharmacopoeia

UV-Vis – Ultraviolet Visible Spectrophotometry

V – Volts

WHO – World Health Organisation
CHAPTER 1

Introduction
CHAPTER 1

1.1 MEDICINES FOR PAEDIATRIC POPULATIONS

The development of age-appropriate formulations for paediatric populations is a major challenge for formulation scientists.\textsuperscript{1,2} Historically, the main focus of pharmaceutical companies has been to only develop adult formulations unless the paediatric market for the drug was particularly large e.g. antibiotics for common childhood infections, asthma treatments and mild pain relief. This has led children to be referred to as ‘therapeutic orphans’ who are exposed to unnecessary dangers when prescribed drugs which do not have a paediatric formulation available.\textsuperscript{3} In recent years both the FDA (Food & Drug Administration) in the USA and the EMA (European Medicines Agency) in Europe have develop specific paediatric regulations to ensure this population is properly catered for.

1.1.1 The Need for Age-Appropriate Formulations

Age-appropriate formulations are essential to ensure the safe and effective treatment of paediatric patients. Due to a lack of age-appropriate formulations, many drugs used to treat children are prescribed either ‘off-label’ or ‘unlicensed’.\textsuperscript{4-6} The license of a medicinal product describes which illness or disease a drug is to be used for, the age range of patients it can be given to, the required dose and the route of administration. ‘Off-label’ use means using the medicine in a way that is different to that described in the license e.g. using a medicine only licensed for adults in paediatric patients. ‘Unlicensed’ can mean using a medicine which is currently not licensed in the patient’s country and must be imported or, most commonly for paediatric patients, the medicine may have a license but needs to be made up to be taken as an unlicensed formulation e.g. a specially prepared liquid for patients unable to swallow a tablet. In the UK it is estimated that in general practice settings approximately 10.8% of medicines prescribed to children are either off-label or unlicensed.\textsuperscript{7} A European study of paediatric in-patient admissions estimates that in hospital settings the proportion of medicines used either off-label or unlicensed increases to approximately 46%.\textsuperscript{8}

When administering an off-label drug to a child, i.e. using a medicine only licensed for adults in paediatric patients, the pharmacist or caregiver may be required to manipulate the adult drug formulation to achieve a paediatric dose. This may involve splitting or crushing a tablet, cutting a patch, opening a capsule and mixing it with a vehicle such as yoghurt or by altering the dose given of a liquid formulation.\textsuperscript{9} This extemporaneous preparation of formulations is fraught with danger as it increases the likelihood of adverse reactions and dosing errors occurring.\textsuperscript{10,11} It also increases the risk of non-adherence of the patient due to poor
palatability of the modified formulation. The development of age-appropriate dosage forms is therefore essential to ensure the successful and safe treatment of paediatric patients.

1.1.2 US FDA Regulatory Initiatives

The FDA Modernisation Act (FDAMA) was passed in 1997. One of the provisions of this act was an incentive which offered pharmaceutical companies an additional six months patent protection on all formulations of a drug if sufficient data was provided supporting the use of the drug in paediatric populations. This was reauthorized in 2002 under the Best Pharmaceuticals for Children Act (BPCA), and again in 2007 as part of the FDA Amendment Act (FDAAA). The BPCA renewed the exclusivity incentives set down in the FDAMA, required public disclosure of clinical study results and also created incentives for research into off-patent medicines for paediatric use through the issuing of government contracts for such studies.

In addition to this, in 1998 the FDA issued a regulation requiring the submission of a paediatric study plan (PSP) for all new drugs (waivers can be granted in the cases of drugs which have no paediatric use, e.g. drugs for age-related neurodegenerative disorders), which was later codified as the Paediatric Research Equity Act (PREA) and reauthorized in 2007 under the FDAAA. In 2012 both the BPCA and PREA were made permanent under the FDA Safety and Innovation Act (FDASIA).

These regulations have had significant success. It has been reported that 41% of new molecular entities approved by the FDA in 2009 were licensed for use in paediatric patients compared to only 20% in 1999, a 105% increase. It has also been reported that the percentage of adult drugs used in children without sufficient tolerability and effectiveness data has decreased from 80% in 1999 to 50% in 2014.

1.1.3 EU Regulatory Initiatives

In 2007 the European Paediatric Regulation was launched by the EMA. The aim of this legislation is: (i) to facilitate the development and accessibility of medicinal products for use in the paediatric population; (ii) to ensure that medicinal products used to treat the paediatric population are subject to ethical research of high quality; (iii) to ensure that medicinal products are appropriately authorized for use in the paediatric population; (iv) to improve the information available on the use of medicinal products in various paediatric populations; (v) to achieve these objectives without subjecting the paediatric population to unnecessary clinical trials and to prevent any delay of the authorization of medicinal products for other age populations.
The legislation is broadly similar to that introduced by the FDA but has some key differences. The EU requires a paediatric investigation plan (PIP) containing detailed information regarding planned studies in all subsets of the paediatric population. The PIP is similar to the PSP required by the FDA, however it must be submitted at the end of Phase 1 trials, whereas a PSP must be submitted by the end of Phase 2. This PIP must be agreed with the Paediatric Committee (PDCO) of the EMA which will assess whether it is appropriate to study the drug in children and ensure that the most suitable and sophisticated methods are used in any such studies.

The legislation also provides incentives for pharmaceutical companies through the issuing of a Supplementary Protection Certificate (SPC) or Paediatric Use Marketing Authorisation (PUMA). If a PIP is accepted by the EMA and rigorously adhered to during the development process an SPC is granted which gives a 6 month exclusivity extension for all formulations of the drug (similar to the incentives offered by the FDA). The PUMA incentivises the development of products containing off-patent drugs (e.g. generics). If an age appropriate formulation is developed and investigated according to a PIP a PUMA may be granted which grants a 10 year exclusivity period for the use of the drug in children. There is no equivalent of this in the FDA legislation.

Since the introduction of this legislation, over 600 PIPs have been approved by the PDCO. By the end of 2012, 33 of these had been completed resulting in approval of new medicines with specific paediatric indications. An SPC was granted for twelve products while a PUMA was granted for just one product (Buccolam). The number of children being recruited for clinical trials in the EU has also increased from 2,840 in 2006 to 39,985 in 2012 which represents at 1300% increase.

1.1.4 Legislation in Other Jurisdictions

Unlike the USA and the EU, very few other jurisdictions have introduced regulatory initiatives to promote the development of paediatric medicines. Japan and Australia, despite having paediatric medicines initiatives through governmental and professional advisory bodies, do not have any formal legislation or regulations to mandate development of paediatric drugs. In Canada, an initiative similar to an SPC is in place which offers a 6 month exclusivity extension to companies which provide evidence supporting a paediatric indication for a given drug.
1.1.5 Challenges in Developing Paediatric Medications

Developing suitable medications for the paediatric population presents a significant challenge for the pharmaceutical industry. As a result there is a severe lack of suitable oral paediatric formulations, which means that stop-gap measures such as extemporaneous or compounded preparations are required. The main challenges associated with developing paediatric medicines are discussed in the following sections.

1.1.5.1 Heterogeneous Population

When developing medications for the adult market (typical age range of 18-65 years) the population is considered to be relatively uniform and thus a one-formulation-fits-all approach is appropriate in the majority of cases. Conversely, the paediatric market is a heterogeneous population ranging from newborns to adolescents (Table 1.1), which represents a very wide spectrum of developmental stages. Thus, a formulation that is suitable for one end of the spectrum, i.e. neonates, is likely to be unsuitable for the other end, i.e. adolescents. For example, if a company decides to only develop a solid oral dosage form such as a mini-tablet, then very young children may be unable to take it. Conversely if a company decided to produce a ‘one-size-fits-all’ single strength liquid formulation then it is likely the volumes required for very young children would be unreasonably small and those for older children would be unreasonably large.

Table 1.1 – Paediatric age categories as designated by the World Health Organisation.17

<table>
<thead>
<tr>
<th>Classification</th>
<th>Age Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature newborn</td>
<td>&lt; 38 weeks gestation</td>
</tr>
<tr>
<td>Term newborn</td>
<td>≥ 38 weeks gestation</td>
</tr>
<tr>
<td>Neonate</td>
<td>0 – 30 days</td>
</tr>
<tr>
<td>Infant</td>
<td>1 – 24 months</td>
</tr>
<tr>
<td>Young child</td>
<td>2 – 6 years</td>
</tr>
<tr>
<td>Child</td>
<td>6 – 12 years</td>
</tr>
<tr>
<td>Adolescent</td>
<td>12 – 18 years</td>
</tr>
</tbody>
</table>

1.1.5.2 Differences in Paediatric Physiology

Physiologically, children differ from adults in terms of their gastrointestinal physiology,18–20, how they metabolise drugs21–24, in their ability to swallow,20 and their taste preferences.25 To further complicate matters, these factors also vary between children of different ages.
Absorption of orally administered drugs from the gastrointestinal (GI) tract is affected by a number of factors including gastric acid secretion, bile salt formation, gastric emptying time, intestinal motility, and microbial flora. Table 1.2 summarises the differences in these factors between different age groups.

It is important that these factors are taken into consideration when developing paediatric formulations. For example, increased gastric pH in neonates and young infants can lead to enhanced bioavailability of weakly basic drugs (e.g. ampicillin) and decreased bioavailability of weakly acidic drugs (e.g. phenobarbital). In neonates and young infants reduced gastric emptying times and intestinal motility can increase the time taken for therapeutic doses to be reached. Immature pancreatic/biliary function in neonates results in reduced bile salt formation which decreases the bioavailability of lipophilic drugs such as diazepam. These are just a few examples of issues that may arise as a result of differences between adult and paediatric GI physiology, but they serve to illustrate the necessity of developing age-appropriate formulations for children of all ages.
Table 1.2 – Comparison of age dependent gastrointestinal physiological factors which influence the absorption of orally administered drugs. 
Adapted from Batchelor et al.\textsuperscript{18}

<table>
<thead>
<tr>
<th>Factor</th>
<th>Neonate (0-28 days)</th>
<th>Infant (1-24 months)</th>
<th>Child (2-5 years)</th>
<th>Child (6 - 11 years)</th>
<th>Adolescent (12-18 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastric fluid pH</strong></td>
<td>At birth, approximately pH 6-8; significant acid secretions between 24-48hrs results in pH 1-3.5. Secretions then decrease and pH is near neutral for 20-30 days before decreasing again.\textsuperscript{19}</td>
<td>Approaching adult levels\textsuperscript{19}</td>
<td>~Adult levels\textsuperscript{19}</td>
<td>~Adult levels\textsuperscript{19}</td>
<td>~Adult levels\textsuperscript{19}</td>
</tr>
<tr>
<td><strong>Gastric emptying time</strong></td>
<td>Reduced\textsuperscript{23}</td>
<td>Delayed in children between 6-8 months and ~ adult after 6 months of age.\textsuperscript{23}</td>
<td>~ Adult\textsuperscript{19}</td>
<td>~ Adult\textsuperscript{19}</td>
<td>~ Adult\textsuperscript{19}</td>
</tr>
<tr>
<td><strong>Stomach capacity</strong></td>
<td>10-100 ml\textsuperscript{19}</td>
<td>90-500 ml\textsuperscript{19}</td>
<td>750-960 ml\textsuperscript{19} (~ Adult)</td>
<td>750-960 ml\textsuperscript{19} (~ Adult)</td>
<td>1500 ml\textsuperscript{19} (~ Adult)</td>
</tr>
<tr>
<td><strong>Intestinal transit time</strong></td>
<td>Reduced (~ 4hr)\textsuperscript{23}</td>
<td>Reduced (~ 4hr)\textsuperscript{23}</td>
<td>3 - 7.5h\textsuperscript{19}</td>
<td>3 – 7.5h\textsuperscript{19}</td>
<td>3 – 7.5h\textsuperscript{19}</td>
</tr>
<tr>
<td><strong>Bacterial flora</strong></td>
<td>Very immature\textsuperscript{23}</td>
<td>Very immature\textsuperscript{23}</td>
<td>Approaching adult\textsuperscript{23}</td>
<td>~ Adult\textsuperscript{23}</td>
<td>~ Adult\textsuperscript{23}</td>
</tr>
<tr>
<td><strong>Pancreatic / biliary function</strong></td>
<td>Very immature\textsuperscript{23}</td>
<td>Approaching adult\textsuperscript{23}</td>
<td>~ Adult\textsuperscript{19}</td>
<td>~ Adult\textsuperscript{19}</td>
<td>~ Adult\textsuperscript{19}</td>
</tr>
</tbody>
</table>
In terms of drug metabolism, children are also significantly different to adults. At birth, both phase I (oxidation, reduction and hydrolysis) and phase II (hydroxylation and conjugation) metabolic enzymes are thought to be immature. There are many different isoforms of phase I and phase II enzymes, all of which have different maturation profiles. Therefore, it is impossible to have a general guideline for young children, as metabolism of a specific drug is dependent on the rate of maturation of the specific enzymes involved.

Typically, when a suitable paediatric dose of a drug is not known, a dose is extrapolated from those found to be safe in adult patients. However, due to variations in maturation of certain enzymes, this may not be suitable. An example of this is ‘grey baby syndrome’ which occurred when the antibiotic chloramphenicol was administered to neonates at doses extrapolated from adult doses. The affected children exhibited vomiting, abdominal distension, abnormal respiration, cyanosis, cardiovascular collapse and death. It was later discovered that this was due to neonates having an immature UDP glucuronosyl transferase system, preventing proper metabolism of the drug and leading to build-up of toxic chloramphenicol metabolites.

Very young infants (0-5 months of age) possess an extrusion reflex which causes them to push any solids placed on the tongue to the front of the mouth, meaning they can only swallow liquids. Physiologically, children are capable of swallowing thick semi-solid formulations (e.g. multiparticulates in soft food) from 6 months of age onwards. There is no general consensus regarding what age children are capable of swallowing solid oral dosage forms. From 2-6 years of age the ability and willingness of a child to swallow a tablet or capsule is highly variable and in many cases is dependent on the individual child, thus liquid formulations are generally favoured for this age group. However, there have been studies which indicate that children younger than six can swallow a tablet with appropriate training or if a ‘mini-tablet’ (i.e. a tablet less than 3mm in diameter) is used. It is generally considered that children over 6 years of age will accept small to medium tablets/capsules while children 12 and over are considered able to take ‘adult-sized’ capsules.

1.1.5.3 Palatability
Palatability can be defined as the ‘overall appreciation of a medicine by organoleptic properties (smell, taste, aftertaste and texture). The vast majority of medicines in use today have a bitter, unpleasant taste that, as humans, we are programmed to avoid. In order for a formulation to be palatable it should not taste or smell unpleasant, it should have an acceptable texture (known as mouthfeel), and be of an appropriate size to enable the patient
to swallow it. However, palatability is not just important for oral dosage forms and needs to be considered for other routes of administration such as inhalation or nasal where the product may indirectly come into contact with taste receptors i.e. via post nasal run off or deposition in the throat.

Children are especially sensitive to the bitter taste of medicines and typically prefer sweet tasting substances. Adults have learned to overcome their innate aversion to medicines and can rationalise that, despite the unpleasant taste/mouthfeel/smell/texture, it is necessary to take the medicine in order to get better. Children however have not yet learned to do this. In a survey of paediatricians, more than 90% stated that the unpleasant taste and poor palatability of a drug were the greatest barriers to treatment completion. Therefore, when considering paediatric formulations, palatability is a very important factor.

1.1.5.4 Choice of Excipients

Excipients are substances used in drug formulations for a range of reasons such as to improve solubility, mask taste, give a suitable form to or improve stability of a drug. They are considered to be inert substances which do not affect the intended therapeutic action of the drug. They include, but are not limited to, diluents, binders, emulsifiers, sweeteners, colourings and solubilising agents.

The selection of appropriate excipients for paediatric formulations is not an easy task. Historically, the safety of excipients has not been well researched as their inertness was taken for granted. However, even when there is extensive and reliable safety data for available for the adult population, it cannot simply be extrapolated to the paediatric population due to the inherent differences in physiology and metabolism between adults and children (and also between children of different ages). The main excipients raising safety concerns in children are parabens, cyclodextrins, mannitol, benzyl alcohol, sorbitol, propylene glycol, polysorbate and ethanol. A commonly cited example is that of ethanol/propylene glycol toxicity in neonates. When ethanol is co-administered with propylene glycol it competitively inhibits the metabolism of propylene glycol, leading to its accumulation and potential toxicity.

When considering excipients for use in adult formulations, formulators can consult the ‘Inactive Ingredients Guide’ database published by the FDA, however for paediatric products a comprehensive database does not exist. A collaboration between European Paediatric Formulation Initiative, the National Institute of Child Health and Development and the US Paediatric Formulation Initiative has resulted in the launch of the Safety and Toxicity
of Excipients in Paediatrics database (STEP). The aim of STEP is to ‘conduct a high-level ongoing scientific literature review of the pharmacology, toxicology and safety data of a prioritized group of excipients likely to be used in paediatric formulations in order to provide a firm foundation and broad resource base for medicine development generally and ultimately highlight the gaps in excipient knowledge’.

1.1.5.5 Manufacturing Considerations

As mentioned previously, the paediatric population represents a relatively small portion of the market compared to the adult population. To supply the paediatric market generally a smaller volume of product is required along with a variety of dosage strengths. Drug manufacturing plants are typically set up to produce extremely large batches of drugs, but this may vastly exceed the amount of dosage units required to supply the market meaning that much of the product could expire before it is needed for use. These factors make the economics of servicing the paediatric market particularly challenging for many pharmaceutical companies.

1.1.5.6 Ethical Considerations

The paediatric population is a particularly vulnerable one and as such there are significant ethical concerns when carrying out clinical trials involving children. However, the physiological differences between adults and children mean that in certain cases it is not appropriate or sufficient to carry out studies in adults and then extrapolate the findings to children.

There has been lengthy discussion regarding ethical issues arising in paediatric research, and regulatory frameworks exist in both the US and EU to ensure this research is carried out appropriately. Issues that have been identified include: levels of acceptable risk, risk/benefit balance, role of parents/carers, informed consent and confidentiality. It is important when carrying out research involving children that the primary objective is to minimise risk to the participant and every effort should be made to ensure that is the case. Before commencing the study it is essential that there is full awareness of the known toxicology, safety and efficacy of the product being tested and the number of participants should be as low as is feasible. Childhood is a formative time and trauma experienced by a child can have long-lasting effects. Therefore it is essential that clinical trials involving children are carried out in a sensitive fashion with minimal disruption or discomfort for the patient.

Clinical trials involving children are a polarising topic and are likely to remain that way, however if children are to have the access they deserve to essential medicines then they are
a necessary part of the formulation development process. By ensuring that all trials are carried out to the highest ethical and medical standards then the benefits will outweigh any potential negatives and allow significant progress to be made in the field of paediatric healthcare.

1.2 CHILDHOOD HEALTH RISKS
According to the World Health Organisation (WHO), over eight million children under 5 die each year from illnesses such as malaria, HIV/AIDS, diarrhoea, pneumonia and tuberculosis. In 2011 the WHO published their priority medicines list for children. This list includes medicines for pneumonia, malaria, diarrhoea, neonatal sepsis, HIV/AIDS, and tuberculosis. Tuberculosis is the second largest killer worldwide caused by a single infectious agent (second only to HIV/AIDS), but it is treatable.

1.2.1 Tuberculosis
Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Figure 1.1). *M. tuberculosis* is a pleomorphic, weakly Gram positive, aerobic, rod-shaped bacteria. It is a disease which has evolved alongside humans for thousands of years, allowing it to develop unique mechanisms to exploit its human host.

*M. tuberculosis* most commonly affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). Transmission of TB is airborne and typically occurs when an infected individual coughs or sneezes, releasing bacteria into the air. In most humans, infection by *M. tuberculosis* does not lead to development of TB disease. Initial exposure to *M. tuberculosis* generally causes an inflammatory response referred to as the Ghon focus situated within the lungs, accompanied by enlargement of the draining lymph nodes in the area, known as the Ghon or primary complex. This is classified as primary pulmonary tuberculosis.

In approximately 95% of patients this primary complex resolves itself and the initial infection is contained. However, while the infection is contained, the bacteria are seldom eradicated and may lay dormant for up to 30 years before reactivation (latent tuberculosis). Of those patients who contain their infection 5% will go on to develop the disease at a later stage. This is most commonly seen in adults and adolescents and is known as post-primary or reactivation disease. In patients with immature or compromised immune systems which cannot mount an adequate inflammatory response, uncontained bacterial replication leads to the development of primary progressive or active TB disease.
1.2.2 Differences Between Adult and Paediatric Tuberculosis

As mentioned above, in general about 95% of individuals exposed to \textit{M. tuberculosis} contain the primary infection and do not develop active TB disease (at least initially). Unfortunately, this is not the case in children. Children have immature immune systems compared to adults and cannot mount a sufficient inflammatory response to prevent the development of TB disease. In the paediatric population the time from initial exposure to development of active disease can be as short as between one and six months. Without adequate treatment the risk of progression to active disease can be as high as 30–40% in infants younger than one year, and 24% in children aged 1-5. The risk reduces to 2% for children aged 5-10 (known as the ‘safe school years’) before increasing again to 10-20% in adolescents.

For many years paediatric TB was neglected. This was due to a number of reasons including difficulty in diagnosing paediatric pulmonary TB, lack of studies and data available on childhood TB, unknown outcomes of children with TB and the belief that childhood TB was not important for TB control as the likelihood of transmission from children is low.

Diagnosis of TB in children is more difficult than in the adult population for many reasons. TB disease is characterised by symptoms such as reduced appetite, weight loss, chronic prolonged cough and fever (lasting more than two weeks), night sweats and fatigue. Some if not all of these symptoms however are also common to many other prevalent children’s diseases such as general bacterial and viral infections, pneumonia and asthma which can lead to delays in diagnosis.

Diagnostic tests used to detect TB disease in adults include the tuberculin skin test (TST), interferon gamma release assays (IGRAs), chest radiography and sputum smear tests to
detect the presence of \textit{M. tuberculosis} in clinical samples (considered the ‘gold standard for TB diagnosis').\textsuperscript{51} These tests are less sensitive in children due to differences in physiology and disease progression, which further complicates the diagnosis of the disease in children.

1.2.3 Prevalence of Tuberculosis

TB is the eighth leading cause of death worldwide.\textsuperscript{45} The WHO Global Tuberculosis Report 2016 states that in 2015 there were 10.4 million reported cases of TB and 1.4 million deaths as a result of the disease. Paediatric TB accounted for approximately 1,000,000 of these cases and approximately 170,000 deaths.\textsuperscript{52} Children living in developing countries or areas affected by poverty, social disruption, and areas in which HIV is endemic are at a significantly higher risk of contracting the disease.\textsuperscript{51} In 2015, 60% of global TB cases occurred in just six countries: China, Indonesia, India, Nigeria, Pakistan and South Africa.\textsuperscript{52}

TB is generally considered eradicated in developed countries, however, in recent years it has been reported as re-emerging. In London, for example, the rate of TB has increased over the last twenty years to 44.4 cases per 100,000 population, which is higher than the WHO high prevalence notification rate (>40 cases per 100,000 population) and accounts for 45% of paediatric TB cases in the UK.\textsuperscript{45} In some London boroughs such as Brent and Newham the rate stands at >80 cases per 100,000 population.\textsuperscript{54} It is generally thought that these increases are as a result of increased international travel and migration from countries where TB is endemic.

1.2.4 Current Treatments for Paediatric TB

Treatment regimens for TB in adults and children are broadly similar. To treat active TB disease biphasic combination regimens are used. The first phase of treatment is an intensive phase with a combination of bactericidal drugs to kill rapidly growing bacilli. This is followed by a continuation phase using fewer drugs that eradicate slower-growing, more persistent bacilli.\textsuperscript{50} It is hoped that by using a combination of drugs it will eradicate both rapid and slow growing bacilli while preventing emergence of drug-resistant organisms.\textsuperscript{55} First line treatment regimens for TB disease typically use a three or four drug combination of isoniazid, pyrazinamide, rifampicin and in certain cases ethambutol. These drugs, which were chosen as model drugs for this project, are described in detail in Chapter 2.

Unlike most childhood bacterial infections, which can usually be cleared up with a week or two of antibiotics, the average TB disease treatment regimen is six months long. Initially the patient takes daily doses of isoniazid, pyrazinamide, rifampicin and ethambutol (if required).
for two months before reducing to daily doses of just rifampicin and isoniazid for four months.

The case of paediatric TB treatment serves particularly well to demonstrate how children are not small adults and require specifically tailored formulations to be designed and tested for this market. Until 2009, recommended dosages in for anti-TB medicines in children were extrapolated from the dosages used in adults. However, due to different rates of drug metabolism, clearance and distribution compared to adults, children were in fact being under-dosed for decades. These dosages have now been re-examined and increased. Table 1.3 compares the recommended dosages of first line TB drugs in adults and children.

Table 1.3 – Comparison of recommended dosages of first line anti-tuberculosis drugs in adults and children.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adult Dosage</th>
<th>Paediatric Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>4-6 mg/kg/day</td>
<td>10-15 mg/kg/day</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>8-12 mg/kg/day</td>
<td>10-20 mg/kg/day</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>20-30 mg/kg/day</td>
<td>30-40 mg/kg/day</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>15-20 mg/kg/day</td>
<td>15-25 mg/kg/day</td>
</tr>
</tbody>
</table>

Unfortunately, in some cases the patient does not respond to first line drugs and in these cases, known as multi-drug resistant TB (MDR-TB), more potent and toxic second line drugs must be used. It is estimated that in 2015 there were 480,000 of MDR-TB and 100,000 cases of rifampicin resistant TB (RR-TB) globally. 45% of these combined resistant cases were reported in India, China and the Russian Federation. Statistics regarding MDR-TB in children are not available, however, since paediatric TB represents 10-20% of total TB cases per year it would suggest that there are at least 48,000 cases of paediatric MDR-TB per year. For the treatment of MDR-TB in children the WHO has the following recommendations: (i) use any first-line medication to which susceptibility is documented or likely, (ii) use of at least four second-line drugs to which the strain is likely to be sensitive (an injectable and a fluoroquinolone to be included), (iii) all doses should be given using directly observed therapy (DOT) to ensure compliance, (iv) the duration of treatment should be at least 18-24 months.

Due to the small size of the market and lack of research in the area, second line anti-TB drugs are rarely produced in paediatric formulations necessitating extemporaneous preparation of
doses. This can lead to inaccurate, sub therapeutic or toxic levels of the drugs being given to children. Table 1.4 gives examples of second line drugs commonly used to treat MDR-TB.

Table 1.4 – Second line drugs used to treat multi-drug resistant tuberculosis. 

<table>
<thead>
<tr>
<th>Injectable agents</th>
<th>Fluoroquinolones</th>
<th>Oral second line bacteriostatic agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin</td>
<td>Moxifloxacin</td>
<td>Ethionamide</td>
</tr>
<tr>
<td>Amikacin</td>
<td>Levofoxacin</td>
<td>Prothionamide</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>Ofloxacin</td>
<td>Cycloserine</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>Terizidone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Para-aminosalicylic acid</td>
</tr>
</tbody>
</table>

1.2.5 Treating Paediatric TB – Issues with Patient Compliance

There are numerous factors which affect patient compliance when treating paediatric TB. Perhaps the most obvious factor is the length of the treatment course (anything from 6-24 months), in many cases patients may significantly improve after 1-2 months and they (or their caregivers) may discontinue treatment. The drugs used to treat TB are extremely potent and as such can have undesirable side effects which can cause patients to discontinue use. Children are especially sensitive to unpleasant tasting medicines and may refuse to take medications due to unpalatable formulations. Another factor to consider is cost which may be a barrier to adherence, particularly in developing countries. Recent advances such as the implementation of directly observed therapy (DOT), in which patients are administered their medicines in the presence of a healthcare professional have led to increased compliance, however, there is still a huge need for the development of cost-effective, palatable, child-friendly formulations of anti-tuberculosis drugs.

1.2.6 Fixed Dose Combinations for the Treatment of Paediatric Tuberculosis

A fixed dose combination (FDC) is a formulation which combines two or more active ingredients into a single dosage form. In 1994 the WHO recommended the use of FDCs for the treatment of TB as they offer a number advantages over conventional therapies, namely simplified treatment and drug management and the reduced probability of monotherapy. A common issue in TB treatment is patients picking and choosing which medicines they want to take, leading to monotherapy. Monotherapy of TB can lead to development of drug resistant bacteria, and should be avoided at all costs. By providing all the required drugs in the same tablet the patient cannot pick and choose which medicines to take, preventing monotherapy and resultant resistance.
There are also significant advantages to using FDCs specifically for treatment of paediatric TB. Liquid formulations of TB medications are often unavailable or difficult to obtain, meaning that children must take multiple tablets per day. By using an FDC the number of tablets the patient is required to take is significantly reduced, which should help improve patient compliance. Reducing the number of tablets also simplifies dose calculations, helping to reduce prescribing errors, improving safety for the patient.

Use of FDCs can also improve the efficiency of the TB drug supply system. When using single drugs in combination, the lack of availability of any one drug disrupts the entire treatment regimen. There can be any number of reasons for lack of availability and it is particularly complicated when different manufacturers, suppliers and supply chains are involved. By using FDCs these variables are reduced, allowing for more efficient supply and preventing disruption of treatment for the patient.

Despite these advantages, however, uptake of FDCs has been very low. Figures quoted by the Global Drug Facility (GDF) state that in 2007 only half of the 136 countries where TB has been reported use FDCs as part of their treatment regimens and globally only 15% of new TB cases are being treated with FDCs.

There are numerous reasons for the low uptake of FDC dosage forms such as perceived inferiority of treatment, potential side effects, higher cost and, in the case of paediatric formulations, lack of dose flexibility. The perceived inferiority of treatment stems from the fact that early versions of FDCs for TB suffered from poor bioavailability of rifampicin which damaged the image of FDCs. The reason for the poor bioavailability of rifampicin has not been definitively elucidated, however a number of potential reasons have been postulated including; poor quality of formulations, adsorption of the drug by excipients, decomposition of the drug within the formulations and in situ decomposition of the drug in the acidic environment of the stomach. It should be noted that improvements in FDC formulations have led to development of FDCs which are fully bioequivalent to single drug reference products and are stable for up to six months in tropical conditions.

Lack of dose flexibility has historically been an issue for the use of FDCs to treat paediatric TB, in recent years however specific paediatric formulations have been developed with more appropriate dosages to address this. The risk of side effects from FDCs is the same as when the drugs are administered separately, however when the drugs are administered together as an FDC the development of side effects may necessitate modification or omission of the entire combination. If the drugs are being administered as individual formulations it is easier
to swap out an individual drug for an alternative. It is important to remember however that this is only likely to occur in a minority of patients and should not prevent the use of FDCs in the general population. Although uptake of FDCs has not been hugely successful to date, the potential benefits of using them has ensured their continued support by the WHO. The advantages and disadvantages of FDC formulation are summarised in Table 1.5.

Table 1.5 – Advantages and disadvantages of the usage of fixed dose combinations for the treatment of paediatric tuberculosis.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduces tablet burden on patient</td>
<td>Lack of dose flexibility</td>
</tr>
<tr>
<td>Prevention of monotherapy</td>
<td>Side effects may necessitate omission of entire formulation</td>
</tr>
<tr>
<td>Improved efficiency of supply chain</td>
<td>Increased cost</td>
</tr>
</tbody>
</table>

1.2.7 Currently Available Paediatric Fixed Dose Combinations

Until recently there were five paediatric FDCs approved by the WHO-UN prequalification program and two FDCs that were available under the GDF Quality Assurance policy. Unfortunately none of these alone were suitable for paediatric patients which necessitated using combinations. This is not ideal as it increases the tablet burden for the child and can result in under/overdosing due to prescribing mistakes.

In 2016 two novel FDCs were released by the Stop TB Alliance and their partners. The first contains isoniazid (50 mg), rifampicin (75 mg) and pyrazinamide (150 mg) and is intended for use in the intensive phase of treatment while the second contains isoniazid (50 mg) and rifampicin (75 mg) and is intended for use in the continuation phase of treatment. The dosages of each tablet are designed to allow for easy dose calculations depending on the body weight of the child being treated (Table 1.6). The formulations are child-friendly, easy to administer (by dispersing in water) and are reported to be well taste-masked. It is hoped that these novel formulations will help to improve adherence to tuberculosis treatment regimens and therefore improve treatment outcomes.
Table 1.6 – Recommended dosing schedules for novel fixed dose combination dispersible
tablets.66

<table>
<thead>
<tr>
<th>Weight range (kg)</th>
<th>Intensive Phase (3 drug FDC)</th>
<th>Continuation phase (2 drug FDC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 - 7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8 - 11</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12 - 15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>16 - 24</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>25 +</td>
<td>Adult dosages recommended</td>
<td></td>
</tr>
</tbody>
</table>

1.3 TASTE

The bitter taste of medicines is one of the greatest barriers to treatment adherence in
paediatric populations.32 Therefore taste assessment and taste-masking are essential steps in paediatric formulation development. The development of bitter taste aversion is believed to have developed as a deterrent to ingesting toxic substances.31 On this basis it could be argued that, since most medicines are toxic substances, it is unsurprising that they have unpleasant, bitter tastes. In fact, when used effectively, bitter compounds can prevent paediatric poisonings.68 For example denatonium benzoate (Bitrex), the most bitter compound known to man, is added to some household cleaning products to prevent children ingesting a sufficient amount of the product to cause poisoning. However, when medicines are concerned it is important to remember that the medicine a child refuses to take has 0% bioavailability and as such palatable medicines are essential to ensure patient compliance and positive treatment outcomes.

1.3.1 Physiology of Taste

When discussing the biology of taste it is important to make the distinction between taste and flavour. When most people talk about taste of medicines they are in fact talking about flavour. Taste is defined as ‘the behavioural and physiologic consequences of stimulating taste receptor cells in the oral cavity’ whereas flavour can be defined as the ‘perceptual integration of signals from the gustatory, olfactory and trigeminal systems’.31

The taste system in humans develops extremely early. Specialised taste cells first appear in the foetus between week seven and eight of gestation and mature receptor cells are recognisable at 13-15 weeks. From birth, infants reject bitter taste and have been shown to
prefer sweet and umami type tastes although early exposure to bitter taste can lead to the development of greater acceptance.  

Humans and many mammals, such as rodents, are capable of sensing and responding to five different taste sensations i.e. sweet, salty, sour, bitter and umami. Mammalian taste recognition is mediated by specialised epithelial cells known as taste receptor cells (TRCs). Historically it was believed that receptors for sweet, salty, sour and bitter were distributed such that sweet tastes were only detected on the front section of the tongue, sour and salty on the lateral sections and bitter on the rear of the tongue (Figure 1.2a), however it has now been demonstrated that taste perception occurs in all areas of the tongue and also throughout the oral cavity (Figure 1.2b).

TRCs are generally organised, in groups of up to 100, into taste buds which are located on the tongue and soft palate. On the tongue taste buds are located within structures known as papillae. There are three types of papillae; fungiform papillae, circumvallate papillae and foliate papillae. Fungiform papillae are located in the anterior of the tongue, circumvallate papillae are found in the posterior of the tongue and foliate papillae are found on the tip and edges of the tongue.

Individual taste cells within taste buds are classified into four types. Type I cells, also known as dark cells, account for approximately 55-75% of all taste cells. They are believed to be supporting cells and may have glial like properties. Type II, also known as light cells, constitute approximately 20% of all cells in taste buds and are the primary sensory receptors in the taste buds. Type III cells are known as intermediate cells. They have been found to express synapse associated proteins and form synaptic junctions with nerve terminals. Finally, type IV cells, are progenitor cells located in the basal parts of the taste bud which
replace the other cell types over the lifespan of the taste bud. Type I, II and III taste cells have microvilli which project into the pore of the taste bud and can interact with solubilised taste molecules.

Each taste attribute has a specific coding mechanism which is mediated by specialised receptors. Bitter taste is mediated by the type 2 taste receptor (T2R) family of G-protein coupled receptors (GPCRs) while sweet and umami tastes are mediated by the type 1 taste receptor (T1R) GPCRs. Salty and sour tastes are on the other hand are believed to be mediated by ion channels (Figure 1.3). The focus of this thesis is bitter taste perception which is detailed in section 1.3.1.1.

Figure 1.3 – Schematic of taste signal transduction pathways of (a) sweet, bitter and umami taste; (b) sour taste; (c) salty taste.

1.3.1.1 Bitter Taste Perception

As discussed previously, the development of bitter taste aversion is believed to have developed as a deterrent to ingesting toxic substances. Bitter taste receptors are encoded by the TAS2R family of genes and humans have approximately 25 different bitter receptors. The number of compounds which elicit a bitter taste is significantly higher than the number
of TAS2R genes, suggesting that each T2R must be capable of recognising multiple tastants. Meyerhof et al.\textsuperscript{79} tested 104 bitter compounds against the 25 known human TAS2Rs and found that while some TAS2Rs have a very selective agonist activation pattern, others are much more promiscuous.

Bitter compounds are extremely structurally diverse, examples include (but are not limited to) fatty acids, peptides, amino acids, ureas, esters, phenols, alkaloids, steroids and crown ethers.\textsuperscript{79} Many studies have tried to correlate the chemical structure of a molecule to its bitterness. A database of bitter compounds (BitterDB) has been developed by Wiener et al.\textsuperscript{80} which has amassed records of over 680 bitter compounds and their associated TAS2Rs in an attempt to facilitate the study of chemical features which cause bitterness. However, to date, it is still extremely difficult to predict the bitterness of a molecule which has not been tasted before. This is further complicated by the fact that is not only the molecular composition of a drug which can contribute to its bitterness but its spatial distribution and even the environment the molecule is in. For example, the amino acid L-tryptophan is bitter whereas D-tryptophan has been reported to have a sweet taste. Another example is linoleic acid which when neat is more or less tasteless, however, when formulated as an emulsion exhibits a bitter taste.\textsuperscript{81}

Bitter (as well as sweet and umami) tastes are mediated by a phosphoinositide-based signalling pathway.\textsuperscript{82} Type II taste cells are the receptors for bitter taste molecules.\textsuperscript{77} These cells express specific components involved in the transduction of bitter taste including taste specific G-protein alpha subunit (α-gustducin), phospholipase C-β2 (PLC-β2), inositol 1,4,5-triphosphate receptor type 3 (IP\textsubscript{3}R3) and transient receptor potential cation channel subfamily M member 5 (TRPM5). It is hypothesised that, on binding of a molecule to a T2R located on the microvilli of Type II cells, heterotrimeric GTP-binding proteins (e.g. α-gustducin) are activated causing the release their β or γ subunits. These interact with phospholipase Cβ2 (PLCβ2) to stimulate synthesis of inositol-1,4,5-triphosphate (IP\textsubscript{3}). IP\textsubscript{3} acts on the IP\textsubscript{3} receptor (IP\textsubscript{3}R\textsubscript{3}) to cause release of calcium ions (Ca\textsuperscript{2+}) from intracellular stores. Elevated [Ca\textsuperscript{2+}] leads to activation of TRPM5 which produces a depolarisation that allows calcium homeostasis modulator 1 (CALMH1) channels to open. This results in release of adenosine triphosphate (ATP), which acts as a neurotransmitter.\textsuperscript{77}

1.3.2 Age Related Changes in Taste Perception
The ability to perceive taste occurs very early in life. Specialised taste cells begin to form in the human foetus at week seven or eight of gestation and mature taste buds are present at
week thirteen to fifteen. Studies of facial expressions have shown that neonates are able to discriminate between taste sensations within hours of birth. When presented with sweet stimuli neonates exhibit relaxation of the facial muscles and retraction of mouth edges in a smile like motion along with eager licking and sucking movements. Sour stimuli elicit lip pursing movements while bitter stimuli induce depression of the mouth edges in a dislike or aversion type motion.

\[\text{Figure 1.4 – Taste response to sweet, sour and bitter taste in newborns.}^{89}\]

Children are born with an innate preference for sweet taste. They prefer more intense sweet tastes than adults, a trend which declines during middle to late adolescence to reach adult levels. It has been suggested that this early preference for sweet taste may be due to the fact that, during growth periods, increased amounts of energy are required and sweet tastes are often associated with high calorie foods. The corresponding age related decrease in sweet preference is thus suggested to be linked to the completion of physical growth. It has also been shown that children have a stronger preference for salty foods than adults which may help to attract them to sodium and (potentially) other minerals needed for bone growth.

Conversely, children dislike and reject bitter tasting substances and in fact many children are more sensitive to bitter tastes than adults. There has been limited research into age related changes in bitter taste perception, however Mennella et al. have demonstrated that the sensitivity of individuals to the bitter taste of 6-n-Propylthiouracil (PROP) is modified.
with age. It was found that children with the bitter sensitive TAS2R38 genotype (AVI/PAV heterozygotes) were more sensitive to the taste of PROP than adults with the same genotype, with the desensitisation occurring during mid-adolescence.

Chemosensory function significantly deteriorates with ageing. This sensory deterioration is generic and happens in normal healthy older people, as demonstrated by the observed increase in taste thresholds after approximately 60 years of age. However this deterioration can be exacerbated by certain medical conditions (e.g. degenerative disease or stroke), medications, radiation and exposure to toxic substances. This deterioration can lead to decreased sense of wellbeing and quality of life in older adults, as well as affecting overall health.

Adult human taste panels (section 1.4.2.3) are generally considered the gold standard for taste assessment. However as discussed, taste sensitivity, in particular for bitterness and sweetness, varies markedly between adults and children. Therefore, adult human taste panels may not be an ideal method for taste assessment of paediatric formulations. Due to the lack of current research on the ontogeny of bitter taste sensitivity, the full extent of age related differences in taste perception is not yet known. Thus, the palatability assessment of paediatric medicines still remains a challenge in formulation development.

1.4 TASTE ASSESSMENT

As discussed in section 1.3, when discussing taste of medicines the concepts of flavour and taste are often used interchangeably. When assessing the palatability of a formulation it is typically referred to as ‘taste assessment’ although it is in fact the overall flavour which is being assessed. In keeping with this convention the term ‘taste assessment’ rather than ‘flavour assessment’ will be used throughout this thesis.

Taste assessment is an essential step when considering paediatric formulation development. Under EU regulations, pharmaceutical companies are required to follow a PIP for paediatric drug development. PIP guidelines define taste-masking and palatability as a key part of the development of paediatric pharmaceutical products. There are various methods of taste assessment, both in vitro and in vivo which will be discussed in the following sections.

1.4.1 In Vitro Taste Assessment Methods

In vitro taste assessment methods are particularly useful for early formulation development as they can be used to assess drugs for which human or animal safety data is not yet available. In some cases, e.g. electronic tongue, initial set up costs can be high, however the
lack of requirement for ethical approval and potential for high throughput screening of molecules or formulations can offset this.

1.4.1.1 In Vitro Drug Release

*In vitro* drug release studies involve measuring the amount of drug released from a formulation under simulated oral conditions. It is generally accepted that only drug molecules which are in solution can interact with the taste buds, thus measuring the amount of drug released from the formulation can give an insight as to whether a formulation is likely to be aversive or not. However, as these tests only look at the concentration of drug released, they neglect any contribution that excipients may make to the overall taste of the formulation.

It is very difficult to accurately model the conditions a dosage form is subjected to within the oral cavity. The oral cavity is a dynamic environment in which saliva is being continuously produced, dosage forms may disintegrate or be broken down by chewing and tastant molecules are being washed away. Key parameters which need to be considered include volume, temperature, pH and osmolarity of dissolution media use, degree of agitation (either through stirring or shaking) and sampling method used. In addition to these challenges, to quantify the aversiveness of the formulation, the threshold bitterness level of the drug must be known. *In vitro* drug release is extremely useful as a screening study for novel formulations, however, to accurately assess the taste of a formulation additional studies are required.

1.4.1.2 Electronic Tongue

Electronic tongues are sensor array based robotic systems which can be used for the assessment of single substances as well as complex mixtures of substances. Electronic tongue systems can be based on a variety of underlying techniques such as potentiometry, amperometry, voltammetry or impedance spectroscopy with potentiometric systems being most commonly used for pharmaceutical applications. Potentiometry measures the potential of a solution between two electrodes; a reference electrode which has a constant potential and an indicator electrode whose potential is dependent on the composition of the sample being assessed. There are two commercially available electronic tongues, the Alpha MOS Astree electronic tongue and the Insent TS-5000Z taste sensing system, both of which employ potentiometric measurements and use a variety of sensors to mimic what happens when molecules interact with taste buds in the human oral cavity. When a molecule interacts with a sensor there is a change in the electrical potential of the sensor. The response of the
sensors depends logarithmically on the activity of the substances which are measured in a way analogous to that of human taste.\textsuperscript{103}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.5.png}
\caption{Commercially available electronic tongue systems (a) Alpha MOS Astree; (b) Insent TS-5000Z.}
\end{figure}

The Astree electronic tongue is composed of a seven sensor probe, an Ag/AgCl reference electrode and an autosampler. There are three types of sensor sets available i.e. food analysis, pharmaceutical analysis and bitterness intensity measurement for new chemical entities. The sensors measure taste in a cross-selective way and are not specific to an individual taste quality.\textsuperscript{100} The sensors are based on chemically modified field effect transistor (ChemFET) technology. The sensors consist of two highly-conducting semiconductor regions, a source and a drain, which are surrounded by an insulator. A coated membrane is deposited between the source and the drain.\textsuperscript{104} Although the exact composition of the membrane is not disclosed by the company, it is suggested that the membrane is capable of forming Van der Waals or hydrogen bonding interactions with tastant molecules.

The Insent TS-5000Z, which is used in this thesis, can be fitted with up to eight lipid membrane sensors, each of which represent a specific taste quality or mouthfeel i.e. sweet, salty, sour, sweet, astringent or bitter. There are four types of bitterness sensors, AC0, AN0, C00 and BT0 which represent basic bitterness, neutral bitterness, acidic bitterness and bitterness of hydrochloride salts respectively. The sensors are composed of an Ag/AgCl electrode which is surrounded by a lipid membrane (Figure 1.6). The composition of the lipid membrane varies depending on the sensor type but always contains an artificial lipid and a plasticiser. It has been reported by Woertz et al.\textsuperscript{100} that the Insent electronic tongue provides
more reliable (in vitro/in vivo correlation) and precise (reproducible) data than the Astree e-
tongue.

![Figure 1.6 – Schematic diagram of sensors used by Insent TS-5000Z taste sensing system.](image)

It is important to remember electronic tongues are not capable of giving an absolute value
regarding the taste of an API or formulation. To do this the data must be compared to human
taste panel results. However, electronic tongues are useful for comparing the taste of a
formulation to that of a placebo or the API alone.

1.4.1.3 Cell Based Taste Assessment Assays

*Dictyostelium discoideum* is a social amoeba which has been investigated as a potential non-
animal model system for taste assessment of APIs.\(^{105-107}\) The change in behaviour of cells, i.e.
speed, shape and direction of movement, following acute exposure to bitter compounds is
assessed using time lapse photography and computer generated quantification.\(^{105,107}\) When
exposed to bitter compounds, the cells lose their typical amoeboid shape and become round
and, in doing so block the formation of membrane protrusions. Cocorocchio *et al.*\(^{105}\) used the
decrease in protrusion formation to calculate an IC\(_{50}\) value i.e. the concentration of drug
which elicited a 50% reduction in cell movement, for each compound under assessment.
Comparison of *D. discoideum* results to those obtained from rodent BATA testing and human
sensory analysis demonstrated a significant positive correlation between these methods,
indicating the utility of this model for bitterness assessment of APIs.\(^{105}\)

Mammalian taste receptor cell lines have also been investigated as taste sensing models. Hui
*et al.*\(^{108}\) demonstrated that human STC-1 cells can be used to identify bitter tasting molecules
using the electrical cell substance impedance sensing technique. The activation of
gustducin/transducin pathways in bovine taste receptor cell membranes in response to bitter tasting molecules was investigated by Ruiz-Avila et al.\textsuperscript{109} It was found that the activation of gustducin in the presence of the taste bud membrane can be measured to identify certain bitter tastants, determine molecular mode of action and screen chemical libraries for potential bitterness inhibitors. However this method is only suitable if the bitter taste response is gustducin/transducating dependent which is not the case for all molecules, e.g. caffeine.

\textbf{1.4.2 In Vivo Taste Assessment Methods}

\textit{In vivo} taste assessment methods are typically used later in the formulation development process as they can only be used to assess drugs for which human or animal safety data is available. Unlike \textit{in vitro} methods, ethical approval must be sought before commencing such studies, however the data obtained, particularly in the case of human taste panels is considered more robust than that obtained from \textit{in vitro} methods.

\textbf{1.4.2.1 Electrophysiological Studies}

Electrophysiological recordings from cats\textsuperscript{110}, primates\textsuperscript{111}, frogs\textsuperscript{112,113}, gerbils\textsuperscript{96,114} and humans\textsuperscript{115} have been used to provide insights into the physiology of taste sensation and screen new molecules. In the experiments the subject is anaesthetised and electrodes are surgically implanted in the chorda tympani and/or glossopharyngeal nerve. The nerve bundles are then stimulated by passing tastant solutions over the tongue of the subject. This enables dose response curves and temporal profiles of taste stimuli to be obtained.\textsuperscript{116} These studies can be extremely costly and difficult to set up, and of course there are significant ethical considerations to be taken, thus these methods are more likely to be used for sensory research than as a taste assessment technique for pharmaceuticals.

\textbf{1.4.2.2 Rodent Brief Access Taste Aversion Model}

In recent years rodent based taste assessment models have been developed as tools to assess the palatability of tastants. These include the conditioned taste aversion\textsuperscript{117} (CTA), operant taste discrimination\textsuperscript{118}, high throughput taste assessment\textsuperscript{119}, two bottle taste preference\textsuperscript{120} and brief access taste aversion\textsuperscript{121} (BATA) models. The BATA model was used for taste assessment in this thesis.

The BATA model has shown great promise in assessing the taste of APIs with comparable results to human taste panel data\textsuperscript{122,123}. In this taste model rodents, most often mice or rats, are mildly water-deprived and then put in an apparatus called ‘lickometer’ (Figure 1.7). The rodents are then randomly presented with API solutions of varying concentration or water
for a short time (typically 8 seconds\textsuperscript{124}). The ‘lickometer’ records the number of licks that the rodents make to different concentrations of the API under assessment. The number of licks taken is then compared to the number of licks the rat takes of pure water. A high number of licks compared to water will indicate that the solution is palatable whereas a low number of licks will indicate an aversive taste. The number of licks obtained for each concentration can be plotted or the data can be expressed in terms of percentage inhibition of licks per concentration, allowing for generation of concentration-response curves.

\textbf{Figure 1.7 – Davis MS – 160 Lickometer.}

A number of studies have investigated the correlation between rodent and human taste data. Devantier \textit{et al.}\textsuperscript{122} assessed the taste of twenty drugs using the mouse BATA model and selected a subset of these (quinine, ciprofloxacin, clarithromycin and nystatin) for assessment using a human taste panel. It was found that the taste intensities of the four drugs reported by human participants matched those found through BATA testing. In addition, the $EC_{50}$ values (i.e. the concentration of drug which elicited half maximal taste response) obtained from both mouse and human testing were within one half log unit of molar concentration of each other. No general assertion could be made as to whether the mice were more or less sensitive to bitterness than humans as it was found that mice were more sensitive to the taste of quinine and clarithromycin and less sensitive to the taste of ciprofloxacin.
Rudnitskaya et al.\textsuperscript{123} similarly assessed the taste of eight drugs (caffeine, paracetamol, quinine hydrochloride, azelastine hydrochloride, chlorhexidine digluconate, potassium nitrate, naratriptan hydrochloride and sumatriptan succinate) using the rat BATA model and a human taste panel. The rank order of drugs in terms of bitterness was found to be the same for both the human panel and rat experiments. Similar to what was found by Devantier et al.\textsuperscript{122} the EC\textsubscript{50} values obtained from both types of testing were within one half log unit of molar concentration of each other, however the rats always rated the bitterness lower than humans did. The authors suggest that this is due to the fact that, as the rats are mildly water deprived, they have an urge to drink, whereas human participants do not. It is important to note however that the concentrations of drug assessed were not always the same for both panels and in fact for quinine hydrochloride they did not even overlap. In addition, for most of the drugs tested the concentrations assessed by humans were lower than those assessed by rats which may explain why rats were found to be less sensitive to the bitter taste of these drugs.

Variations on the BATA model methodology have been used to assess formulations of bitter tasting drugs. The taste of an iron EDTA complex formulated as both chewable and orodispersible tablets was assessed using a human taste panel and a rat BATA model.\textsuperscript{125} Instead of the traditional BATA method described above, rats were water deprived for 24 hours and then presented with a bottle containing either water or one of three concentrations of drug under assessment for five minutes. The number of licks taken of each solution was measured and percentage inhibition of licks was calculated. The correlation coefficient between the rat and human responses for each formulation under assessment was calculated and found to be above 0.6 in all cases which was deemed a good correlation.

Tiwari et al.\textsuperscript{126} also used a similar method to assess the taste of caffeine citrate formulations prepared by hot melt extrusion. Rats were water deprived for 22 hours before being presented with a bottle containing either water, a sweet tasting solution (fructose in water) or one of the test solutions (pure drug or formulated drug) for thirty minutes. The volume of liquid consumed was recorded and compared to the volume of water consumed. A clear concentration dependent decrease in consumption was observed for the pure drug solutions. A significant increase in consumption of formulation test solutions compared to those containing pure drug was observed indicating the bitter taste of the drug was effectively masked by the formulations. The results of the \textit{in vivo} testing were compared to \textit{in vitro} drug release in simulated salivary fluid and found that the rank order of formulations in terms of predicted aversiveness was identical by both methods.
1.4.2.3 Human Taste Panels

Psychophysical evaluation of taste by human taste panels is considered the ‘gold standard’ for taste assessment of pharmaceutical formulations. Human taste panels can evaluate many characteristics of a formulation, such as taste quality, taste intensity, texture, flavour and aftertaste, which cannot easily be achieved with alternative methods. In human taste panels trained healthy adult volunteers are typically used to evaluate the taste of formulations using an objective scale (e.g. visual analogue scale).

Despite being considered the ‘gold standard’ for taste assessment, human taste panels have many limitations. Recruitment, training and retention of panellists can be challenging and costly. Ethical and/or safety concerns limit what can be assessed in human taste trials. For example, assessment of new chemical entities (NCEs) can only be done when human toxicological data is available. In addition, it would be considered unethical to enrol healthy volunteers to assess the taste of drugs which may be toxic to them e.g. cytotoxic drugs. Taste trials are specifically designed to minimise bias and variability of responses between volunteers. However, it should be noted that even with specially trained subjects, it is still a subjective evaluation method.

As mentioned, human taste assessment of NCEs can only be carried out when extensive human toxicological data is available, which can be problematic when we consider paediatric formulation development. PIPs (section 1.1.3) must be submitted at the end of Phase I trials, when human toxicity data can be quite limited. Thus, human taste data and defined paediatric dose(s) may not be available at the time of writing the PIP which can result in limited information about paediatric dosage forms in the PIP. Additionally, for paediatric formulations ideally taste should be assessed in children as there are differences in taste perception between adults and children (section 1.3.2). However, there are ethical, safety and methodological limitations which can prevent the use of children in taste assessment panels.

1.5 Taste-Masking

Taste-masking of bitter APIs is an essential part of formulation development, particularly for paediatric medicines as palatability is the most important parameter governing paediatric treatment adherence. There are four general taste-masking strategies (Figure 1.8) which are commonly used namely; overpowering the taste of the API e.g. with flavourings or sweeteners, API modification through salt or prodrug formation, creating a molecular barrier around the API e.g. with cyclodextrins or ion exchange resins or by applying a polymeric
coating around the API. In some cases, a combination of these techniques are used, for example, Fini et al.\textsuperscript{127} developed orally disintegrating ibuprofen tablets consisting of a lipid matrix coated in a film forming agent and combined with a sweetener to achieve sufficient taste-masking of the API.

Figure 1.8 – Overview of commonly used taste-masking methods.

1.5.1 Taste-Masking Techniques

Perhaps the simplest form of taste-masking is the combination of an API with a sweetener or flavouring agent to overpower its bitter taste. However, these sweeteners and flavours are subject to regulations which restrict their usage in the paediatric population. Natural sweeteners such as sucrose, glucose or fructose are known to be cariogenic and should be avoided in medicines which must be taken long term. They are also not suitable for diabetic children/adolescents and should be avoided completely in medicines for this group. On the other hand, the use of synthetic sweeteners is considered controversial by some. For example, aspartame, a synthetic sweetening agent has been reported to cause hyperactivity in children, although this has yet to be proven conclusively.\textsuperscript{41} Unfortunately the addition of sweeteners/flavourings is not a platform technology and as such has to be assessed on a case by case basis which can complicate matters, however it still remains an important technique in the formulation scientist’s tool kit.

API modification through salt or prodrug formation masks the taste of a drug by either decreasing solubility or increasing hydrophobicity to reduce the interaction of a drug with the taste buds. However, as this technique involves producing a new compound the
solubility, stability, bioavailability and toxicity of this new compound must be considered. The bitter taste of aspirin can be very effectively masked by forming its magnesium salt which is tasteless. The antibiotic erythromycin can be taste-masked by forming alkylxoyalkyl carbonates at the 2' position, a modification which also increases oral bioavailability.

Cyclodextrins or ion exchange resins can be used to form a molecular barrier around the API or by reducing its oral solubility to prevent it from coming into contact with the taste buds and eliciting a taste response. Cyclodextrins form inclusion complexes with APIs whereby the drug molecule fits into the cavity of the cyclodextrin to form a stable complex. β-cyclodextrin (βCD) is most commonly used for pharmaceutical applications. The marketed product Nicorette Microtabs contain nicotine complexed with βCD to mask its unpleasant taste.

Jagdale et al. produced taste-masked complexes of diltiazem hydrochloride with βCD by a variety of methods i.e. kneading, co-evaporation, co-grounding, freeze-drying and melting. It was found that the bitter taste of diltiazem hydrochloride was best masked by the freeze-dried formulation which was subsequently incorporated into an orodispersible tablet for ease of administration.

Ion exchange resins are water insoluble high molecular weight polymers with salt forming groups at repeating positions on the polymer chain which can exchange ions with the surrounding medium. The API is adsorbed onto the surface of the resin to form a resin-drug complex which masks the taste of the drug. Drug molecules are then released from the complex by exchanging with appropriately charged ions in the GI tract. The cation exchange resin Indion 204 was used to form a resin-drug complex with Etoricoxib which successfully masked the bitter taste of the drug, as demonstrated by a human taste panel.

Polymeric or lipidic coatings may be used to create a physical barrier around the dosage form or API. Solid dosage forms such as tablets, minitablets, multiparticulates or granules may be taste-masked by applying a saliva resistant barrier on the outside of the particle, pellet or tablet. Polymer coating of solid dosage forms may be achieved by techniques such as fluidised bed coating, drum coating or microencapsulation. Conversely lipids may be simply melted and applied directly to the substrate. Polymers commonly used for taste-masking coatings include Eudragit E-PO, Kollicoat IR and hydroxypropylmethylcellulose (HPMC). Lipids typically used for coatings are glycerides such as glyceryl tristearate or hard waxes such as cannauba wax. Guhman et al. produced taste-masked granules of diclofenac by coating the API with an aqueous dispersion of Eudragit E-PO using a fluidised bed coater. The utility of using lipidic coatings for taste-masking was
demonstrated by Suzuki *et al.* who used a mixture of Witepsol H-15 (hydrogenated coco-glycerides), Benecocat BMI-40 and sucrose to mask the taste of paracetamol without influencing the desired immediate release profile of the drug.\textsuperscript{135}

**1.6 HOT MELT EXTRUSION**

Hot melt extrusion has recently emerged as a novel technique for taste-masking of bitter APIs. Hot melt extrusion was first used in the late eighteenth century to manufacture lead pipes. Since then the technique has found uses in the plastic, rubber and food manufacturing industries producing items such as pipes, rubber sheeting and even pasta.\textsuperscript{136} Hot melt extrusion is a very versatile technology which has already been used by the pharmaceutical industry to produce a range of formulations, including pellets, oral-fast dissolving films, controlled release tablets, transdermal/-mucosal delivery systems and implants.\textsuperscript{137} Hot melt extrusion was extensively used in this thesis to produce taste-masked formulations, thus it is described in detail in Chapter 2.

**1.6.1 Hot Melt Extrusion as a Taste-Masking Technique**

Hot melt extrusion has only recently been investigated as a technique for the production of taste-masked dosage forms, but despite this various research groups have demonstrated its validity. One of the first studies carried out in this field was by Gryczke *et al.* in 2003, which used hot melt extrusion to generate taste-masked granules of verapamil HCl – a drug used to treat hypertension and cardiac arrhythmia. The researchers extruded basic salts of verapamil with Eudragit L100 and Eudragit L100-55 polymers in various ratios. Complete taste-masking was observed when drug loading was in the range of 30-50\% wt/wt while a slight bitter taste was observed when loadings above 70\% wt/wt were used.\textsuperscript{138} It was concluded that in this case, hot melt extrusion processing of cationic drugs and anionic polymers results in the formation of strong drug-polymer complexes during the melt extrusion process which contributes to taste-masking by preventing the interaction of the free drug with taste buds in the mouth.

This concept was applied in reverse, again by Gryczke *et al.*, to develop taste-masked granules of ibuprofen.\textsuperscript{139} Anionic ibuprofen was extruded with cationic Eudragit E-PO in various ratios. Effective taste-masking of the ibuprofen granules was observed at drug loadings of up to 25\% wt/wt, with mild bitterness being detected at loadings of 33\% and above. Again, it was concluded that the reduced bitterness of ibuprofen was as a result of the formation of strong drug-polymer interactions. In a subsequent study 40\% ibuprofen was processed with E-PO (50\%) and talc (10\%).\textsuperscript{140} These extruded materials were ground up and
compressed into tablets which were compared to the commercially available ‘Nurofen Meltlets Lemon’ orally dissolving tablets (ODTs). The extruded tablets were found to have better taste-masking efficiency and also exhibited a 5-fold increase in dissolution of ibuprofen compared to the commercially available formulation.

Maniruzzaman et al.\textsuperscript{141} conducted a study which compared the taste-masking effect of extruding paracetamol with either Eudragit E-PO or cross-linked polyvinypyrrolidone (Kollidon VA64). Drug loadings ranging from 30-60% wt/wt were used. The taste-masking properties of the formulations were analysed using both an Astree e-tongue sensor system and an \textit{in vivo} study of six healthy human volunteers. The results obtained from both the \textit{in vitro} and \textit{in vivo} studies indicated that excellent taste-masking was achieved with both polymers for drug loadings up to 50%.

More recently, Maniruzzaman et al.\textsuperscript{142} carried out a study in which cetirizine HCl and verapamil HCl were extruded with either Eudragit L100 or Eudragit L100-55 polymers. A 10:90 wt/wt drug to polymer ratio was used for all formulations. The formulations obtained were analysed using both \textit{in vitro} (Astree e-tongue) and \textit{in vivo} (human taste panel) methods. The \textit{in vitro} study indicated that both polymers effectively masked the bitter taste of the drugs, correlating with the results of the \textit{in vivo} studies in which no bitter taste was reported by the panellists.

1.7 Aims and Objectives

The overall aim of the research detailed in this thesis was to investigate the feasibility of using hot melt extrusion to mask the bitter taste of anti-tuberculosis drugs and develop a prototype paediatric appropriate, taste-masked FDC formulation containing isoniazid and rifampicin. The work detailed in this thesis is composed of three main areas which are explored in the following chapters. The aims of each of these areas were:

(i) To quantify the bitterness of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride using a human taste panel and investigate the feasibility of using non-human tools for the assessment of these drugs.

(ii) To produce taste-masked polymeric formulations containing isoniazid, rifampicin and a combination of the two drugs by HME.

(iii) To use the taste-masked extrudate to produce a FDC dispersible tablet suitable for paediatric administration.
As mentioned above, the overall aim was to develop and age-appropriate taste-masked FDC. However, in order to effectively taste-mask, the bitterness levels of these drugs must be understood. There is a wealth of anecdotal information which states that the bitter taste of these drugs is a major barrier to treatment compliance but to date there have been no studies which quantify exactly how bitter these drugs are. The first objective of this work was therefore to quantify the bitterness of isoniazid, rifampicin, pyrazinamide and ethambutol. This was done by assessing these drugs using a human taste panel. While human taste panels remain the ‘gold standard’ for taste assessment they can be very time consuming and costly to run, as well as having significant ethical considerations. Non-human techniques for taste assessment are therefore extremely useful, particularly for screening prototype formulations during early stage development. Thus the second objective of this section was to investigate the utility of non-human tools for taste assessment of these drugs. The ability of two non-human techniques to detect and assess the taste of isoniazid, rifampicin, pyrazinamide and ethambutol hydrochloride were investigated, the rodent BATA model and the electronic tongue. The results obtained from these experiments were compared to those from the human taste panel to determine what, if any correlation there was between the techniques.

The second area of this thesis had two main objectives. The first was to use HME to produce polymeric formulations of isoniazid, rifampicin and a fixed dose combination containing isoniazid and rifampicin in a dose ratio suitable for paediatric patients. The second objective was to determine the taste-masking efficiency of these formulations.

The final area of this thesis focused on the development of an FDC dispersible tablet which would be suitable for paediatric administration. The first objective in this area was to mill the FDC extrudates produced by HME and determine whether this powder, when combined with appropriate excipients would be suitable for the production of a tablet by direct compression. The final objective was to determine the taste-masking efficiency of this formulation which was carried out by determining the amount of drug released from the formulation under simulated administration conditions.
CHAPTER 2

Materials and Methods
Chapter 2

2.1 MATERIALS

2.1.1 Model drugs

2.1.1.1 Isoniazid

Isoniazid (Figure 2.1) is a highly bactericidal drug which is the cornerstone of modern anti-
tuberculosis regimens. It is used in both the intensive phase and continuation phase of
tuberculosis treatment.\(^{143}\) It is a borderline BCS Class I/III drug\(^{144}\), with an aqueous solubility
of approximately 125 mg/mL.\(^{145}\) Isoniazid is generally well tolerated, however common side
effects of this drug can include nausea, vomiting, constipation, dry mouth and skin rashes.
Less common side effects include peripheral neuritis, optical neuritis and hepatitis.\(^{146}\)

![Figure 2.1 – Chemical structure of isoniazid.](image)

The mechanism of action of isoniazid has been extensively studied and it is now suggested
that isoniazid may kill \textit{M. tuberculosis} through a combination of synergistic mechanisms.\(^{147}\)
Isoniazid is a prodrug which enters the mycobacterial cell via passive diffusion.\(^{148}\) Once inside
the cell, isoniazid is activated by an oxidant (e.g. superoxide, hydrogen peroxide) and the
heme catalase-peroxidase enzyme KatG to form an isonicotinoyl radical (Figure 2.2). This
isonicotinoyl radical inhibits InhA (2-trans-enoyl-acyl carrier protein reductase), an enzyme
involved in fatty acid elongation, by covalently binding to NADH within the active site of the
protein.\(^{149}\) Inhibition of this enzyme prevents the production of long chain fatty acids, known
as mycolic acids, which are major components of the mycobacterial envelope.\(^{150}\)

The oxidative activation of isoniazid by KatG results in the production of a variety of carbon,
oxogen and nitrogen centred free radical species which can then go on to attack key cellular
components such as lipids, proteins and nucleic acids.\(^{147}\) This combination of mechanisms
makes isoniazid extremely effective as it attacks multiple targets in the bacterial cell,
however resistance to this drug can still occur. \textit{M. tuberculosis} resistance to isoniazid
primarily occurs through mutations in KatG which cause the enzyme to be unable to activate
the prodrug to the reactive isonicotinoyl radical.\(^{151}\)
2.1.1.2 Rifampicin

Rifampicin (Figure 2.3), like isoniazid, is used in both the intensive and continuation phase of tuberculosis therapy.\textsuperscript{55} Rifampicin is a bactericidal drug which also has ‘sterilising’ effects.\textsuperscript{152} Rifampicin, along with pyrazinamide, is known as a ‘sterilising’ drug because they are capable of clearing difficult to eradicate, dormant, bacilli.\textsuperscript{153} It is a borderline BCS Class II drug\textsuperscript{154} (high permeability, low solubility). It is zwitterionic and as a result its solubility, permeability and lipophilicity are pH dependent.\textsuperscript{155} The solubility of rifampicin decreases from approximately 125 mg/mL at pH 1 to only 0.85 mg/mL at pH 7.\textsuperscript{156} Common side effects of rifampicin include nausea, vomiting, diarrhoea, headache and orange-red staining of bodily secretions such as saliva, urine and sweat. Less common side effects include haemolytic anaemia, renal failure and jaundice.\textsuperscript{146}
Rifampicin is an inhibitor of bacterial RNA polymerase, an enzyme responsible for DNA transcription.\textsuperscript{157} Most drugs which act on RNA polymerase typically interact with the DNA template, whereas rifampicin specifically inhibits the enzyme by forming a stable drug-enzyme complex, thus preventing transcription from occurring.\textsuperscript{158} This specificity of action means that, unlike RNA polymerase inhibitors which interact with the DNA template, rifampicin is not toxic to mammalian RNA polymerase.\textsuperscript{159} Resistance to rifampicin typically occurs due to mutations which alter the residues of the rifampicin binding site, resulting in a decreased affinity for the drug.\textsuperscript{160}

2.1.1.3 Pyrazinamide

Pyrazinamide (Figure 2.4) is a ‘sterilizing’ drug which is only used in the intensive phase of treatment.\textsuperscript{153} The use of pyrazinamide in the intensive phase of treatment results in the killing of persistent \textit{M. tuberculosis} bacilli and allows the total treatment course to be shortened from 9 - 12 months to 6 months.\textsuperscript{161} It is a BCS Class III drug with an aqueous solubility of approximately 15 mg/mL.\textsuperscript{162} Side effects of pyrazinamide include nausea, vomiting hepatotoxicity, jaundice and occasionally photosensitivity.\textsuperscript{146}

\textbf{Figure 2.4 – Chemical structure of pyrazinamide.}

Pyrazinamide is an unusual drug as, despite its exceptional sterilizing activity \textit{in vivo}, it is inactive against \textit{M. tuberculosis} under standard \textit{in vitro} culture conditions.\textsuperscript{163} Extensive studies have been carried out to elucidate the mechanism of action of pyrazinamide since its discovery in the 1950's, however, it is still the least well understood of the first line tuberculosis drugs. Similar to isoniazid, pyrazinamide is a prodrug which must be activated after entering the cell via passive diffusion.\textsuperscript{161} Once inside the cell, pyrazinamide is converted
to pyrazinoic acid by the amidase enzyme nicotinamidase/pyrazinamidase (PZase) (Figure 2.5).\textsuperscript{164} Pyrazinoic acid then accumulates in the cell which is suggested to cause cell death by disrupting the proton motive force required for essential membrane transport functions.\textsuperscript{165}

\begin{center}
\begin{tikzpicture}
  \node at (0,0) {Pyrazinamide} edge[->] node[above] {PZase} (1,0) node {Pyrazinoic acid} + node {Ammonia} (0.5,0);
\end{tikzpicture}
\end{center}

\textit{Figure 2.5 – Schematic representation of the conversion of pyrazinamide to pyrazinoic acid by nicotinamide/pyrazinamidase enzyme (PZase).}

Resistance to pyrazinamide is primarily attributed to mutation in the \textit{pncA} which encodes the PZase enzyme. Resistance may also occur as a result of deficient uptake of pyrazinamide by the bacterial cell, however this is less common.\textsuperscript{166}

2.1.1.4 Ethambutol Dihydrochloride

Ethambutol dihydrochloride (Figure 2.6) is a bacteriostatic drug which is only used in the intensive treatment phase of tuberculosis. It does not contribute to sterilisation but acts to prevent resistance developing to its companion drugs or to prevent broadening of the resistance spectrum in cases where the bacillus is resistant to one of the first line drugs being used.\textsuperscript{143} It is a BCS Class III drug which is freely soluble in water (up to 1000 mg/mL).\textsuperscript{167} Side effects of ethambutol include optical neuritis, peripheral neuritis and red/green colour blindness.\textsuperscript{146}

\begin{center}
\begin{tikzpicture}
  \node {OH} edge[->] node[above] {} (1,0) node {N} edge[->] node[above] {} (1.5,0) node {N} edge[->] node[above] {} (2,0) node {H} edge[->] node[above] {} (2.5,0) node {H} edge[->] node[above] {} (2.8,0) node {2HCl} edge[->] node[above] {} (0,0);
\end{tikzpicture}
\end{center}

\textit{Figure 2.6 – Chemical structure of ethambutol dihydrochloride.}

The primary mechanism of action of ethambutol is proposed to be inhibition of arabinogalactan (a mycobacterial cell wall component) biosynthesis.\textsuperscript{168} Resistance to ethambutol primarily occurs due to mutations in the \textit{embB} gene.\textsuperscript{169}

2.1.2 Excipients

2.1.2.1 Soluplus

Soluplus (Figure 2.7) is a polyvinyl caprolactam-polyvinyl acetate – polyethylene glycol graft copolymer specifically developed for solid solutions. It has an approximate molecular weight
of 118,000 g/mol and a glass transition temperature ($T_g$) of 70°C.\textsuperscript{170} It is presented as a free-flowing white to slightly yellowish granule and has practically no taste.\textsuperscript{171} Soluplus has been widely used for solubility enhancement of poorly water soluble drugs such as carbamazepine\textsuperscript{172}, artemether\textsuperscript{173} and meloxicam\textsuperscript{174} via hot melt extrusion. It has not however been extensively studied as a taste-masking polymer.

![Chemical structure of Soluplus.](image)

**Figure 2.7 – Chemical structure of Soluplus.**

### 2.1.2.2 Eudragit E-PO

Eudragit E-PO (Figure 2.8) is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate\textsuperscript{175} which is widely used in the pharmaceutical industry as a taste-masking polymer. It has a $T_g$ of approximately 45 ± 5°C. It is soluble in gastric fluid up to pH 5 and is insoluble but swellable and permeable at pH values > 5.\textsuperscript{176} This pH dependent solubility makes it ideal for taste-masking as it will not dissolve in saliva which has been reported to have a pH range of 5.3-7.8.\textsuperscript{98} However, as mentioned, it is still swellable and permeable under these conditions so it is possible for some drug release to occur in the oral cavity.

![Chemical structure of Eudragit E-PO.](image)

**Figure 2.8 – Chemical structure of Eudragit E-PO.**

Eudragit E-PO has been used with a variety of formulation techniques to both increase the dissolution rate of poorly water soluble drugs\textsuperscript{177,178} and mask the taste of bitter drugs.\textsuperscript{141,179,180}
2.1.2.3 Starch 1500

Starch 1500 is a partially pregelatinized maize starch which is used as a binder, disintigrant, filler and flow aid for tablet production. Starch is composed of amylose and amylopectin (Figure 2.9). Amylose is a helical polymer consisting of α-D-glucose units bonded through α(1 – 4) glycosidic bonds. Amylopectin on the other hand is a branched macromolecule composed of short chains of α(1 – 4) linked α-D-glucose with α(1-6) linked branches. On exposure to water amylose swells and amylopectin dissolves, thus aiding disintegration of the tablet. Starch 1500 is pregelatinized meaning that the bonds between portions of the two polymers are broken. This imparts enhanced flow and compression characteristics making it a particularly useful excipient for direct compression.

![Chemical structure of (a) amylose; (b) amylopectin.](image)

2.1.2.4 Sodium Stearyl Fumarate

Sodium stearyl fumarate (Figure 2.10) is used as a lubricant in capsule and tablet formulations, typically at 0.5 – 2.0% w/w concentration. Lubricants are used in tableting to reduce friction between the tablet and die and to prevent adhesion of the powder blend to the punches or die wall. It is water soluble and generally regarded as a non-toxic and non-irritant material.
Figure 2.10 – Chemical structure of sodium stearyl fumarate.

2.1.3 Raw Materials Sourcing

Table 2.1 lists the suppliers of all APIs, polymeric carriers and other chemicals detailed in this chapter.

Table 2.1 – Sources of chemicals used in this study.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Sigma Aldrich (UK)/Macleods (India)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Fagron Ltd. (UK)/Macleods (India)</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Sigma Aldrich (UK)/Macleods (India)</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>Sigma Aldrich (UK)/Macleods (India)</td>
</tr>
<tr>
<td>Soluplus</td>
<td>BASF (Germany)</td>
</tr>
<tr>
<td>Eudragit EPO</td>
<td>Evonik GmbH (Germany)</td>
</tr>
<tr>
<td>Starch 1500</td>
<td>Colorcon (UK)</td>
</tr>
<tr>
<td>Sodium stearyl fumarate</td>
<td>JRS Pharma (Germany)</td>
</tr>
</tbody>
</table>

2.2 METHODS

2.2.1 Rodent BATA Model

The rodent BATA model has shown great promise in assessing the taste of APIs with comparable results to human taste panel data. In this thesis rats were the rodent of choice for taste assessment. A typical rat BATA experiment has a time frame of 5 days. The experiment begins with a short water deprivation period. This period can range from 16 – 24 hours. The water deprivation step is required in order to acquire adequate lick detection and has not been shown to adversely impact on the welfare of the animals. To ensure the welfare and safety of animals in this study the weight and hydration of each rodent on a water deprivation schedule was checked every day. If the weight of an animal dropped below 85% of their free feeding weight the animals would receive adequate rehydration to recover their normal weight or be excluded from the study.

After a sufficient period of water deprivation, the rat is placed in the lickometer device. The lickometer enables the counting of the number of licks the rat takes of each sample they are
presented with. There are three main types of lickometer i.e. optical, force and electrical. Optical lickometers detect licks by measuring how many times the animal’s tongue interrupts a beam of light. Force lickometers detect the pressure of an animal’s tongue on the licking surface. In electrical lickometers the spout of the sample bottle and the floor of the test cage are connected to an electrical circuit and, when the animal completes the circuit with its tongue, the change in voltage or capacitance enables detection of the lick. The current used in these devices is kept in the sub-μA range to ensure the animal is not adversely affected by it.

The Davis MS – 160 lickometer (DiLog Instruments, Tallahassee, Florida, USA) was used in this study (Figure 2.11a). It consists of a transparent cage with a wire mesh floor and a front metal wall containing a shutter which can be raised or lowered electronically. Up to sixteen glass drinking tubes may be mounted on a sliding rack at the front of the cage. When the shutter is raised, the animal has access to one of the drinking tubes. The rack is controlled by a computer which allows for the position of the shutter, the tube being presented and the stimulus interval to be controlled. When the animal’s tongue makes contact with the drinking tube the circuit is completed and the lick is detected. The Davis MS – 160 lickometer has a low open – circuit input voltage (0.75 V) and a very low short – circuit current (0.06 μA) which is low enough to be undetected by the rodents and has no impact on their health or behaviour.

Each rat is placed in the lickometer for a session length of 30 – 60 minutes. The shutter opens and a drinking tube is presented to the rat for 5 – 10 seconds and the number of licks taken is recorded. The shutter then closes and the rat has a brief break before the next drinking tube is presented. After each sample is tested a brief water rinse is provided. Upon completion of the testing session the rat is allowed free access to water for at least 15 minutes to ensure sufficient rehydration.
2.2.2 Human Taste Panel

The human taste panel contained in this thesis was carried out using the ‘swirl and spit’ technique. This is a single blind technique in which participants are asked to assess the taste of solutions of drug which are presented at random. Participants rinse their mouth with the solution for 5 seconds, covering all oral surfaces before expectorating the sample. Immediately upon expectoration participants rate the taste on a bipolar visual analogue scale. They then rinse their mouths with water and can eat an unsalted cracker to neutralise
the taste. A 10 minute break is given between samples and sessions typically last no longer than two hours in order to minimise taste fatigue. The short exposure time of participants to the samples coupled with the fact that samples are not ingested means that risks of adverse reactions is minimised. A full description of the technique used for the human taste panel detailed in this thesis is given in Section 3.3.3.

2.2.3 Electronic Tongue

The Insent TS-5000Z electronic tongue was used in this thesis to assess the taste of both drugs and novel formulations. As discussed in Chapter 1 this device can be fitted with up to eight lipid membrane sensors, each of which represent a specific taste quality or mouthfeel i.e. sweet, salty, sour, sweet, astringent or bitter.

The mechanism of detection of the Insent e-tongue is based on the Gouy Chapman theory which states that when an object is immersed in an aqueous solution, an electrical double layer is formed at the surface of the object. In this case, formation of the double layer is due to dissociation of the acid groups of the lipid membrane of the sensor.\textsuperscript{187} The first layer, known as the Stern layer, is composed of ions (either positive or negative) adsorbed onto the membrane. The second layer is composed of ions attracted to the surface charge of the Stern layer, which act to electrically screen the Stern layer. The membrane potential can then be calculated using the Poisson-Boltzmann equation.\textsuperscript{188} The addition of drug to the aqueous solution affects the membrane potential by changing the environment surrounding the membrane. For example, addition of an acidic molecule prevents dissociation of the acid groups, thus leading to a change in membrane potential.\textsuperscript{187}

2.2.3.1 Sensors

Sensors and reference electrodes were purchased from PPM instruments (West Sussex, UK). The TS-5000Z (Insent Inc., Atsugi-shi, Japan) was equipped with four lipid membrane sensors and two corresponding reference electrodes. Three of the sensors represent bitterness, bitterness sensor 1 (SB2AC0), bitterness sensor 2 (SB2AN0) and bitterness sensor 3 (SB2C00). The fourth sensor represents astringency (SB2AE1). Before beginning experiments 0.2ml inner solution (0.33M potassium chloride and saturated silver chloride) was filled into each sensor. Reference electrodes were completely filled with inner solution. Lipid membrane sensors were preconditioned in standard solution for 24 hours before measurement. Reference sensors were preconditioned in 3.33M potassium chloride for 24 hours before measurement.
2.2.3.2 Measurement Procedure

A schematic diagram of the measurement procedure is given in Figure 2.12. Initially sensors are washed in reference solution twice, with each washing lasting 120 seconds. The reference solution contains 30mM KCl and 0.3mM tartaric acid and is designed to mimic human saliva. Up to ten pairs of reference solution are available and a different pair is used for each washing cycle. After the initial washing step sensors are placed into a separate reference solution and the voltage is recorded. It is expected that the voltage reading will remain constant when the sensor is placed in reference solution ($V_r$). The sensors then move on to the first sample where they are immersed for 30 seconds and the voltage is recorded ($V_s$). The voltage reading for the initial taste is given by $V_s - V_r$. The sensors are then briefly washed (3 seconds) in two different pairs of reference solution. The sensors are then immersed again in reference solution for 30 seconds and another voltage reading is recorded ($V_{r'}$). $V_{r'} - V_r$ then denotes the aftertaste or change in membrane potential after adsorption (CPA). Finally the sensors are rinsed in either a positively or negatively charged washing solution (as appropriate) before moving onto the next sample.

![Schematic diagram of TS-5000Z electronic tongue measurement procedure cycle.](image)

2.2.3.3 Maintenance Measurement

A maintenance measurement was carried out once a month when the machine was in continuous use and every time the sensors were changed. Sensors are exposed to standard solutions which represent salty, sour, bitter and astringent taste attributes (Appendix B). Each sensor is expected to respond only to the taste attribute for which it is designed. If any
sensor responds to a standard solution for which it is not designed, then it is considered defective and must be replaced.

2.2.3.4 Sensor Check

Prior to commencement of each experiment a sensor check was carried out. This involves washing the sensors in the positive and negative washing solutions (as appropriate) for 2 x 120 seconds before washing for 90 seconds in reference solution. There are up to 10 pairs of reference solutions which can be used. Following the 90 second wash sensors are immersed in a different pair of reference solutions and the voltage readings of each sensor is recorded. The voltage readings must be within a certain range (Table 2.2) and the procedure is repeated up to twenty times until the sensor gives the required reading on three consecutive occasions. If the required voltage reading is not achieved after twenty attempts then the sensor is marked as defective. Please note it is possible for the sensors to pass the sensor check and fail the maintenance measurement, thus routine maintenance measurements (section 2.2.3.3) are still essential.

Table 2.2 – Required voltage readings to be achieved on three consecutive occasions by sensors during sensor check.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Voltage Reading (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN0</td>
<td>-63 ± 2.0</td>
</tr>
<tr>
<td>AC0</td>
<td>-65 ± 2.0</td>
</tr>
<tr>
<td>C00</td>
<td>47 ± 2.0</td>
</tr>
<tr>
<td>AE1</td>
<td>119 ± 2.0</td>
</tr>
</tbody>
</table>

2.2.4 Hot Melt Extrusion

Hot melt extrusion, as discussed in Chapter 1, is a technique which is widely used in the pharmaceutical industry for the production of drug delivery systems. The hot melt extrusion process (Figure 2.13a) can essentially be broken down into four stages (i) feeding the mixture to be extruded through a hopper, (ii) mixing, grinding, size reduction, melting and kneading of the mixture, (iii) flow of mixture through the die, (iv) extrusion from the die and downstream processing (e.g. pelleting/granulation). Extruders used for pharmaceutical applications are typically of the twin-screw type as they allow for both dispersive and distributive mixing to occur (Figure 2.13b). Distributive mixing refers to content uniformity of an ingredient within the extrudate. Dispersive mixing is related to size reduction and
Combining both types of mixing ensures that the HME product is uniform throughout.

A typical twin extrusion set up is composed of a motor that rotates the screws, an extrusion barrel, two rotating screws, an extrusion die and an electronic control unit. The material to be extruded (e.g. an API and appropriate polymer) is fed via a hopper above the feeding zone into the screw. The extrusion barrel contains different zones, which are pre-set to the required temperature before extrusion begins. The turning motion of the screw conveys the material to be extruded through the barrel. As the material is moved along the barrel thermal energy is generated by the action of the turning screw. This, coupled with the thermal energy supplied by the heaters in the machine, causes the material to melt and start to homogenise. This melted material is then pushed towards the die at the end of the barrel which moulds the resultant extrudate. The control unit controls the process parameters such as temperature within the barrel and screw speed. Downstream processing equipment which cools and collects the extrudate may also be employed.

The processing parameters used for hot melt extrusion play a key role in determining the properties of the final extrudate. The most commonly adjusted parameters are processing temperature and screw speed. The processing temperature must be determined based on the melting temperature ($T_m$) of the drug, the glass transition temperature ($T_g$) of the polymeric carrier and the thermoplastic properties of the drug-polymer mixture as well as the degradation temperature of all materials used. Plasticizing agents may be used to lower the processing temperature required in order to prevent thermal degradation of the API. A plasticizer is typically a low molecular weight compound which lowers the $T_g$ and increases the elongation and softness of the polymer.

![Figure 2.13](image)

*Figure 2.13 – (a) Schematic diagram of hot melt extrusion process; (b) Comparison of dispersive versus distributive mixing.*
There are many advantages to using HME over other processing techniques. Removal and disposal of potentially harmful organic solvents is a common issue with many pharmaceutical processing techniques. HME is a solvent free process, thus removing the need for this step as well as making it an environmentally friendly process. It is a continuous non-ambient process which is easy to scale up, increases the stability of the API within the carrier matrix and is not time consuming.\textsuperscript{193} It also meets the goals of the FDA process analytical technology (PAT) scheme for quality by design in pharmaceutical products.\textsuperscript{194} One drawback of HME is that since it is a thermal process it is not suitable for extremely heat sensitive molecules, although low to medium heat sensitive molecules can be used as the time exposure of the APIs to the heat extrusion process is quite short.

In this thesis, hot-melt extrudates were prepared using a Thermo Scientific Process 11 co-rotating twin screw extruder (ThermoScientific, UK) fitted with a round die (die diameter: 2mm). The temperature and screw speed used varied depending on the formulation being extruded. Details of these parameters are given in the appropriate chapters.

### 2.2.5 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a thermal analysis technique which is widely used within the pharmaceutical sciences. In a typical DSC experiment a linear heating or cooling signal is applied to a sample and the subsequent flow of energy into or out of the material is recorded. This allows the characterisation of a range of thermal events such as melting, glass transitions and recrystallisation.\textsuperscript{195} There are two types of DSC, heat flux and power compensation. Heat flux DSC is the most commonly used and was the type used in this thesis.

In heat flux DSC (Figure 2.14) a sample pan and an empty reference pan are placed in a furnace and a common heat sources heats both pans at the same rate. If a transition occurs within the sample a temperature difference will occur between the sample and reference pan. The heat flow (\( \frac{dq}{dt} \)) is proportional to the temperature difference.\textsuperscript{196}

\[
\frac{dq}{dt} \sim \Delta T \quad \text{Equation 2.1}
\]

Conversion of \( \Delta T \) to power is done by multiplication by an experimentally determined cell constant \( k \):

\[
\frac{dq}{dt} = k\Delta T \quad \text{Equation 2.2}
\]
Chapter 2

Figure 2.14 – Schematic diagram of heat flux differential scanning calorimetry.

DSC experiments must be carried out in an inert atmosphere which may be achieved by purging the cell with an inert gas e.g. nitrogen. A constant flow of purge gas enhances heat flow between the cell and the sample pan while also allowing removal of volatile compounds, e.g. water vapour.

To ensure the accurate performance of the instrument routine calibration processes were carried out each time an experimental parameter (e.g. heating rate, pan type) was changed. In this case calibrations were carried out using high purity indium, n-octadecane, benzoic acid and tin.

DSC thermograms were recorded using a TA Instruments Q2000 calorimeter (TA Instruments, New Castle, Delaware, USA). For analysis 4-6 mg of sample was accurately weighed and sealed in an aluminium pan (hermetic and pinhole pans used as appropriate). Samples were heated under nitrogen gas (flow rate 50mL/min) at a rate of 10°C/min.

2.2.6 Modulated Temperature Differential Scanning Calorimetry

Modulated temperature differential scanning calorimetry (MTDSC) is an extension of conventional DSC in which a perturbation is applied to the heating program. This perturbation is typically a sinusoidal wave and, when combined with a mathematical procedure, allows deconvolution of the different types of sample heat flow. The separation procedure is represented as follows:

\[
\frac{dq}{dt} = C_p \frac{dT}{dt} + f(t, T)
\]

Equation 2.3
where $C_p$ represents heat capacity, $T$ is absolute temperature, $t$ is time and $f(t, T)$ is a function of time and temperature which governs the response associated with the physical or chemical transformation. $C_p \frac{dT}{dt}$ describes the heat flow associated with heat capacity of the sample and represents reversible processes e.g. glass transitions. $f(t, T)$ is related to the enthalpy of the reaction and represents irreversible events e.g. relaxation and crystallisation. Overall MTDSC gives three signals in a single heating run which allows, heat capacity, kinetic and total heat flow to be delineated.

![Figure 2.15 – Schematic diagram of modulated temperature differential scanning calorimetry thermogram.](image)

There are three key parameters for MTDSC experiments namely; underlying heating rate, temperature modulation amplitude and reciprocal period. The heating rate used for MTDSC is much slower than that used for conventional DSC ranging from 1 – 5°C/min, compared to 10 – 20°C/min. This low underlying heating rate is combined with large modulation amplitudes, typically in the range of ± 0.1 to ± 1°C. The modulation period is generally 30 – 80 seconds long. In order to enable complete separation it is necessary to have at least six modulation cycles throughout the duration of each thermal event. MTDSC calibration was carried out in an analogous way to that of conventional DSC, with the additional step of heat capacity calibration using a sapphire disc. Modulated temperature DSC (MTDSC) thermograms were recorded using a TA Instruments Q2000 calorimeter (TA Instruments, New Castle, Delaware, USA). For analysis 4-6 mg of sample was accurately weighed and sealed in an aluminium pan (hermetic and pinhole pans used as appropriate). Samples were
heated under nitrogen gas (flow rate 50mL/min) at a rate of 2°C/min, amplitude ± 0.212°C and a period of 40 seconds. Calibration was performed using n-octadecane, benzoic acid, indium, and tin.

2.2.7 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is a technique which measures the change in weight of a sample as a function of temperature (non-isothermal experiments) or time (isothermal experiments). TGA is typically used in the pharmaceutical sciences to assess the water/solvent content and degradation temperature of a solid drug or formulation. It can also be used to determine the stoichiometry of solvates and indicate the strength of water binding on the basis of the temperature at which the solvent is lost.\textsuperscript{195}

TGA instruments are generally equipped with sample holder, a high sensitivity balance, a furnace and a recorder. Similar to DSC, the furnace is purged with a gas such as air or nitrogen to maintain a controlled atmosphere around the sample and ensure removal of volatile substances. In this study TGA was carried out using a TA Instruments Hi-Res 2950 thermogravimetric analyser (TA Instruments, New Castle, Delaware, USA). Samples were analysed in open aluminium pans with a heating rate of 10°C/min.

2.2.8 Powder X-Ray Diffraction

Powder X-ray diffraction (PXRD) is a fundamental technique for structural characterisation of materials. In an X-ray diffractometer X-rays are generated by heating a filament (the cathode) which acts as a source of electrons. These electrons are accelerated and hit a target (the anode) composed of a transition metal such as copper, molybdenum, chromium or silver to produce a characteristic X-ray spectrum.\textsuperscript{199} When X-rays pass through the sample being assessed they interact with the electron cloud of atoms contained within the sample and the intensity and position of the resultant X-rays are recorded by a detector. The diffraction of X-rays as a result of interaction with crystalline material is governed by Braggs Law:\textsuperscript{200}

\[ n\lambda = 2d\sin\theta \quad \text{Equation 2.4} \]

where \( n \) is the order of the diffracted beam, \( \lambda \) is the wavelength of the incident X-ray beam, \( d \) is the distance between adjacent planes of atoms and \( \theta \) is the angle of incidence of the X-ray beam. By altering \( \theta \) during scanning, the distance between adjacent planes of atoms, or d-spacing, can be varied. By plotting beam intensity against angle of emergence a diffractogram with peaks corresponding to lattice spacing is obtained which gives an insight into the molecular arrangement and orientation of atoms within a sample.\textsuperscript{201}
Materials which are crystalline and thus have long range order produce diffractograms with clearly defined sharp peaks of varying intensities which correspond to the uniform lattice spacing, arrangement and orientation of molecules within the sample. On the other hand, materials which do not have long range order, i.e. are amorphous, produce a broad signal with no defined peaks known as an amorphous halo.

In this study PXRD was carried out using a Rigaku 600 Miniflex diffractometer (Rigaku, Tokyo, Japan), CuKα radiation, operating power: 40mV, 15mA. Patterns were recorded over the 2θ range 3°-40° at a scan rate of 5°/min.

2.2.9 Dissolution Testing

Dissolution testing is a fundamental aspect of formulation development which provides an insight into the rate and extent of drug absorption while assessing the effect of drug and formulation properties on the release profile of the drug. In this thesis, the dissolution profiles of formulations in both gastric and oral conditions were assessed.

The dissolution rate of a solid in a liquid can be described by the Noyes – Whitney equation\textsuperscript{202,203}:

\[
\frac{dc}{dt} = \frac{DA}{Vh}(C_s - C_x)
\]

\textbf{Equation 2.5}

where \(\frac{dc}{dt}\) represents dissolution rate, \(D\) is the diffusion coefficient, \(A\) is the surface area, \(V\) is the volume of the dissolution medium, \(C_s\) is the saturation solubility of the drug, \(C_x\) is the drug concentration of the bulk solution and \(h\) is the hydrodynamic boundary layer thickness. It should be noted that in this equation it is assumed that the surface area and thickness of the hydrodynamic layer remain constant.

Gastric dissolution studies in this thesis were carried out in 900 mL 0.1N hydrochloric acid at 37°C using either US Pharmacopoeia (USP) apparatus Type I (basket) or II (paddle) as appropriate with the aid of a Pharmatest PTWS 120D dissolution apparatus (Pharma Test Apparatebau AG, Hainburg, Germany). There are no dissolution techniques recommended by either USP or British Pharmacopoeia (BP) for taste-masked formulations which mimic the conditions experienced in the oral cavity. In this thesis, oral dissolution studies were designed to mimic the oral cavity as closely as possible in terms of dissolution media, volume, agitation and residence time of sample. 5 mL of simulated salivary fluid (SSF) at 37°C was used as dissolution media and samples were gently stirred using a magnetic stirrer. Samples were withdrawn at set time periods (up to 5 minutes), filtered and analysed by either UV-Vis spectrometry or HPLC.
2.2.10 Direct Compression Tabletting

Powder compression is the most commonly used method of tablet production. Powder compression may be defined as ‘the reduction in volume of a powder due to application of a force’. The tabletting process can be divided into three stages (i) die filling; (ii) tablet formation and (iii) tablet ejection. Powder is filled into the die before being compressed. During compression, the powder particles are forced together by the upper and lower punches of the tablet press. This increased proximity promotes bond formation between the particles resulting in formation of the compacted tablet which is then ejected from the tablet press.

There are two types of tablet press: the single punch press and rotary (multi-station) press. In this study, a single punch press was used. A single punch press (Figure 2.16) has one die and one pair of punches (lower and upper). The powder is filled into a hopper shoe which moves backwards and forwards over the die, allowing the powder blend to be fed into the die. During compression, the lower punch is stationary while the upper punch descends and enters the die, compressing the powder into a tablet. The lower punch then moves up, ejecting the tablet which is moved away by the hopper shoe.

In this thesis tablets were produced using a Manesty Type F3 Tablet Press (Manesty, Liverpool, UK) fitted with 10 mm diameter round flat faced punches.

Figure 2.16 – Schematic diagram of single punch tablet press.
CHAPTER 3

In Vivo Taste Assessment of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol
CHAPTER 3

3.1 INTRODUCTION

The aversive taste of medicines is a major cause of non-adherence to treatment regimens, particularly in the case of anti-tuberculosis treatment. It has been reported in the literature that isoniazid, rifampicin, pyrazinamide, and ethambutol, the four first-line drugs used for the treatment of tuberculosis, have aversive tastes. However, despite many reports of this, there is no quantitative data available as to the bitterness levels of these drugs.

The palatability of drugs and formulations can be assessed in a number of ways, both in vitro and in vivo. In vitro methods include electronic taste sensors, known as electronic tongues, drug release and dissolution tests. In vivo methods include human taste panels and animal taste preference tests such as the rodent brief access taste aversion (BATA) test.

Human taste panel studies involve evaluating the taste of medicines or dosage forms by estimating the gustatory sensation responses in healthy adult human volunteers. Typically, subjects are required to assess the taste stimulus on an intensity scale where 0 represents no taste at all and the maximum point on the scale represents extremely aversive, unbearable taste. Human taste panels are generally sensitive measures of taste and are specifically designed to minimise bias and variable responses between the volunteers. However, it should be noted even with specially trained subjects, it is still a subjective evaluation method.

Animal models such as mice, rats, and dogs may also be used to assess taste. The rodent Brief-Access Taste Aversion (BATA) model is an in vivo taste assessment tool that has shown great promise in assessing the taste of Active Pharmaceutical Ingredients (APIs) with comparable results to human taste panel data. In this animal taste model, rodents, most often mice or rats, are mildly water-deprived and then put in an apparatus called a ‘lickometer’ that records the number of licks that the rodents make to different concentrations of the API under assessment. A high number of licks will indicate that the solution is palatable whereas a low number of licks compared to water will indicate an aversive taste. With this procedure, a full concentration-response curve of lick rate can be obtained over a short period of time with very few animals.

Despite numerous reports of the bitter taste of tuberculosis medicines being a barrier to treatment adherence, no studies have been carried out to quantify the bitterness of these drugs. To address this, in the present study, the taste of isoniazid, rifampicin,
pyrazinamide and ethambutol was assessed using a human taste panel and the rodent brief access taste aversion model.

3.2 AIMS AND OBJECTIVES

The aim of this study was to assess taste of isoniazid, rifampicin, pyrazinamide and ethambutol using both a human taste panel and the rodent BATA model to quantify the bitterness of these drugs and determine whether the BATA model is a suitable model for taste assessment of formulations containing these drugs.

3.3 MATERIALS AND METHODS

3.3.1 Materials

For rodent BATA experiments isoniazid, pyrazinamide and ethambutol dihydrochloride were obtained from Sigma Aldrich (Sigma Aldrich, Dorset, UK). Rifampicin was obtained from TOKU-E (Bellingham, WA, USA). For human experiments GMP grade isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride were obtained from Macleods Pharmaceuticals (Mumbai, India). Deionised water was used for rat experiments. Bottled drinking water (Waitrose Essential, Waitrose Ltd, Bracknell, UK) was used for all human experiments. All substances were used as received.

3.3.2 BATA Testing

3.3.2.1 Animals

A total of ten naïve adult male Sprague–Dawley rats aged 70-77 days on arrival (Charles-River, Kent, UK) were used for these experiments. Each rat was assigned a unique number which was marked on their tail before being weighed and housed in pairs in standard cages. The rats were allowed to acclimatise to their new environment for a minimum of 7 days. They were housed in a room that was maintained at 21 ± 2°C with 55 ± 10% relative humidity and with a 12:12 hour light/dark cycle. All training and testing occurred during the ‘light’ period of the cycle. Animals were allowed free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a water-restriction schedule occurred (section 2.3.2.2). Daily food and water consumption were monitored throughout the course of the experiments. As a safety and welfare measure, each rat was weighed daily at different time points; prior to commencement of water deprivation, before and after each lickometer session and after rehydration. This was to ensure that their weight did not drop below 85% of their free-feeding weight. A one week wash-out period was observed for each rat between BATA experiments to ensure rats became naïve again and could be re-used. All the
procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668).

3.3.2.2 Taste Solutions

Solutions of drug for assessment were freshly prepared on the day of the study and presented to the rats at room temperature. Solutions of varying concentration were prepared in deionised water by dissolving the exact amount of the corresponding API in a fixed volume of water. Sonication (XUBA3 ultrasonic bath, Grant Instruments Ltd., Cambridgeshire, UK) was used to facilitate dissolution of APIs when required. The concentrations of each drug used are given in Table 3.1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>9.11 18.23 36.46 72.92 145.84 291.67</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.24 0.73 1.22 1.70 2.19 2.67</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>3.55 7.11 14.21 28.43 56.89 113.72</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>15.52 30.59 61.18 122.36 244.73 489.45</td>
</tr>
</tbody>
</table>

3.3.2.3 BATA Experiment

BATA experiments were carried out using the protocol developed by Soto et al. which is summarised in Table 3.2. Each rat was water-deprived for 22 hours before each session (training and testing) and then placed in the lickometer “Davis MS-160” (DiLog Instruments, Tallahassee, Florida, USA) for a maximum session-length of 40 minutes. After each session, the rats received tap water for rehydration in their home cage. During the first week of experiments rats received an initial training session where the shutter was continuously open presenting a single tube containing deionised water. This session was followed by a second training session where they were presented with sixteen tubes containing deionised water. On subsequent weeks they received only the second training session. The training sessions were followed by two testing sessions during which each rat was presented with either deionised water or one of the six concentrations of drug in a randomised order. Each concentration was presented for 8 seconds a total of four times per session. Each trial was intercepted by a 2 second water rinse to minimise carry over effects. One session per day was carried out and different drugs were assessed on separate days.
Chapter 3

Table 3.2 – Experimental protocol used for BATA experiments, developed by Soto et al.

<table>
<thead>
<tr>
<th>Day</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Commence water deprivation (22 hours). Free access to food.</td>
</tr>
<tr>
<td>2</td>
<td>Training session 1 (first week only): rat placed in lickometer with free access to one stationary water bottle.</td>
</tr>
<tr>
<td>3</td>
<td>Training session 2 (all weeks): rat placed in Davis Rig with access to moving water bottles.</td>
</tr>
<tr>
<td>4</td>
<td>Test session 1: randomised presentation of API solutions being assessed.</td>
</tr>
<tr>
<td>5</td>
<td>Test session 2: randomised presentation of API solutions being assessed.</td>
</tr>
</tbody>
</table>

3.3.2.4 Data Analysis

The normality of the data (lick numbers) was checked with the Shapiro-Wilk test. As the data were not normally distributed, the Kruskal-Wallis test was performed followed by post-hoc analysis carried out with Gao et al.’s non-parametric multiple test to check which concentrations elicited a number of licks significantly different compared to deionised water. The IC\textsubscript{50} value which corresponds to the concentration of drug which suppresses 50% of licks, were calculated using the E\textsubscript{max} model developed by Soto et al.:\[ y = BL \times \left(1 - E_{\text{max}} \times \frac{X}{IC_{50} + X}\right) \] where \( y \) represents ‘lick numbers’ or ‘lick ratios’, \( X \) represents the concentration of drug, \( BL \) is the number of licks taken of deionised water, \( IC_{50} \) is the concentration of drug which produces the half maximum taste response (i.e. a 0.5 lick ratio value or 50% suppression of lick numbers) and \( E_{\text{max}} \) is the maximum fraction of \( BL \) when concentration is extremely high. When \( y \) represents lick ratios, the value of \( BL \) is set to 1.

All statistical analyses were done with R (R Core Team, Vienna, Austria, version 3.3.1). OriginPro (OriginLab, Northampton, MA, USA, version 9.0.0) was also used for the generation of any additional graphs. Non-linear mixed effects modelling was performed using NONMEM® (ICON, Ellicott City, Maryland, version 7.3) in conjunction with a gfortran (64-bit).
compiler using Perl-Speaks NONMEM® (PSN, version 4.2.0) as an interface to run NONMEM®.

3.3.3 Human Taste Assessment

The study was approved by UCL Research Ethics Committee (REC) Project ID 4612/009. The UCL REC application can be found in Appendix A. All procedures were carried out in accordance with the UK Data Protection Act 1998.

3.3.3.1 Participants

20 (11 female, 9 male) healthy adults in the age range 18-40 were recruited (mean age 26) via an email circulated to UCL School of Pharmacy staff and students and by word of mouth. Individuals with antecedent deterioration of taste or smell, smokers, those who had undergone dental care or medicinal treatment (excluding contraceptives) up to 15 days before the test or those with known drug allergies were excluded from participating. Neutral breakfast or lunch were advised to be taken at least 30 minutes before the session began. Participants were given an information leaflet and a consent form to sign. Participants were reminded that their participation in the study was completely voluntary and that withdrawal from the study would not affect their relationship with the research team. Participants received £7.50 per session as a thank you gesture for their time committed to the project.

3.3.3.2 Taste Solutions

Solutions of drug for assessment were prepared on the day of the study and presented to the participants at room temperature. Solutions of varying concentration were prepared in bottled water by dissolving the exact amount of the corresponding API in a fixed volume of bottled water. Sonication (XUBA3 ultrasonic bath, Grant Instruments Ltd., Cambridgeshire, UK) was used to facilitate dissolution of APIs when required. All extemporaneous preparations were carried out under the supervision of a registered UK pharmacist following standard operating procedures. The concentrations of each drug used are given in Table 3.3.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>18.23  36.46  72.92  145.84</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.73    1.22    1.70    2.19</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>7.11    14.21   28.43   56.89</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>15.52  30.59  61.18  122.36</td>
</tr>
</tbody>
</table>

Table 3.3 – Concentration ranges of each drug used in human taste assessment study.
3.3.3.3 Taste Assessment

A single blind, cross over, single centre study was conducted using the ‘swirl and spit’ method. Different drugs were assessed on separate days and thus participants were asked to attend four sessions, each of which lasted two hours. A 72 hour ‘washout’ period was respected between sessions to reduce the burden on participants. Each participant was assigned a random code for identification. ‘Taste stations’ (Figure 3.1) were set up which consisted of a computer, bottled water (Waitrose Essential) to rinse the mouth between each sample, a plastic cup to spit samples into after assessment, unsalted crackers (Rakusen’s Matzo crackers, Rakusen’s Ltd., Leeds, UK) to neutralise the taste in the mouth and tissues. Each station was isolated from surrounding stations to ensure that participants could not see each other during the study. This was to prevent participant’s reactions from introducing bias in other participants.

Figure 3.1 – Taste station set up for human taste panel.

10 mL samples of drug solution were randomly presented to participants in opaque brown plastic tubes labelled with a three-digit code. Participants rinsed their mouths with the solution for 5 seconds to cover all oral surfaces, before spitting the sample into a receptacle provided. Immediately upon expectoration, participants rated the taste on a bipolar visual
analogue scale (Qualtrics, Provo, USA) (Figure 3.2). Participants were also encouraged to leave a comment on the taste of the sample if they wished. Participants then washed their mouths out with water and ate an unsalted cracker to neutralise the taste. A 10 minute interval was observed between each sample. All participants tasted 12 samples per sessions (n = 4 concentrations of one drug x 3 times/day) plus the negative control. The negative control was presented first and consisted of a sample of bottled water indicating ‘no taste’. Participants were allowed to re-taste each sample once if required.

Figure 3.2 – Survey used to collect taste assessment date from participants during human taste assessment of isoniazid, rifampicin, pyrazinamide and ethambutol (Qualtrics, USA).
Annex 1: Bitterness Assessment of Commonly Used Anti-Tuberculosis Drugs

<table>
<thead>
<tr>
<th>Day</th>
<th>Compound</th>
<th>Concentrations</th>
<th>Randomisation</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isoniazid</td>
<td>4</td>
<td>R1</td>
<td>2h</td>
</tr>
<tr>
<td>2</td>
<td>Rifampicin</td>
<td>4</td>
<td>R2</td>
<td>2h</td>
</tr>
<tr>
<td>3</td>
<td>Pyrazinamide</td>
<td>4</td>
<td>R3</td>
<td>2h</td>
</tr>
<tr>
<td>4</td>
<td>Ethambutol dihydrochloride</td>
<td>4</td>
<td>R4</td>
<td>2h</td>
</tr>
</tbody>
</table>

Wash out minimum 72h

Swirl and Spit = SS

UCL Ethics project ID number 4612/009

Figure 3.3 – Flow diagram of procedure used for human taste assessment study.
3.3.3.4 Data Analysis
The taste ratings obtained from the visual analogue scale were automatically converted to a score between 0 (not aversive) and 100 (extremely aversive) by the Qualtrics software. For each drug, concentration and participant, the average taste rating value obtained from the three replicates was calculated as well as the standard deviation. The normality of the data was checked with the Shapiro-Wilk test.\textsuperscript{215} As the data were not normally distributed, the Kruskal-Wallis test\textsuperscript{216} was performed followed by post-hoc analysis carried out with Gao et al’s non-parametric multiple test\textsuperscript{217} to check which concentrations elicited a response significantly different compared to deionised water. The same statistical tests were performed to verify if a significant difference was observed between participants of different gender. The EC\textsubscript{50} value, which corresponds to the concentration of the drug that elicits half the maximum taste response compared to the reference, deionised water, was calculated with the following $E_{\text{max}}$ model:

\[
E = \frac{E_{\text{max}} \times C^{Hill}}{EC_{50}^{Hill} + C^{Hill}} + \epsilon \tag{Equation 3.2}
\]

where $E$ is the taste rating, $E_{\text{max}}$ represents the maximum taste rating (i.e. 100), $C$ is the concentration of the drug, $Hill$ is the slope factor affected by the gradient of the curve, $EC_{50}$ represents the concentration which elicits a half-maximal taste rating and $\epsilon$ is the intra-participant variability.

A 95% confidence interval (CI), variance values for each of the parameters as well as the error value were obtained from this model as a reflection of inter- and intra-participant variability. All statistical analyses were done with R (R Core Team, Vienna, Austria, version 3.3.1). OriginPro (OriginLab, Northampton, MA, USA, version 9.0.0) was also used for the generation of any additional graphs. Non-linear mixed effects modelling was performed using NONMEM® (ICON, Ellicott City, Maryland, version 7.3) in conjunction with a gfortran (64-bit) compiler using Perl-Speaks NONMEM® (PSN, version 4.2.0) as an interface to run NONMEM®.

3.3.4 Human Taste Panel – BATA Model Correlation
The correlation between the results of the human taste panel and the BATA studies was assessed by comparing the IC\textsubscript{50} and EC\textsubscript{50} values. The taste intensity ranking of the four drugs by both the rodents and human participants was also examined.
3.4 RESULTS

3.4.1 Rodent BATA Testing

The results of BATA testing of the four drugs are given in Figure 3.4. For each API the number of licks obtained in 8 seconds for each concentration was converted to percentage inhibition of licks. Isoniazid, rifampicin and ethambutol dihydrochloride show a clear aversive taste that could be quantified by an IC$_{50}$ value (Table 3.4). The IC$_{50}$ value is the concentration of drug which suppresses 50% of licks compared to water. None of the concentrations tested for either ethambutol dihydrochloride or rifampicin were as palatable as water (all p-values <0.05). For isoniazid, all except the two lowest concentrations (9.11 mM and 18.23 mM) were statistically different from water (p = 0.53 and p = 0.41). However, for pyrazinamide only the highest concentration, 113.72 mM, had a significantly different number of licks compared to water (p = 0.0013). This indicated that the drug was not aversive at the concentrations tested for the rat taste panels and thus was the most palatable agent among the four under assessment. IC$_{50}$ values (Table 3.4) were calculated for isoniazid, rifampicin and ethambutol dihydrochloride using the E$_{max}$ model.$^{121}$ Since all except the highest concentration of pyrazinamide were not found to be significantly different to water by the rats, an IC$_{50}$ value could not be determined.

![Figure 3.4 - Percentage inhibition of licks (± SEM) as a function of concentration for isoniazid, rifampicin, pyrazinamide and ethambutol (n = 10 rats).]
Table 3.4 – IC\textsubscript{50} values obtained for isoniazid, rifampicin, pyrazinamide and ethambutol calculated from BATA testing results using E\textsubscript{max} model.\textsuperscript{121}

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC\textsubscript{50} Value (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>80.94</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>1.31</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Could not be determined</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>13.63</td>
</tr>
</tbody>
</table>

3.4.2 Human Taste Panel

20 participants were recruited for this study. The sample size of \( n = 20 \) was chosen as this was within the range used for previous human sensory assessment studies and is large enough to apply appropriate statistical tests. All participants successfully completed all four sessions and did not report any discomfort as a result of the study.

The results of the human testing of the four drugs are given in Figure 3.5. The taste intensities of each concentration of drug were calculated for each participant by calculating the mean of the ratings obtained for the three replicates. The mean rating obtained from all volunteers was then calculated for each concentration. For isoniazid and ethambutol dihydrochloride all concentrations were found to be significantly different to each other and the negative control (\( p < 0.05 \)). For rifampicin, all concentrations were significantly different to water however, the higher concentrations (1.22 mM – 2.19 mM) were not significantly different to each other. Finally, for pyrazinamide all concentrations were found to be significantly different to water but the two lower concentrations (7.11 mM and 14.21 mM) were not significantly different to each other.
Figure 3.5 – Average taste ratings (± SEM) as a function of concentration for isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride (n = 20 participants).

The ratio of male and female participants was selected with n = 9 and n = 11 respectively. The Kruskal-Wallis test was used to determine if there were any differences in taste perception between genders (Figure 3.6). It can be seen that males are slightly more sensitive to the taste of isoniazid, rifampicin and ethambutol while females are slightly more sensitive to the taste of pyrazinamide. However, these differences were not found to be statistically significant (p < 0.05).
Figure 3.6 – Boxplots of taste ratings as a function of gender for (a) isoniazid; (b) rifampicin; (c) pyrazinamide; (d) ethambutol. The middle box represents the middle 50% of scores of the group, i.e. the interquartile range. The median of the data is represented by the line which divides each box into two parts. The upper and lower whiskers represent scores outside the middle 50%. The dots represent outliers which correspond to any value which is more than 1.5x the interquartile range.
The data obtained was fitted to the $E_{\text{max}}$ model which allowed the calculation of EC$_{50}$ and 95% confidence interval value and inter- and intra - participant variability (Table 3.5). It should be noted that as the maximum taste intensity is not reached for isoniazid, rifampicin and pyrazinamide the EC$_{50}$ values are estimated. Ranking the drugs using the EC$_{50}$ values it is found that rifampicin > ethambutol > pyrazinamide > isoniazid.

Table 3.5 – EC$_{50}$, 95% confidence interval, inter-subject and intra-subject variability obtained from the $E_{\text{max}}$ model. Please note that as the maximum taste intensity is not reached for isoniazid, rifampicin and pyrazinamide the EC$_{50}$ values are estimated.

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC$_{50}$ (mM)</th>
<th>95% Confidence Interval</th>
<th>Inter-subject variability</th>
<th>Intra-subject variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>259</td>
<td>80.05 – 437.95</td>
<td>0.843</td>
<td>95.7</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>3.6</td>
<td>1.19 – 6.01</td>
<td>1.36</td>
<td>211</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>158</td>
<td>90.77 – 225.23</td>
<td>0.336</td>
<td>161</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>27</td>
<td>18.77 – 35.23</td>
<td>0.335</td>
<td>227</td>
</tr>
</tbody>
</table>

3.4.3 Human Taste Panel – BATA Model Correlation

The IC$_{50}$ values obtained from BATA testing and the EC$_{50}$ values obtained from human taste panel assessment are given in Table 3.6. The IC$_{50}$ values indicate that most aversive API is rifampicin followed by ethambutol dihydrochloride, which is in agreement with the rank order seen in human testing. However, unlike the rats, humans found pyrazinamide to be somewhat aversive with each sample being deemed significantly different to water. Humans ranked isoniazid as the least aversive of the four drugs. For isoniazid, rifampicin and ethambutol dihydrochloride, rats were found to be more sensitive to the taste of the API compared to humans. Humans were more sensitive to the taste of pyrazinamide. Participants were able to distinguish even the lowest concentration as significantly different to water while in BATA studies, only the highest concentration of pyrazinamide was deemed significantly different to water. For this reason an IC$_{50}$ value could not be obtained for pyrazinamide from BATA experiments. For the three drugs for which both IC$_{50}$ and EC$_{50}$ values could be calculated the values obtained from rats and humans were within one-half log unit of molar concentration of each other.
Table 3.6 – Comparison of rat IC\textsubscript{50} and human EC\textsubscript{50} values obtained from fitting of data to $E_{\text{max}}$ model.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC\textsubscript{50} (mM)</th>
<th>log(IC\textsubscript{50})</th>
<th>EC\textsubscript{50} (mM)</th>
<th>log(EC\textsubscript{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>80.94</td>
<td>1.91</td>
<td>259</td>
<td>2.41</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>1.31</td>
<td>0.12</td>
<td>3.6</td>
<td>0.56</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>-</td>
<td>-</td>
<td>158</td>
<td>2.20</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>13.63</td>
<td>1.13</td>
<td>27</td>
<td>1.43</td>
</tr>
</tbody>
</table>

The variability in responses from both taste panels was assessed by comparing the standard error of the mean for each concentration of drug in rats and humans (Table 3.7). In general, the SEM of the rat data is lower than that of the human data, demonstrating that the overall variability is lower in rats than in humans.

Table 3.7 – Comparison of Standard Error of the Mean (SEM) for human and rat taste data.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mM)</th>
<th>SEM of rat data</th>
<th>SEM of human data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>9.11</td>
<td>1.40</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>18.23</td>
<td>1.36</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>36.46</td>
<td>2.02</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>72.92</td>
<td>1.99</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>145.84</td>
<td>1.24</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td>291.67</td>
<td>0.42</td>
<td>-</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.24</td>
<td>1.94</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>2.55</td>
<td>3.08</td>
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<td>2.30</td>
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<tr>
<td></td>
<td>2.67</td>
<td>2.44</td>
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</tr>
<tr>
<td>Pyrazinamide</td>
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<td>1.63</td>
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<td>1.13</td>
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<td></td>
<td>360.71</td>
<td>1.76</td>
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<td>-</td>
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<td>30.59</td>
<td>2.32</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>61.18</td>
<td>1.70</td>
<td>3.12</td>
</tr>
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<td></td>
<td>122.36</td>
<td>0.87</td>
<td>2.36</td>
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<td></td>
<td>244.73</td>
<td>0.41</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>489.45</td>
<td>0.41</td>
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</tbody>
</table>
3.5 DISCUSSION

In this study the taste of the four main anti-tuberculosis drugs was assessed via two in vivo methods; rodent BATA model and human taste panel. It was found that the EC_{50} values obtained in humans were higher than the IC_{50} values obtained from the BATA model indicating that rats are more sensitive to the taste of these medicines than humans are. In all cases the IC_{50} and EC_{50} values obtained were within one half-log unit of molar concentration of each other which is in accordance with previous studies of this type.\textsuperscript{122–124}

Rudnitskaya et al.\textsuperscript{123} used the rat BATA model and a trained human taste panel to assess eight drugs; paracetamol, azelastine hydrochloride, caffeine, quinine hydrochloride, chlorhexidine digluconate, potassium nitrate, naratriptan hydrochloride and sumatriptan succinate. The same rank order of bitterness was observed for the human panels as for the BATA experiments and the bitterness intensity scores obtained from both in vivo methods for each drug were within one half-log unit of molar concentration of each other. Similarly, Devantier et al.\textsuperscript{122} used a trained human taste panel and a mouse BATA model to assess taste of quinine, clarithromycin, nystatin and ciprofloxacin. The measured taste intensities of the drugs obtained from human panellists and the mice were again within one half-log unit of molar concentration of each other and were not found to differ statistically.

While the bitter taste of these drugs is commonly mentioned as a cause of non-adherence to anti-tuberculosis medicines\textsuperscript{206–208,212,213}, to the best of the author’s knowledge, this was the first attempt to quantify the taste intensities of the drugs using in vivo methods. In a study of adherence to isoniazid preventive therapy in Indonesian children by Rutherford et al.\textsuperscript{208} it was found that all respondents stated difficulty in administering the medicine due to the bitter taste of isoniazid. Le Roux et al.\textsuperscript{207} investigated adherence to isoniazid prophylaxis among HIV-infected children in tertiary paediatric care centres in South Africa and found that adherence was much higher in children older than 4 years than it was in toddlers and infants. They attributed this to the fact that the drug was administered in tablet form which older children could swallow, whereas the tablets had to be crushed for younger children giving them an unpleasant taste and texture.

A study by the Hong Kong Chest Service\textsuperscript{206} into the acceptability and compliance of isoniazid, rifampicin and pyrazinamide states that uncoated pyrazinamide tablets have a bitter taste which can cause difficulty swallowing and can lead to nausea or vomiting in some patients. The taste of rifampicin was evaluated in comparison to eight other anti-bacterial and antifungal drugs such as cephalexin, clindamycin and linezolid by Steele et al. and found to
Chapter 3

be the least aversive. However in this study the taste of all the drugs was just compared to one another without quantifying the taste intensity of each drug.\textsuperscript{218}

It is important to note that the EC\textsubscript{50} values calculated from the human taste panel data (except for that of ethambutol) are only estimates as the maximum taste value was not achieved during the study. The concentrations used in the study were chosen based on a number of factors including water solubility of the drug, clinical dose of the drug and rat LD\textsubscript{50} and human acceptable daily intake (ADI) limits. To enable a direct comparison of taste intensity the concentrations used in both panels were overlapping, however, fewer concentrations were tested in humans (four compared to six in rats) to ensure that the risk of taste fatigue was minimised.\textsuperscript{209} The concentrations used were well below the level causing no toxicological effects in humans to ensure that, in the unlikely event of accidental ingestion of a single sample the amount of drug the participant would be exposed to would be well below the ADI limit.

For rifampicin the maximum concentration of drug used was limited by the low water solubility of rifampicin, approximately 2.5 mg/mL.\textsuperscript{152} It would be impossible to use the concentrations of rifampicin required to achieve maximum taste response without adding a solubilising agent or changing the pH of the solution which in turn could affect the taste of the solution. For isoniazid and pyrazinamide increasing the concentrations to the values required to achieve maximum taste response would require giving very large doses of drugs to the participants which could cause unwanted side-effects. While the human EC\textsubscript{50} values are estimates they are still useful guidelines as to the taste intensity of the drugs, which may be useful for future formulation development.

For isoniazid and ethambutol both rat and human participants were able to identify each concentration tested as significantly different to water and each other. For pyrazinamide rats could only identify the highest concentration as being significantly different to water while humans were able to identify all as significantly different to water but could not differentiate between the lowest two concentrations. Finally for rifampicin in both rats and humans we see a plateauing of responses at the highest concentrations. For human taste sensation, the Weber-Fechner law states that the threshold of discrimination between two stimuli increases logarithmically with the intensity of the stimulus.\textsuperscript{219} In practice this means that upon reaching a certain threshold value, differences in taste intensity can no longer be detected which may be what is happening in the case of rifampicin. Alternatively it may be that as the concentrations of drug used are too close to each other to allow for full
differentiation between samples. Unfortunately due to the solubility issues with rifampicin a larger concentration range could not be used, and the risk of using two fold dilutions, as per the other three drugs tested, was that the lower concentrations would be too small to be detected.

In previous studies\(^\text{124}\), drug solutions were presented to participants in transparent plastic universal tubes, however in this study opaque brown tubes were used. Rifampicin is a bright red coloured compound. Solutions of rifampicin of varying concentration can be distinguished visually as the colour intensity of the solution increases with increasing concentration (Figure 3.7). Therefore opaque tubes were used to prevent the colour of the solution introducing bias in the participant’s responses. The other drugs assessed in this study produce clear solutions which cannot be distinguished visually, however opaque tubes were also used for these solutions to keep the study parameters uniform.

![Figure 3.7 – Rifampicin solutions of increasing concentration from 0.73 – 2.19 mM (left to right). As concentration increases the colour intensity of the solution increases which could introduce bias in participant responses.](image)

Each concentration was tested in triplicate to increase the accuracy and reliability of the data obtained. A negative control consisting of bottled water representing ‘no taste’ was given to participants at the start of each session to help them ‘calibrate’ their taste. In this study no positive control was used. Positive controls, i.e. a known bitter compound such as quinine, may be used either blinded or open label in taste panels. When the control is presented blind during the session it can be used to calibrate responses from session to session. When the control is presented open label then participants can use it to ‘calibrate’ their taste. However this can also introduce bias into the participants which can lead to skewed results. Given that this was the first study of this type using these four drugs we did not even have an approximate idea of how bitter they are likely to be. Thus, there was a chance that they may
in fact have been more bitter than a positive control used, which may have confused participants. Not using a positive control, as in this study, does present a disadvantage as participants do not have any reference against which to rate the sample, however it does allow participants to rate the samples in a relatively unbiased fashion.

Results were recorded using a computerised visual analogue scale (Figure 3.2). Participants were asked to rate the taste of the sample by moving the marker along the scale from ‘least aversive’ to ‘most aversive’. The scores were then automatically converted to a score out of 100 by the software. A continuous scale was chosen rather than anchored scale as it allows participants more flexibility to rate the samples independently rather than being confined to discrete categories.

In the current study no statistically significant difference was observed in the taste responses from male or female participants. However, this may be due to the relatively small sample size. It is possible that if larger sample sizes were used then a statistical difference would be observed, however this was unfeasible for this study given the resources available. In the BATA experiments only male rats were used to avoid introducing a gender effect into the experiments.

A larger variability is seen in human responses compared to those of the rats. This is most likely due to different perceptions of taste which can be as a result of many factors including gender, age, diet, culture and genetics. 6-n-Propylthiouracil (PROP) and phenythiocarbamide (PTC) are two examples of compounds, the perception of which varies hugely among people due to genetic variation. Incidence rates of taste blindness to PTC/PROP vary around the world ranging from ~3% in western Africa to 40% in India, while in the adult Caucasian population of North America roughly 30% are taste blind to PTC/PROP. In this study a panel size of 20 was recruited to decrease variation amongst the volunteers.

While human participants came from a variety of backgrounds and cultures, with associated differences in taste perception, rats used for BATA testing are much more uniform in terms of environment and diet, which may explain their lower variability in taste perception. Another factor to consider is that panellists were not screened for bitterness sensitivity before being selected for the study. Pre-screening of participants may have led to lower inter-subject variability, however to do this would have required extra commitment from participants, and a larger initial sample size, which was unfeasible.
Compared to a known bitter compound, such as quinine which have been found to have a very low EC$_{50}$ value (0.257 mM$^{124}$) these drugs are not strongly bitter. It is likely that there is more variability in responses to less strongly bitter drugs due to individual differences in taste perception than there would be to a strongly bitter drug such as quinine.

Overall it was found that the rats were more sensitive to the bitter taste of isoniazid, rifampicin and ethambutol than human adults. For this reason they may be especially useful for paediatric formulation development as it is generally considered that children are more sensitive to bitter taste than adults.$^{31}$ On the other hand the rats were insensitive to all but the highest concentration of pyrazinamide tested, while human participants were able to distinguish all concentrations from water. This indicates that the BATA model is not suitable for assessing the taste of pyrazinamide containing formulations.

### 3.6 Conclusions

In this study the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was assessed using two different in vivo methods, the rat BATA model and a human taste panel. To the best of the authors’ knowledge this is the first quantitative in vivo assessment of the taste of these four commonly used anti-tuberculosis drugs. The four drugs were found to be mildly bitter compared to standard bitter compounds such as quinine, based on IC$_{50}$ and EC$_{50}$ values obtained from these studies. Rats were found to be more sensitive to the taste of the bitter taste of isoniazid, rifampicin and ethambutol dihydrochloride than human participants. However, calculated IC$_{50}$ and EC$_{50}$ values were within one half-log unit of molar concentration to each other, which is in line with previous studies. Unfortunately, the rats were shown to be relatively insensitive to the taste of pyrazinamide compared to human panellists, indicating that the BATA model is not suitable for assessing this drug. In summary, estimated human EC$_{50}$ values for these four drugs have been determined and it has been shown that the BATA model can be useful for assessment of formulations containing isoniazid, rifampicin and ethambutol dihydrochloride.
CHAPTER 4

Investigation of the Insent E-Tongue for Taste Assessment of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol
4.1 INTRODUCTION
The taste of medicines can be assessed using *in vivo* or *in vitro* methods. As discussed in Chapter 3, *in vivo* taste assessment methods involve using either humans or animals to assess the taste of substances. In recent years *in vitro* methods such as electronic tongue systems have been developed as novel methods for taste assessment. Electronic tongue systems are sensor array based robotic systems which can be used for the assessment of single substances as well as complex mixtures of substances. There are two commercially available electronic tongues, the Alpha MOS Astree electronic tongue and the Insent TS-5000Z taste sensing system. These sensors attempt to imitate and represent what happens when molecules interact with taste buds in the human oral cavity. When a molecule interacts with a sensor there is a change in the electrical potential of the sensor. The response of the sensors depends logarithmically on the activity of the substances which are measured in a way analogous to that of human taste. It has been reported that the Insent electronic tongue provides more reliable (*in vitro*/*in vivo* correlation) and precise (reproducible) data than the Astree e-tongue.

There has been much discussion as to whether electronic tongues can be used as a substitute for human taste panels in formulation development, however, to date no definitive conclusions have been drawn. In this study the ability of the Insent TS-5000Z electronic tongue to detect and assess the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was investigated to determine whether the electronic tongue would be useful for taste-masking assessment of formulations containing these drugs.

4.2 AIMS AND OBJECTIVES
The aim of this study was to investigate whether the taste of isoniazid, rifampicin, pyrazinamide and ethambutol could be detected and assessed using the Insent TS-5000Z electronic tongue and to determine whether it is a suitable technique for taste assessment of formulations containing these drugs.

4.3 MATERIALS AND METHODS
4.3.1 Materials
Isoniazid, pyrazinamide and ethambutol dihydrochloride were obtained from Sigma Aldrich (Sigma Aldrich, Dorset, UK). Rifampicin was obtained from Fagron UK Ltd (Newcastle, UK). Potassium chloride, potassium hydroxide and tartaric acid were obtained from Sigma Aldrich.
(UK). Hydrochloric acid was obtained from Fisher Chemicals (Loughborough, UK). All substances were used as received. Deionised water was used for all experiments.

4.3.2 Methods

4.3.2.1 Preparation of Standard Solutions

*Reference solution* used for cleaning and as a reference solution was prepared by dissolving 30 mM/L potassium chloride and 0.3 mM/L tartaric acid in distilled water.

*Negatively charged washing solution* used for washing the negatively charged sensors (SB2AC0 and SB2AN0) was prepared by diluting absolute ethanol to 30% with distilled water and adding 100 mM/L hydrochloric acid.

*Positively charged washing solution* used for washing the positively charged sensors (SB2C00 and SB2AE1) was prepared by diluting absolute ethanol to 30% and adding 100mM/L potassium chloride and 10mM/L potassium hydroxide.

4.3.2.2 Measurement Procedure

All measurements were performed using the taste sensing system TS-5000Z (Insent Inc., Atsugi-shi, Japan). Each measurement cycle consisted of measuring a reference solution ($V_r$), followed by the sample solution ($V_s$), a short (2 x 3 seconds) cleaning procedure and measurement of the aftertaste ($V_r'$) and finally a 330 second cleaning procedure. The sensor output for taste (relative value, R value) was calculated relative to the preliminary sensor response to the reference solution ($V_r$).

$$R = V_s - V_r \quad \text{Equation 4.1}$$

The entire measurement procedure was performed for all samples and repeated afterwards up to six times. For further data treatment the first run was discarded (as recommended by Insent) to enable conditioning of sensors.

4.3.2.3 Taste Solutions

Solutions of drug of varying concentration were prepared in distilled water by dissolving the exact amount of the corresponding API in a fixed volume of water. Sonication (XUBA3 ultrasonic bath, Grant Instruments Ltd., Cambridgeshire, UK) was used to facilitate dissolution of APIs when required. Concentrations of drugs used are given in Table 4.1. The pH of each solution was measured using pH meter (SciQuip 902, SciQuip, Wem, UK) and the ratio of ionised to unionised drug present in solution was calculated using the Henderson Hasselbalch equation\textsuperscript{223} (Equation 4.2).
Chapter 4

\[ pH = pK_a - \log_{10} \frac{[A^-]}{[HA]} \]  

Equation 4.2

Table 4.1 – Concentrations of drugs assessed using electronic tongue.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>9.11 18.23 36.46 72.92 145.84 291.67</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.24 0.73 1.22 1.70 2.19 2.67</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>3.55 7.11 14.21 28.43 56.89 113.72</td>
</tr>
<tr>
<td>Ethambutol dihydrochloride</td>
<td>15.52 30.59 61.18 122.36 244.73 489.45</td>
</tr>
</tbody>
</table>

4.3.2.4 Data Analysis

The normality of the data was checked with the Shapiro-Wilk test.\(^{215}\) As the data were normally distributed, one-way analysis of variance (ANOVA) was performed followed by post-hoc analysis with Tukey’s honest significant difference test\(^{224}\) to check which concentrations elicited a sensor response significantly different to that elicited by deionised water. Dose response curves were created by plotting the mean sensor response (± standard deviation) as a function of concentration. All statistical analyses on electronic tongue data were carried out using OriginPro (OriginLab, Northampton, MA, USA, version 9.0.0).

4.4 RESULTS

To assess whether the e-tongue was a suitable tool for assessing these drugs, drug solutions of varying concentrations (identical to those used in BATA testing in Chapter 3) were tested. The normality of the resulting data was checked using the Shapiro-Wilk test. In all cases the data were found to be normally distributed. One-way analysis of variance (ANOVA) was then performed followed by post-hoc analysis with Tukey’s honest significant difference test to assess whether each concentration of drug tested elicited a response significantly different to that of water (p < 0.05).

4.4.1 Isoniazid

The response of all four sensors to isoniazid are given in Figure 4.1. ANOVA was used to determine whether sensor responses are significantly different (p < 0.05) to the response obtained for water or from each other. The results of this are given in Table 4.2. It can be seen that only the C00 sensor is capable of differentiating all concentrations of isoniazid from water.
Figure 4.1 – Sensor response curve for isoniazid showing normalised sensor response as a function of concentration (n = 6, mean ± S.D.).
Table 4.2 – Results of ANOVA testing to determine if sensor response for each concentration of isoniazid were significantly different to that of water (indicated by 0) and each other (p < 0.05). Y indicates significant difference, N indicates no significant difference.

<table>
<thead>
<tr>
<th>Concentration 1 (mM)</th>
<th>Concentration 2 (mM)</th>
<th>AC0</th>
<th>AN0</th>
<th>CO0</th>
<th>AE1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.11</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>0</td>
<td>18.23</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>0</td>
<td>36.46</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>0</td>
<td>72.92</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>0</td>
<td>145.82</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>0</td>
<td>291.67</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>9.11</td>
<td>18.23</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>9.11</td>
<td>36.46</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>9.11</td>
<td>72.92</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>9.11</td>
<td>145.84</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>9.11</td>
<td>291.67</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>18.23</td>
<td>36.46</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>18.23</td>
<td>72.92</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>18.23</td>
<td>145.84</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>18.23</td>
<td>291.67</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>36.46</td>
<td>72.92</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>36.46</td>
<td>145.84</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>36.46</td>
<td>291.67</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>72.92</td>
<td>145.84</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>72.92</td>
<td>291.67</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>145.84</td>
<td>291.67</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>

It might be expected that if a sensor were to be unable to differentiate between water and a drug solution, it would be the lowest concentrations that are indistinguishable, however this is not what is seen with isoniazid. The AC0 sensor cannot distinguish the 18.23 mM solution and 36.46 mM solutions from water, whereas the AN0 cannot distinguish the two highest concentrations from water. For the AE1 sensor the three lowest concentrations are
not distinguished from each other by the sensors, however this sensor detects astringency rather than bitterness so it is likely that the three lowest concentrations of drug solution are not significantly more or less astringent than water alone.

The sensors also struggle to distinguish varying concentrations of drugs from each other. For example, all except the C00 sensor are capable of distinguishing the two highest concentrations of drug from each other, which may be due to saturation of the sensors. However in other cases the sensors are unable to distinguish seemingly random concentrations of drug, e.g. the AC0 sensor cannot distinguish 9.11 mM from 72.92 mM isoniazid, even though these concentrations are significantly different from each other.

Isoniazid has three pK\text{a} values (Figure 4.2) the hydrazine nitrogen has a pK\text{a} of 1.8, the pyridine nitrogen has a pK\text{a} of 3.5 and the deprotonation of the hydrazide group to a mesomerism stabilised anion has been reported to have a pK\text{a} of 10.8 (all measured at 25°C).\textsuperscript{225}

![Figure 4.2 – pK\text{a} values of isoniazid.](image)

Typically drug molecules interact more easily with the lipid membranes of the sensors when they are ionised. The pH of all solutions of isoniazid was measured as 6.85. The percentage ionised of each ionisable group was calculated at this pH, these values are given in Table 4.3. Overall, less than 0.05% of the drug will be ionised at this pH, thus making it less easy for the drug molecules to interact with the lipid membrane.

<table>
<thead>
<tr>
<th>Group</th>
<th>pK\text{a}</th>
<th>Percentage Ionised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrazine nitrogen</td>
<td>1.8</td>
<td>8.95 x 10^{-4} %</td>
</tr>
<tr>
<td>Pyridine nitrogen</td>
<td>3.5</td>
<td>0.032 %</td>
</tr>
<tr>
<td>Hydrazide</td>
<td>10.8</td>
<td>0.011 %</td>
</tr>
</tbody>
</table>
4.4.2 Rifampicin

The sensor response of all four sensors to isoniazid are given in Figure 4.3. ANOVA was used to determine whether sensor responses are significantly different (p < 0.05) to the response obtained for water or from each other. The results of this are given in Table 4.4.

![Sensor response curve for rifampicin showing normalised sensor response as a function of concentration (n = 6, mean ± S.D.)](image)

In the case of rifampicin, it is only the AN0 sensor which cannot distinguish all concentrations of drug from water. Similar to what is seen for isoniazid, it is not the lowest concentration of drug which the sensor cannot distinguish from water. For the AN0 sensor it is the 2.19 mM solution which is not deemed significantly different to water. Interestingly, similar to what was observed in human and BATA testing of rifampicin, a decrease in response is seen at the highest concentrations. Similar to what was observed for isoniazid the sensors were also unable to distinguish between certain concentrations of rifampicin e.g. the C00 sensor is unable to distinguish between 0.73 mM and 2.19 mM solutions of rifampicin.
Table 4.4 – Results of ANOVA testing to determine if sensor response for each concentration of rifampicin were significantly different to that of water (indicated by 0) and each other (p < 0.05). Y indicates significant difference, N indicates no significant difference.

<table>
<thead>
<tr>
<th>Concentration 1 (mM)</th>
<th>Concentration 2 (mM)</th>
<th>Ac0</th>
<th>An0</th>
<th>C00</th>
<th>Ae1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.24</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
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<td>1.22</td>
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<td>1.70</td>
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<tr>
<td>0.24</td>
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</tbody>
</table>

Rifampicin is amphoteric and thus has two $pK_a$ values (Figure 4.4), the 4-hydroxyl group has a $pK_a$ of 1.7 while the 3-piperazine nitrogen has a $pK_a$ of 7.9. It has an isoelectric point at pH 4.8 in aqueous solution.\textsuperscript{226}
Figure 4.4 – $pK_a$ values of rifampicin.

The pH of the rifampicin solutions was measured to determine if the compound was likely to be ionised in the test solutions. The pH of all solutions was measured and found to decrease from 6.02 for the 0.24 mM solution to 5.75 for the 2.67 mM solution. The percentage ionised of each ionisable group was calculated at these pH values and was found to be 98–99% ionised for each group at all concentrations. This suggests that the drug should interact well with the charged lipid membrane. Looking more closely at Figure 4.3 it can be seen that from 0.24 mM to 1.7 mM a reasonably linear response is observed for each sensor. At concentrations above this the response becomes erratic, possibly due to saturation of the sensor. Another possibility to consider is that while each ionisable group is found to be ionised under the experimental conditions, they have opposite charges. The 4-hydroxyl group loses a proton to become negatively charged and the 3-piperazine nitrogen gains a proton to become positively charged. It is possible that these opposing charges largely cancel each other out which may account for the erratic response observed from the sensors.

4.4.3 Pyrazinamide

The response of all four sensors to pyrazinamide are given in Figure 4.5. ANOVA was used to determine whether sensor responses are significantly different ($p < 0.05$) to the response obtained for water or from each other. The results of this are given in Table 4.5. It can be seen that both the AC0 and C00 sensors are capable of differentiating all concentrations of pyrazinamide from water.
Figure 4.5 – Sensor response curve for pyrazinamide showing normalised sensor response as a function of concentration (n = 6, mean ± S.D.).

Again we see that it is seemingly random concentrations of pyrazinamide that cannot be distinguished from water with the 56.89 mM solution and 14.21 mM solution not being recognised by the AN0 and AE1 sensor respectively. In terms of differentiating between the solutions of drugs themselves the AN0 sensor in particular struggles to distinguish between individual concentrations of drug. Notably, none of the sensors are able to differentiate 14.21 mM and 28.43 mM solutions of pyrazinamide from each other.
Table 4.5 – Results of ANOVA testing to determine if sensor response for each concentration of pyrazinamide were significantly different to that of water (indicated by 0) and each other (p < 0.05). Y indicates significant difference, N indicates no significant difference.

<table>
<thead>
<tr>
<th>Concentration 1 (mM)</th>
<th>Concentration 2 (mM)</th>
<th>AC0</th>
<th>AN0</th>
<th>C00</th>
<th>AE1</th>
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<tr>
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</tr>
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<td>7.11</td>
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<td>14.21</td>
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<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>0</td>
<td>28.43</td>
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<tr>
<td>0</td>
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</tr>
<tr>
<td>0</td>
<td>113.72</td>
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</tr>
<tr>
<td>3.55</td>
<td>7.11</td>
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</tr>
<tr>
<td>3.55</td>
<td>14.21</td>
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<tr>
<td>3.55</td>
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<td>3.55</td>
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<td>3.55</td>
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<td>113.72</td>
<td>N</td>
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Pyrazinamide is a very weak base which has been reported to have a pKₐ value of 0.5, thus it is extremely unlikely that the drug will be protonated under the test conditions, again making it less easy for the drug to interact with the lipid membrane of the sensors.
4.4.4 Ethambutol Dihydrochloride

The response of all four sensors to ethambutol dihydrochloride are given in Figure 4.6. The sensor responses were all found to be significantly different to each other and the sensor response for water \( (p < 0.05) \).

![Sensor response curve for ethambutol dihydrochloride showing normalised sensor response as a function of concentration \( (n = 6, \text{mean} \pm \text{S.D.}) \).](image)

All concentrations of ethambutol dihydrochloride were deemed significantly different from water and each other by all four sensors \( (p < 0.05) \). It can be seen from Figure 4.6 that, unlike the previous three drugs, a clear linear dose response is seen for ethambutol dihydrochloride. As it is a chloride salt, this drug will be 100% ionised under the test conditions. The charged ethambutol molecule will be able to easily interact with the lipid membrane of the sensors, thus eliciting a clear dose response.

4.4.5 Correlation Between Human Taste Panel Scores and Electronic Tongue

The correlation between human taste panel scores and the electronic tongue response for each drug was assessed by choosing the individual sensor for which the most linear response was observed and comparing these values to human taste panel scores. For isoniazid the C00 sensor was chosen while the AC0 sensor was used for both rifampicin and pyrazinamide. For ethambutol dihydrochloride, since all sensors responded in a linear fashion, the response of all four sensors was compared to human taste panel scores. The results of this are shown in Figure 4.8.
Figure 4.7 – Correlation between human taste panel scores and electronic tongue response for (a) isoniazid; (b) rifampicin; (c) pyrazinamide; (d) ethambutol.
Chapter 4

Figure 4.8 – Correlation between human taste panel scores and electronic tongue response for (a) isoniazid; (b) rifampicin; (c) pyrazinamide; (d) ethambutol.

It can be seen that for isoniazid and rifampicin in particular that the correlation between responses is quite low at 0.4368 and 0.02291 respectively. For pyrazinamide the correlation is higher at 0.79785. The correlation between sensor responses and human taste scores for ethambutol is very high with all sensors having a correlation coefficient in excess of 0.99. The highest correlation is observed for the AN0 sensor with a $R^2$ value of 0.99997.
4.5 DISCUSSION

In this study the utility of the Insent TS-5000Z electronic tongue for assessing the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was investigated. Previous work carried out within the group had established the factors which can affect the detection of a drug by the taste sensor as: (i) interaction between detecting sensors and ions; (ii) extent of dissociation/ionisation of electrolyte; (iii) concentration of drug; (iv) effect of solvent.\textsuperscript{228}

Each sensor membrane is composed of a different artificial lipid and plasticiser which are designed to detect different taste attributes. Four sensors were used in this study; bitterness sensor 1 (SB2AC0), bitterness sensor 2 (SB2AN0) and bitterness sensor 3 (SB2C00) and astringency (SB2AE1). The composition of each sensor is given in Table 4.6. The acidic bitterness and astringency sensors have positively charged membranes and the basic bitterness sensors have negatively charged membranes.

\textbf{Table 4.6 – Composition of sensor membranes.}\textsuperscript{228}

<table>
<thead>
<tr>
<th>Sensor Name</th>
<th>Characteristic</th>
<th>Composition</th>
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<tbody>
<tr>
<td>C00</td>
<td>Acidic Bitterness</td>
<td>Tetradecylammonium bromide</td>
</tr>
<tr>
<td>AE1</td>
<td>Astringency</td>
<td>Tetradecylammonium bromide</td>
</tr>
<tr>
<td>AC0</td>
<td>Basic Bitterness</td>
<td>1–Hexadecanol</td>
</tr>
<tr>
<td>AN0</td>
<td>Basic Bitterness</td>
<td>Phosphoric di-n-decyl ester</td>
</tr>
</tbody>
</table>

As discussed in Section 1.4.1.2, according to the Gouy-Chapman theory upon which the Insent electronic tongue is based, when the artificial membrane is immersed in aqueous solution an electrical double layer (stern and diffuse layer) is formed at the surface of the membrane. For the positively charged membranes it is expected that the stern layer will be composed of primarily of anions and the diffuse layer of cations, whereas for the negatively charged membranes the opposite will be true. As the membranes are charged we can thus assume that the drug must dissociate and be in its ionised form in order to elicit a change in membrane potential. Strong electrolytes which are completely ionised or dissociated, e.g. ethambutol in this study, have more ions in solution which can interact with the electronic tongue. This is in contrast to the human tongue which is also capable of detecting dissolved...
molecules which are not ionised. The Henderson – Hasselbalch equation was used in this study to calculate the degree of ionisation of the drugs being assessed (section 4.3.2.5). This equation describes the link between pH, $pK_a$ and degree of ionisation for a weak acid or base. Previous work carried out within the group had indicated that when a drug is unionised the molecule will not be detected by the taste sensor. Gondongwe investigated the dose response of a variety of drugs including theophylline, theobromine, caffeine, caffeine citrate and quinine.\textsuperscript{228} It was found that drugs such as caffeine, theophylline and theobromine, which were unionised under the experimental conditions, showed no dose response while caffeine citrate and quinine, which were ionised, showed a linear dose response. Uchida \textit{et al.}\textsuperscript{229} also reported the same phenomenon with caffeine and theophylline not eliciting a response from the electronic tongue, despite being classed as aversive in human sensory panels.

In the current study, ethambutol dihydrochloride was the only drug to show a true linear dose response. As it is a hydrochloride salt it will be fully ionised in aqueous solution, thus allowing the molecule to interact easily with the lipid membrane of the taste sensors. According to the Henderson – Hasselbalch equation, isoniazid and pyrazinamide will be not be ionised under the experimental conditions however, they do elicit a response (albeit an erratic one) from the sensors which is contrary to the findings reported by Uchida \textit{et al.} and Gondongwe. Ito \textit{et al.} demonstrated that a potentiometric response may be elicited from lipid membranes by neutral molecules under certain conditions. If a molecule is sufficiently lipophilic it may partition into the membrane and then dissociate, releasing an ion into the aqueous phase and generating a potentiometric response.\textsuperscript{230} This is unlikely to be what is happening in the case of isoniazid and pyrazinamide however as these molecules are not lipophilic, as evidenced by their low logP values of -0.64\textsuperscript{231} and -1.884\textsuperscript{232} respectively. Further work will need to be done to investigate how these drugs are interacting with the membranes of the electronic tongue sensors, although this is outside the scope of this thesis.

Rudnitskaya \textit{et al.}\textsuperscript{123} investigated whether changing the pH of test solutions could improve the detection of drugs by ensuring they are ionised (although it should be noted these experiments were carried out on a different electronic tongue system). Three drugs: paracetamol, caffeine and naratriptan were assessed at five different pH values i.e. 2, 4, 6, 8 and 10. Paracetamol and naratriptan have a $pK_a$ value of 9.67 while caffeine has a $pK_a$ of 0.18. Paracetamol, with its high $pK_a$ value is only ionised at pH 8 and 10, thus it only elicited a potentiometric response at these values. Conversely caffeine only elicits a potentiometric response at pH 2 and 4 due to its low $pK_a$ value. Interestingly, naratriptan in spite of its high
The authors postulate that the significantly higher lipophilicity of naratriptan compared to the other two drugs (similar to the observations of Ito et al.\textsuperscript{230}). The pH dependency of sensor response was not investigated in this study as the aim was to attempt to correlate the results to that of the human/rodent experiments and thus altering the pH of the test solutions would not have been representative of what was presented to the human/rodent participants.

It is interesting to note that high concentrations of rifampicin elicit erratic responses from the electronic tongue, similar to those observed from human and rodent testing. As discussed in Chapter 3, with human participants this may be due to the fact that upon reaching a certain threshold value, differences in taste intensity can no longer be detected or alternatively the concentrations of drug used were too close to each other to allow for full differentiation between samples which may also be the case for the electronic tongue. Rifampicin is a lipophilic molecule with a logP of 3.719 thus it is likely that the drug is partitioning into the lipid membrane of the sensor (further discussed below). This may also contribute to the erratic results observed at higher concentrations.

The results above, combined with the results of Gondongwe\textsuperscript{228} and Uchida et al.\textsuperscript{229} demonstrate that it is essential to first assess the dose response of the drug being analysed before assessing any formulations containing the drug. Formulations containing drugs which exhibit a linear dose response such as ethambutol are ideal for assessment using the electronic tongue, while those such containing drugs such as caffeine, which produce no response, must be assessed via different means. It may still be possible to assess formulations containing drugs such as pyrazinamide and isoniazid, which do produce a response albeit not a linear one, using the electronic tongue. The concentration of drug contained within the formulation could be assessed and compared to the response obtained from water. If it is found to have a significantly different response then it may still be possible to assess formulations containing the drugs.

Little has been published on the longevity of the sensors employed by the electronic tongue however, given their significant cost, it is a pertinent issue to consider. When assessing rifampicin it was observed that, after the experimental run, the basic bitterness sensors AC0 and AN0 were stained red indicating that rifampicin molecules had adsorbed onto the lipid membrane. The AC0 sensor in particular was the most badly affected. No colour change was observed for C00 and AE1 sensors. Despite extensive washing in both reference and negatively charged washing solution the red colour could not be removed and calibration.
testing of the sensors indicated they were outside of their calibration limits. On this basis rifampicin is not suitable for assessment on the electronic tongue as it adversely affects the AC0 and AN0 sensors, requiring them to be replaced. This also highlights the importance of periodically testing whether the sensors are within their calibration limits as it is possible that this phenomenon occurs with other drugs but may go unnoticed as they are not highly coloured like rifampicin.

The correlation between human taste panel responses and electronic tongue sensor responses for each drug was compared. A poor correlation was observed for isoniazid and rifampicin, while a reasonable correlation was observed for pyrazinamide. An excellent correlation was observed between human and electronic tongue responses for ethambutol dihydrochloride with all sensors having a $R^2$ value in excess of 0.99. It is unsurprising that ethambutol dihydrochloride has the highest correlation coefficient, given that it is the only drug which exhibited a fully linear response from each sensor, however it is both surprising and encouraging that the correlation is so good.

There has been much discussion in the literature about whether electronic tongues can be used in place of human panels. Eckert et al. investigated the utility of the electronic tongue, HPLC and a human panel for the detection of herbal products in lozenges. They concluded that, while electronic tongues are useful, they cannot replace human panels. Conversely Maniruzzaman et al. assessed taste-masked granules of paracetamol using both a human taste panel and electronic tongue and concluded that the electronic tongue can be used to replace human panellists in formulation development. Based on the results of these experiments carried out in this chapter, it does not seem that the electronic tongue can be used to replace human panellists for the assessment of formulations containing these drugs.

A major limitation of the electronic tongue is that, due to the nature of the sensors, only liquid formulations/solutions of drug can be assessed. For solid formulations a pre-dissolution step must be carried out in order to assess the taste of the formulation. The procedure recommended by Inset involves stirring the formulation in 100mL of 10mM potassium chloride solution for a set time period (e.g. 1 minute), filtering the resulting solution and analysing the taste of this ‘taste extracted liquid’. It can be seen that this dissolution step is not representative of the conditions a formulation will experience in the oral cavity. It also begs the question as to whether, in these cases, the electronic tongue is merely acting as a surrogate dissolution apparatus.
Due to the nature of the electronic tongue it cannot be used to determine human threshold bitterness values for drugs, it can only be used to compare the difference in taste between pure drugs and formulations. For drugs which elicit a linear response from the electronic tongue this will be a relatively simple comparison. However, it may also still be possible to use the electronic tongue to assess formulations containing isoniazid, rifampicin and pyrazinamide which do not elicit a linear response. The concentration of drug contained in the formulation being assessed could first be assessed separately to ensure that the drug elicits a response significantly different to that of water. If it is found to be significantly different, then the electronic tongue may still be used to determine differences in taste between pure drug and formulations.

### 4.6 Conclusions

In this study, the taste of isoniazid, rifampicin, pyrazinamide and ethambutol was assessed using the Insent TS-5000Z electronic tongue. It was found that for isoniazid, rifampicin and pyrazinamide a non-linear response was obtained from the sensors indicating that the electronic tongue may not be ideal for taste assessment of formulations containing these drugs. For ethambutol dihydrochloride a linear response is observed for all sensors, most likely due to the fact ethambutol dihydrochloride (as it is a salt) is fully ionised under the experimental conditions, unlike the other drugs being tested. A poor correlation was observed between human taste responses and electronic tongue responses for isoniazid, rifampicin and pyrazinamide, indicating that this technique is not suitable as a replacement for human taste panels of formulations containing these drugs. Ethambutol showed an excellent correlation between human and electronic tongue responses, however, given the relatively small sample size used for the human taste panel further studies should be carried out to fully elucidate the strength of this correlation. In summary, while the electronic tongue may be a useful tool for early screening studies of novel formulations, it cannot be used to replace in vivo methods of taste assessment for these drugs.
CHAPTER 5

Production of Taste-Masked Formulations of Isoniazid and Rifampicin by Hot Melt Extrusion
CHAPTER 5

5.1 INTRODUCTION

As discussed in Chapter 1, a large number of drugs currently on the market or under development have poor organoleptic properties which, unless formulated appropriately, can be very unpalatable. Therefore, taste-masking has become an important part of the formulation development process, particularly for paediatric medications. There are a wide range of formulation strategies that can be used either alone or in combination to mask the bitter taste of a drug including, but not limited to: use of flavours and sweeteners, lipophilic vehicles, salt formation, solid dispersions, salting out layers, ion exchange resins, complexation with cyclodextrins, or film coating.

In recent years hot melt extrusion has emerged as a novel processing method for taste-masking of bitter drugs. Hot melt extrusion is a very versatile technique which has already been used by the pharmaceutical industry to produce a variety of formulations including pellets, oral fast dissolving films, controlled release tablets, transdermal/mucosal delivery systems and implants.

There are many advantages of using hot melt extrusion over traditional formulation methods. Removal and disposal of potentially harmful organic solvents is a common issue with many pharmaceutical techniques, in particular when producing medicines for children. HME is a solvent free process, thus avoiding the need for this step and also making it an environmentally friendly process. It is also a continuous process which is easy to scale up, and can be adapted to meet the goals of the FDA process analytical technology (PAT) scheme for quality by design in pharmaceutical products.

As seen in Chapter 3, isoniazid and rifampicin (Figure 5.1) are moderately bitter compounds, with rifampicin having a significantly lower EC$_{50}$ than isoniazid (3.6 mM compared to 259 mM). Isoniazid is a borderline BCS Class I/III drug, with an aqueous solubility of approximately 125 mg/mL, which has been found to be 100% bioavailable under most circumstances. Rifampicin on the other hand is a borderline BCS Class II drug with a pH dependent solubility ranging from approximately 125 mg/mL at pH 1 to only 0.85 mg/mL at pH 7. It has a bioavailability of roughly 70%.
Figure 5.1 – Chemical structure of (a) isoniazid and (b) rifampicin.

It is important that any taste-masking strategy used does not adversely affect the bioavailability of either of these drugs. Therefore, it is essential that the formulations undergo rapid dissolution in the stomach. For this reason, two polymers which are known to have rapid gastric release profiles were chosen, i.e. Eudragit E-PO and Soluplus. Eudragit E-PO is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate which is insoluble above pH 5.\textsuperscript{175} Soluplus is a polyvinyl caprolactam - polyvinyl acetate - polyethylene glycol graft copolymer which is freely water soluble.\textsuperscript{173,241,256,257} It has not yet been widely used for taste-masking applications. Eudragit E-PO has already been widely used for taste-masking due to its selective release properties\textsuperscript{176}, while Soluplus has not been studied in this regard but provides a useful comparator of a polymer that should release the drug reasonably efficiently. By using these two matrix systems we intend to examine the correlation between polymer water miscibility, solid state structure, in vitro drug release and taste-masking efficiency.

Figure 5.2 – Chemical structure of (a) Soluplus and (b) Eudragit E-PO.
5.2 AIMS AND OBJECTIVES

The aim of the work described in this chapter was to assess whether hot melt extrusion could be used to produce taste-masked formulations of isoniazid and rifampicin which undergo rapid dissolution in the stomach.

5.3 MATERIALS AND METHODS

5.3.1 Materials

Isoniazid, potassium chloride, sodium chloride, potassium phosphate monobasic, sodium hydroxide, calcium chloride, potassium hydroxide and tartaric acid were obtained from Sigma Aldrich (UK). Rifampicin was obtained from Fagron UK Ltd (Newcastle, UK). Soluplus was kindly donated by BASF (Ludwigshafen, Germany). Eudragit EPO was obtained from Röhm GmbH & Co. (Sontheim/Brenz, Germany). Hydrochloric acid was obtained from Fisher Chemicals (Loughborough, UK). Distilled water was used for all experiments. All substances were used as received unless otherwise stated.

5.3.2 Methods

5.3.2.1 Calculation of Hansen Solubility Parameters

The Hansen solubility parameters of drugs and polymers were calculated from their chemical structures using the van Krevelen and Hoftyzer method\textsuperscript{258} according to following equation:

\[
\delta_i = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2}
\]

(Equation 5.1)

where:

\[
\delta_d = \frac{\sum F_{di}}{V_i}, \quad \delta_p = \sqrt{\frac{\sum F_{pi}^2}{V_i}}, \quad \delta_h = \sqrt{\frac{\sum E_{hi}}{V_i}}
\]

\(\delta_d\) refers to dispersion forces; \(\delta_p\) refers to polar interactions; \(\delta_h\) refers to hydrogen bonding; \(i\) refers to the structural groups within the molecule; \(F_{di}\) is the molar attraction constants due to molar dispersion forces; \(F_{pi}\) is the molar attraction constant due to molar polarisation forces; \(E_{hi}\) is hydrogen bonding energy; \(V_i\) represents the group contribution to molar volume.

The solubility parameter model suggests that compounds with similar \(\delta_i\) values will be miscible. It is reported that compounds with a \(\Delta\delta_i < 7\text{MPa}^{1/2}\) are likely to be miscible whereas compounds with \(\Delta\delta_i > 10\text{MPa}^{1/2}\) are likely to be immiscible.\textsuperscript{259}

5.3.2.2 Hot Melt Extrusion

Hot-melt extrudates were prepared using a Thermo Scientific Process 11 co-rotating twin screw extruder (ThermoScientific, UK) fitted with a round die (die diameter: 2mm). The
formulations and optimised extrusion parameters used are given in Table 5.1 and Table 5.2. Post extrusion the extrudates were cut into ~1 cm pieces using a Pharma 11 Varicut Pelletizer (Thermo Fisher Scientific, U.K.).

**Table 5.1 – Isoniazid formulations and associated processing parameters used for hot melt extrusion.**

<table>
<thead>
<tr>
<th>Isoniazid (% w/w)</th>
<th>Soluplus (% w/w)</th>
<th>Eudragit E-PO (% w/w)</th>
<th>Temperature (°C)</th>
<th>Speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>80</td>
<td>-</td>
<td>140</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>-</td>
<td>140</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>80</td>
<td>130</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>70</td>
<td>130</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 5.2 – Rifampicin formulations and associated processing parameters used for hot melt extrusion.**

<table>
<thead>
<tr>
<th>Rifampicin (% w/w)</th>
<th>Soluplus (% w/w)</th>
<th>Eudragit E-PO (% w/w)</th>
<th>Temperature (°C)</th>
<th>Speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>75</td>
<td>-</td>
<td>150</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>-</td>
<td>160</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>75</td>
<td>140</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>-</td>
<td>60</td>
<td>150</td>
<td>20</td>
</tr>
</tbody>
</table>

**5.3.2.3 Determination of Drug Loading**

A 0.1% w/v solution of each extrudate was prepared by dissolving 10 mg of ground extrudate in 10 mL ethanol. The mixtures were sonicated for 30 minutes to ensure complete dissolution of the extrudate. Each solution was diluted 1 in 10 before the UV absorbance (Jenway 6305 Spectrophotometer, Bibby Scientific, Staffordshire, UK) was recorded at 263 nm (isoniazid extrudates) or 475 nm (rifampicin extrudates) where the polymer showed no absorbance. Standard solutions of isoniazid and rifampicin within the concentration range of 1.5625 to 50 µg/mL were prepared in ethanol and the absorbance measured at 263 nm or 475 nm as appropriate. Standard curves were plotted using the acquired data. The concentration of drug in each extrudate was determined from the absorbance of the filtered sample using the values of the standard curve.
5.3.2.4 Differential Scanning Calorimetry
Standard DSC and modulated temperature DSC (MTDSC) thermograms were recorded using a TA Instruments Q2000 calorimeter (TA Instruments, New Castle, Delaware, USA). For analysis, 4-6 mg of sample was accurately weighed and sealed in an aluminium pan (hermetic and pinhole pans used as appropriate). For standard DSC, samples were heated under nitrogen gas (flow rate 50 mL/min) at a rate of 10°C/min. For MTDSC, samples were heated under nitrogen gas (flow rate 50 mL/min) at a rate of 2°C/min, amplitude ± 0.212°C and a period of 40 seconds. Calibration was performed using n-octadecane, benzoic acid, indium, and tin. Samples were analysed in triplicate unless stated otherwise. Data analysis was carried out with TA Universal Analysis software.

5.3.2.5 Thermogravimetric Analysis
Thermogravimetric analysis (TGA) was carried out using a TA Instruments Hi-Res 2950 thermogravimetric analyser (TA Instruments, New Castle, Delaware, USA). Samples were analysed under nitrogen in open aluminium pans with a heating rate of 10°C/min. Data analysis was carried out with TA Universal Analysis software.

5.3.2.6 Powder X-Ray Diffraction
Powder X-Ray Diffraction (PXRD) was carried out using a Rigaku 600 Miniflex diffractometer (Rigaku, Tokyo, Japan), CuKα radiation, operating power: 40mV, 15mA. Patterns were recorded over the 2θ range 3°- 40° at a scan rate of 5°/min.

5.3.2.7 In Vitro Dissolution Testing
In vitro drug release was tested using British Pharmacopoeia method 2.9.3 dissolution test for solid dosage forms with the aid of Pharmatest PTWS 120D dissolution apparatus (Pharma Test Apparatebau AG, Hainburg, Germany). The pure drug and extrudates were all assessed using this method. Samples were loaded into Size 4 gelatine capsules (Qualicaps Europe SA, Madrid) and placed into a metallic sinker. The sinker was placed in a dissolution bath containing 900 mL 0.1N HCl (pH 1.2 ± 0.2) at 37.0 ± 0.5°C and a paddle speed of 50 rpm. At predetermined time intervals, a 10 mL sample was withdrawn from each vessel and replaced with the same amount of fresh media to ensure constant volume of medium within the vessel. The concentration of drug in the dissolution medium was measured by UV-Vis spectrometry (Jenway 6305 Spectrophotometer; Bibby Scientific, Staffordshire, UK) at 263 nm or 475 nm as appropriate. Experiments were carried out in triplicate and dissolution profiles are plotted as mean percentage drug release ± standard deviation.
5.3.2.8 Solubility Studies

The saturated solubility of isoniazid was assessed in both distilled water and simulated salivary fluid (SSF) at pH 7.4 adapted from Hughes et al.\textsuperscript{260} (Table 5.3). An excess amount of pure drug was added to 30 mL distilled water or SSF at 37 ± 0.5°C and shaken for 72 hours until equilibrium was reached. The samples were then filtered using 0.22 µm filters (Merck-Millipore, Cork, Ireland) and the absorbance of the samples were recorded at 263 nm using a Jenway 6305 UV-Vis Spectrophotometer (Bibby Scientific, Staffordshire, UK).

\textit{Table 5.3 – Composition of Simulated Salivary Fluid}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium phosphate monobasic</td>
<td>12 mM</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>40 mM</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>to pH 7.4</td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 1 L</td>
</tr>
</tbody>
</table>

5.3.2.9 Simulation of Drug Release in Oral Cavity

Dissolution testing was carried out to simulate the dissolution of pure drugs and the polymeric formulations in the oral cavity. Exposure of the drug/formulations to oral cavity conditions was mimicked by 5 minutes drug contact with 5 mL of SSF at pH 7.4. The unit dose of isoniazid was set at 150 mg however, to have sufficient volume to allow sampling during the experiment, an amount of formulation equal to 10 unit doses of drug was added to 50 mL SSF. 2 mL samples were manually withdrawn at 0.5, 1, 1.5, 2, 2.5, 3 and 5 minutes and replaced with fresh media. A temperature of 37 ± 0.5°C and a rotational speed of 50 rpm were maintained during the experiment. Samples were filtered through 0.22 µm filters (Merck-Millipore, Cork, Ireland) and analysed by UV-Vis Spectrometry (Jenway 6305 Spectrophotometer; Bibby Scientific, Staffordshire, UK) at 263 nm. Drug release was compared to the human EC\textsubscript{50} and rodent IC\textsubscript{50} for isoniazid to determine whether the formulations were likely to be aversive or not.

5.3.2.10 Taste Assessment using Electronic Tongue

5.3.2.10.1 Sensors

Sensors and reference electrodes were purchased from PPM instruments (West Sussex, UK). The TS-5000Z (Insent Inc., Atsugi-shi, Japan) was equipped with four lipid membrane sensors
and two corresponding reference electrodes. Three of the sensors represent bitterness, bitterness sensor 1 (SB2AC0), bitterness sensor 2 (SB2AN0) and bitterness sensor 3 (SB2C00). The fourth sensor represents astringency (SB2AE1). Before beginning experiments, 0.2 mL inner solution (saturated AgCl solution) was filled into each sensor. Reference sensors were completely filled with inner solution. Lipid membrane sensors were preconditioned in standard solution for 24 hours before measurement. Reference sensors were preconditioned in 3.33 M potassium chloride before measurement.

5.3.2.10.2 Preparation of Standard Solutions

Reference solution used for cleaning and as a reference solution was prepared by dissolving 30 mM/L potassium chloride and 0.3 mM/L tartaric acid in distilled water.

Negatively charged washing solution used for washing the negatively charged sensors (SB2AC0 and AB2AN0) was prepared by diluting absolute ethanol to 30% with distilled water and adding 100 mM/L hydrochloric acid.

Positively charged reference solution used for washing the positively charged sensors (SB2C00 and SB2AE1) was prepared by diluting absolute ethanol to 30% and adding 100 mM/L potassium chloride and 10 mM/L potassium hydroxide.

5.3.2.10.3 Measurement Procedure

All measurements were performed using the taste sensing system TS-5000Z (Insent Inc., Atsugi-shi, Japan). A sensor check was conducted routinely before each measurement to ensure that the sensors were working within the correct mV range. Each measurement cycle consisted of measuring a reference solution (\(V_r\)), followed by the sample solution (\(V_s\)), a short (2 x 3 seconds) cleaning procedure and measurement of the aftertaste (\(V_r'\)) and finally a 330 second cleaning procedure. The sensor output for taste (relative value, \(R\) value) was calculated relative to the preliminary sensor response to the reference solution (\(V_r\)).

\[
R = V_s - V_r
\]  \hspace{1cm} Equaion 5.2

The entire measurement procedure was performed for all samples and repeated afterwards up to six times. For further data treatment the first run was discarded (as recommended by Insent) to enable conditioning of sensors.

5.3.2.10.4 Analysis of Polymers, Physical Mixtures and Formulations

The e-tongue can only assess the taste of liquid solutions. Therefore when assessing the taste of solid formulations (as is the case with hot melt extrudates) it is necessary to carry out a pre-dissolution step. Only compounds which are dissolved in the oral cavity will be able to
interact with taste buds and give a taste response. The dose unit of isoniazid was set at 150 mg. For taste evaluation 20 dose units (equivalent to 3 g isoniazid) were added to 100 mL of 10 mM potassium chloride solution at 37°C and gently stirred for 1 minute. This represents a concentration of one dose in 5 mL which is suitable for taste assessment as there is only slight dilution of the sample. The mixture was then filtered through 0.33 µm filters, (Merck-Millipore, Cork, Ireland) remove any suspended particles. The taste of this liquid was then assessed using the measurement procedure described in section 5.3.2.10.3.

5.3.2.10.5 Data Treatment

The sensor signal results were evaluated either univariately or multivariately. Principal component analysis (PCA) was used to reduce the multidimensional space (i.e. responses from four independent sensors) without losing information. In PCA, the dataset is projected onto the space spanned by the vectors called principal components which correspond to the maximum variance of the dataset. Using PCA the most important information contained in the raw data could be transformed into the first principal component (PC-1) and the second most important is transformed into the second principal component (PC-2). Plotting of PC-1 versus PC-2 gives a map which allows the assessment of similarities and differences between different samples. Differences between samples were assessed by determining the Euclidean distance between them after multivariate data analysis. Euclidean distances were calculated according to the following equation:

\[ d(p, q) = \sqrt{\sum_{i=1}^{n} (p_i - q_i)^2} \]  

Equation 5.3

All data analysis was carried out using OriginPro 9.1 (Origin Lab, Massachusetts, USA).

5.4 RESULTS

5.4.1 Estimation of Drug Polymer Miscibility

The theoretical miscibility of the components of the extrudates was assessed by determining the Hansen solubility parameters for each component (pure drug and pure polymers) using the method described by van Krevelen and Hoftyzer. The solubility parameter model suggests that compounds with similar \( \delta \) values are miscible since, in such a system, the energy of mixing from intermolecular interactions is balanced by the energy of mixing from intramolecular interactions. It is reported by Greenhalgh et al. that compounds with a \( \Delta \delta \)
Chapter 5

< 7MPa$^{1/2}$ are likely to be miscible whereas compounds with $\Delta \delta_{t} > 10$MPa$^{1/2}$ are likely to be immiscible.$^{259}$ The detailed calculation for isoniazid is given in Table 5.4.

Table 5.4 – Calculation of Hansen solubility parameter for isoniazid using group contribution method outlined by van Krevele and Hoftyzer.$^{258}$

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>$V_i$</th>
<th>$F_{di}$</th>
<th>$F_{pi}$</th>
<th>$E_{hi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-NH$_2$</td>
<td>1</td>
<td>19.2</td>
<td>280</td>
<td>-</td>
<td>8400</td>
</tr>
<tr>
<td>-NH-</td>
<td>1</td>
<td>4.5</td>
<td>160</td>
<td>210</td>
<td>3100</td>
</tr>
<tr>
<td>-CO-</td>
<td>1</td>
<td>10.8</td>
<td>290</td>
<td>770</td>
<td>2000</td>
</tr>
<tr>
<td>-N=</td>
<td>1</td>
<td>5.0</td>
<td>20</td>
<td>800</td>
<td>5000</td>
</tr>
<tr>
<td>=C=</td>
<td>1</td>
<td>-5.5</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>=CH-</td>
<td>4</td>
<td>13.5</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ring</td>
<td>1</td>
<td>16.0</td>
<td>190</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Molar volume $V_i = 19.2 + 4.5 + 10.8 + 5.0 - 5.5 + (13.5 \times 1) + 16.0 = 104$

$\delta_d = \frac{\Sigma F_{di}}{\Sigma V_i}$

$\Sigma F_{di} = 280 + 160 + 290 + 20 + 70 + (4 \times 200) + 190 = 1810$

$\delta_d = \frac{\Sigma F_{di}}{\Sigma V_i} = \frac{1810}{104} = 17.40$ MPa$^{1/2}$

$\delta_p = \sqrt{\frac{\Sigma F_{pi}^2}{\Sigma V_i}}$

$\Sigma F_{pi}^2 = (210)^2 + (770)^2 + (880)^2 = 1411400$

$\delta_p = \sqrt{1411400/104} = 11.42$ MPa$^{1/2}$

$\delta_h = \sqrt{\frac{\Sigma E_{hi}}{\Sigma V_i}}$

$\Sigma E_{hi} = 8400 + 3100 + 2000 + 5000 = 18500$

$\Sigma E_{hi}/\Sigma V_i = 18500/104 = 177.88$

$\delta_h = \sqrt{177.88} = 13.34$ MPa$^{1/2}$

135
\[ \delta_t = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \]

\[ \delta_t = \sqrt{[(17.40)^2 + (11.42)^2 + (13.33)^2]} \]

\[ \delta_t = 24.7 \text{ MPa}^{1/2} \]

Hansen solubility parameters for the polymers were obtained from the literature. These values along with the calculated \( \Delta \delta \) for each drug/polymer combination are given in Table 5.5. It can be seen that the \( \Delta \delta \) values for isoniazid with Soluplus and Eudragit E-PO are 5.3 and 5.8 MPa\(^{1/2} \) respectively. This indicates that isoniazid will be miscible with these polymers.

**Table 5.5 – Hansen solubility parameters for pure polymers, isoniazid and calculated \( \Delta \delta \) for drug-polymer combinations.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \delta_d ) (MPa(^{1/2} ))</th>
<th>( \delta_p ) (MPa(^{1/2} ))</th>
<th>( \delta_h ) (MPa(^{1/2} ))</th>
<th>( \delta_{\text{total}} ) (MPa(^{1/2} ))</th>
<th>( \Delta \delta_{\text{total}} ) (MPa(^{1/2} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>17.4</td>
<td>11.4</td>
<td>13.3</td>
<td>24.7</td>
<td>-</td>
</tr>
<tr>
<td>Soluplus (^{262} )</td>
<td>17.4</td>
<td>0.3</td>
<td>8.6</td>
<td>19.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Eudragit E-PO (^{141} )</td>
<td>17.9</td>
<td>0.7</td>
<td>6.1</td>
<td>18.9</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Hansen solubility parameters for rifampicin were calculated as per isoniazid. These values, along with the calculated \( \Delta \delta \) for each drug/polymer combination are given in Table 5.6.

**Table 5.6 – Hansen solubility parameters for pure polymers, rifampicin and calculated \( \Delta \delta \) for drug-polymer combinations.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \delta_d ) (MPa(^{1/2} ))</th>
<th>( \delta_p ) (MPa(^{1/2} ))</th>
<th>( \delta_h ) (MPa(^{1/2} ))</th>
<th>( \delta_{\text{total}} ) (MPa(^{1/2} ))</th>
<th>( \Delta \delta_{\text{total}} ) (MPa(^{1/2} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>12.7</td>
<td>3.4</td>
<td>6.6</td>
<td>14.7</td>
<td>-</td>
</tr>
<tr>
<td>Soluplus (^{262} )</td>
<td>17.4</td>
<td>0.3</td>
<td>8.6</td>
<td>19.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Eudragit EPO (^{141} )</td>
<td>17.9</td>
<td>0.7</td>
<td>6.1</td>
<td>18.9</td>
<td>5.4</td>
</tr>
</tbody>
</table>

The \( \Delta \delta \) values for rifampicin and Soluplus and rifampicin and Eudragit EPO are 4.7 MPa\(^{1/2} \) and 5.4 MPa\(^{1/2} \) respectively, below the cut off of 7MPa\(^{1/2} \), indicating that rifampicin is miscible with these two polymers. It is important to note that while solubility parameters have been successfully used to predict drug/polymer miscibility,\(^{172,259,263,264} \) they are only a theoretical model and need to be validated by experimental observations.
5.4.2 Solid State Characterisation of Drugs and Polymers

When carrying out HME the optimum extrusion parameters must be determined based on the melting temperature ($T_m$) of the drug, the glass transition temperature ($T_g$) of the polymeric carrier, the thermoplastic properties of the drug-polymer mixture and the degradation temperature of the materials used.\textsuperscript{191} Thus, it is essential to know the thermal properties of the model drugs and excipients prior to extrusion.

Standard DSC measurements were carried out to determine the melting point ($T_m$) of isoniazid and rifampicin. Isoniazid (Figure 5.3a) was found to have a melting point with an onset temperature of $170.8 \pm 0.1^\circ$C and peak at $173.1 \pm 0.2^\circ$C. This is in close agreement with the reported melting point of isoniazid ($171.4^\circ$C).\textsuperscript{231} For rifampicin (Figure 5.3b) a broad melting endotherm is observed with an onset temperature of $165.9 \pm 4.1^\circ$C and a peak at $181.2 \pm 2.9^\circ$C which corresponds to the reported melting point of rifampicin ($183^\circ$C).\textsuperscript{152}

In addition to knowing the thermal transitions of the drugs, it is also essential to know the degradation profile of the drugs to ensure that HME processing temperatures are kept below the degradation temperature ($T_d$) of the drug. This was analysed using TGA.
Figure 5.3 – DSC thermogram (10°C/min) of (a) isoniazid; (b) rifampicin; (hermetic pans).

For isoniazid (Figure 5.4a) no weight loss was observed in between 30-120°C indicating that no water is contained in the sample. A sharp drop in weight is observed between 175-280°C with a mass loss of 87.45 ± 0.88% corresponding to degradation of isoniazid. Rifampicin (Figure 5.4b) shows a small % weight loss (0.57 ± 0.15%) between 30-120°C corresponding to loss of water from the sample. Decomposition of rifampicin was observed in two steps, the first from 190-240°C and the second from 240-270°C, which amounted to 4.16 ± 0.29% and 14.50 ± 0.34% weight loss respectively.
Figure 5.4 – TGA curve (10°C/min) of (a) Isoniazid; (b) Rifampicin showing the weight loss and associated derivative loss curve.

It can be seen from the DSC and TGA data that isoniazid and rifampicin have a very small margin between their respective melting and degradation temperatures. Therefore it is unfeasible to carry out extrusion at temperatures higher than the $T_m$ of the drug. In general hot melt extrusion is carried out above the melting point of the drug, although there are examples in the literature of solid dispersions of drugs being successfully formed at temperatures below the melting point of the drug\textsuperscript{141,174} so this may not be as much of a challenge as it seems on initial inspection.
PXRD was carried out to validate the physical nature of the drugs. The diffraction pattern of isoniazid (Figure 5.5a) shows numerous sharp peaks, indicating that the drug is in the crystalline state. They key characteristic peaks of isoniazid occur at 15.6°, 16.8°, 19.6° and 25.2°. The diffraction pattern of rifampicin is given in Figure 5.5b. Rifampicin is reported to have two crystal forms, Form I and II, and an amorphous form. Form I and Form II can be differentiated in PXRD by characteristic peaks at 13.65° and 14.35° for Form I and 9.93° and 11.10° for Form II. The rifampicin used in this study is predominantly in Form II, as evidenced by the sharp peaks at 9.93° and 11.10°. However less intense peaks are also observed at 13.65° and 14.35° and the baseline has a halo appearance, suggesting that both Form I and amorphous rifampicin are also present in the sample.

Figure 5.5 – Powder X-Ray diffraction pattern of (a) isoniazid and (b) rifampicin.
The thermal properties of the polymeric carriers were also investigated using DSC and TGA. In order to accurately determine the glass transition temperature of the polymers, modulated temperature DSC (MTDSC) was carried out. In addition, the samples were subjected to a heat-cool-heat cycle in order to erase any thermal history and obtain an accurate $T_g$ value. TGA was carried out to determine the degradation profile of the polymers.

The $T_g$ of Soluplus was determined to be 70.4 ± 2.3°C which is in close agreement with the values quoted in the literature (~70°C). The $T_g$ of Eudragit E-PO was determined to be 55.3°C ± 0.5°C, which is in accordance with reference values (57°C). In all cases, no melting peaks were observed for the polymers, suggesting that they are in the amorphous state. Representative thermograms for the polymers are given in Figure 5.6.
TGA was carried out to assess the water content of the polymers and to determine their degradation profiles. Soluplus (Figure 5.7a) shows a small % weight loss (2.25 ± 0.05%) between 30-120°C indicating that water is contained in the sample. A second small weight loss is observed beginning at 185°C with 1.47 ± 0.07% of the sample being lost. This weight loss can be attributed to decomposition of Soluplus. Similarly, for Eudragit E-PO (Figure 5.7b) a small % weight loss (0.22 ± 0.01%) was observed in between 30-120°C corresponding to loss of water from the sample. Decomposition of Eudragit E-PO was observed in a single step from approximately 180°C, with 11.96 ± 0.29% of the sample being lost.
Figure 5.7 – TGA curve (10°C/min) of (a) Soluplus and (b) Eudragit EPO showing the weight loss and associated derivative loss curve.

The PXRD patterns of polymeric carriers are given in Figure 5.8. It can be seen that in both cases the diffraction patterns take the form of a halo, indicating that they are in the amorphous state. This is in agreement with the DSC results which showed no melting peak for either of the polymers.
Figure 5.8 – Powder X-Ray diffraction pattern of (a) Soluplus and (b) Eudragit E-PO.

The physicochemical properties of drugs and polymers are summarised in Table 5.7

Table 5.7 – Physicochemical properties of drugs and polymers.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Crystalline / Amorphous</th>
<th>T_m (°C)</th>
<th>T_g (°C)</th>
<th>T_d (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Crystalline</td>
<td>173.1 ± 0.2</td>
<td>-</td>
<td>175</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Crystalline</td>
<td>181.2 ± 4.1</td>
<td>-</td>
<td>190</td>
</tr>
<tr>
<td>Soluplus</td>
<td>Amorphous</td>
<td>-</td>
<td>70.4 ± 2.2</td>
<td>185</td>
</tr>
<tr>
<td>Eudragit E-PO</td>
<td>Amorphous</td>
<td>-</td>
<td>55.3 ± 0.5</td>
<td>180</td>
</tr>
</tbody>
</table>
5.4.3 Hot Melt Extrusion

5.4.3.1 Preparation of Placebo Polymer Extrudates
To determine appropriate extrusion parameters for the polymer/drug mixture, the polymers alone were first processed using the hot-melt extruder to assess their extrusion properties. As mentioned previously, the extrusion parameters should be chosen based on the thermoplastic properties of the material to be extruded. Specifically, the extrusion temperature should be higher than the $T_g$ of the carrier being used to decrease viscosity and ensure proper flow of the material through the extruder.

The optimum processing parameters for Soluplus were determined to be 150°C and 50 rpm. Under these parameters clear, dark yellow extrudates were formed. These extrudates had a uniform diameter and flowed well from the extruder. Extrusion of Eudragit E-PO at 140°C and 50 rpm produced opaque, pale yellow extrudates. Again, these flowed well from the extruder and had a uniform diameter.

5.4.3.2 Preparation of Isoniazid Loaded Extrudates
Initial extrusion experiments were carried out with a drug loading of 20% w/w. Optimum processing parameters for Soluplus alone were found to be 150°C and 50 rpm. When extruding the Soluplus/isoniazid 20% w/w mix under these conditions yellow, grainy extrudates were formed. Bubbling was also observed as the extrudates left the die head. It appears that isoniazid has a plasticizing effect on Soluplus, lowering the $T_g$ of the polymer. It was decided to lower the temperature to attempt to improve the flow of extrudates from the extruder. At 140°C and 50 rpm opaque, yellow extrudates were formed which flowed well from the extruder, were not sticky and had a uniform diameter. Based on the success of the experiments involving the 20% w/w drug loaded mixture it was decided to increase the drug loading to 30% to see what effect this would have on the extrusion properties of the mixture. At this higher drug loading opaque yellow extrudates were once again formed. The yellow colour is as a result of the colour of the polymer, as described in section 5.4.3.1 above. Increasing the drug loading did not appear to have any adverse effect on the processability of the mixture.

Optimum processing parameters for Eudragit E-PO were determined to be 140°C and 50 rpm. When the Eudragit E-PO/isoniazid mix was extruded at 140°C and 50 rpm yellow, semi-opaque extrudates were obtained. The extrudates were significantly thinner and less viscous than those formed by Eudragit E-PO alone. As with the Soluplus based system it was decided to lower the temperature to attempt to improve the flow of extrudates from the extruder.
At 130°C and 50 rpm opaque, off white/yellowish extrudates were formed which flowed well from the extruder, were not sticky and had a uniform diameter. Again, it was decided to increase the drug loading of the mixture to see what effect this would have on its extrusion properties. At 30% w/w drug loading uniform, opaque off-white/yellowish extrudates were formed. As per the Soluplus based systems, the yellow colour is as a result of the polymer and increasing the drug loading did not appear to have any effect on the processability of the mixture.

5.4.3.3 Preparation of Rifampicin Loaded Extrudates

Initial extrusion experiments were carried out with 25% w/w drug loading. Lower drug loadings were not investigated as the required dose of rifampicin is quite high and lower drug loadings would mean that final formulations developed from these extrudates would be extremely large.

Based on work previously carried out with the isoniazid formulations, the initial extrusion parameters used for the Soluplus/rifampicin 25% w/w mix were 140°C and 50 rpm. However under these conditions the torque values were too high to allow extrusion to occur. The temperature was increased to 150°C and the screw speed reduced to 30 rpm to allow the mix to soften and reduce torque within the machine. Under these conditions extrusion was able to occur; however torque was still high (up to 95%). Dark red, brittle extrudates were produced which flattened on exit from the die. Extrusion of the Eudragit E-PO/rifampicin 40% w/w mix was first attempted under the same conditions of 150°C and 30 rpm however, again, the torque was too high for extrusion to occur. The temperature was raised by to 160°C and screw speed reduced to 20 rpm which allowed extrusion to occur, although torque values still remained high. Similar to the 25% w/w extrudates, dark red, brittle extrudates were formed which flattened on exit from the die.

Extrusion of the Eudragit E-PO/rifampicin 25% w/w mix was first attempted at 140°C and 30 rpm. This produced smooth, dark red, uniform extrudates which flowed well from the extruder. Extrusion of the Eudragit E-PO/rifampicin 40% w/w mix was first attempted under the same conditions however, the torque was too high for extrusion to occur. The temperature was raised by 10°C to 150°C to further soften the mix and this allowed the production of smooth, uniform extrudates. In all cases torque values within the machine were quite high, which suggests that the rifampicin may be exerting an antiplasticisation effect on the polymer. Antiplasticisation occurs when low molecular weight materials retard motions in the polymer chains thus changing the mechanical behaviour of the material.268
5.4.4 Physicochemical Characterisation

5.4.4.1 Isoniazid Loaded Extrudates

After production of the extrudates the first step was to test the drug loading of the formulations to ensure that the drug was not being degraded during the extrusion process. The drug loading was assessed by dissolving a fixed amount of extrudate in ethanol and analysing the drug content of the resulting solution by UV-Vis spectroscopy. The drug loading of the formulations is given in Table 5.8. It can be seen that in all cases the drug loading is within the expected range for the formulations which demonstrates that the drug is not being degraded during the extrusion process.

Table 5.8 – Drug loading of isoniazid containing formulations. Drug loading is given as mean percentage ± standard deviation.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Expected Drug Load (% w/w)</th>
<th>Actual Drug Load (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluplus</td>
<td>20</td>
<td>20.38 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.47 ± 1.58</td>
</tr>
<tr>
<td>Eudragit E-PO</td>
<td>20</td>
<td>20.13 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.72 ± 0.32</td>
</tr>
</tbody>
</table>

Figure 5.9 shows the DSC thermograms of the Soluplus based isoniazid loaded extrudates. The $T_g$ of each of these formulations was lower than the $T_g$ of Soluplus alone (approximately 70°C\(^1\)) indicating the presence of amorphous drug in these formulations. As drug loading is increased, the $T_g$ of the formulations decreases i.e. 49.6 ± 2.8°C and 42.2 ± 3.6°C for the 20% and 30% drug loaded systems respectively. The lower $T_g$ of the drug loaded systems implies that the system has been plasticized\(^2\) by the presence of isoniazid, which in turn indicates that the drug is molecularly dispersed within the carrier.

For the 20% extrudates a single $T_g$ was observed indicating that the drug is in a fully amorphous form. The 30% extrudates on the other hand show a cold crystallisation of amorphous material with an onset of 90.4 ± 1.9°C and peak at 98.2 ± 1.3°C followed by melting of crystalline material with an onset of 143.38 ± 0.18°C and peak at 156.1 ± 0.4°C. It is likely that at higher drug loadings the polymer is unable to stabilise the amorphous drug leading to recrystallisation.
Figure 5.9 – Modulated temperature DSC (2°C/min, 0.212°C/40s) of (a) 20% w/w SLP/I2D; (b) 30% w/w SLP/I2D extrudates (pinhole pans).

Figure 5.10 shows the DSC thermograms of the Eudragit E-PO based isoniazid loaded extrudates. The $T_g$ of the 20% and 30% Eudragit E-PO based extrudates were determined to be 52.9 ± 0.4°C and 53.9 ± 0.7°C respectively. This represents a very similar value to that of the polymer alone (55.3 ± 0.5°C). A melting peak is observed at approximately 170°C for both formulations which can be attributed to melting of crystalline isoniazid, which has a $T_m$ of 170.8 ± 0.1°C. The sharpness of the melting peak and the similarity of the melting point to the drug alone indicate little or no interaction between the drug and polymer.
Figure 5.10 – Modulated temperature DSC (2°C/min, 0.212°C/40s) of (a) 20% w/w EPO/IZD; (b) 30% w/w EPO/IZD extrudates (pinhole pans).

The PXRD pattern of Soluplus based extrudates and isoniazid are given in Figure 5.11. The diffraction pattern of the 20% w/w drug loaded extrudate shows and amorphous halo with no distinct crystalline peaks. This is in agreement with the results of the DSC experiments which show no melting peak associated with crystalline isoniazid. The 30% w/w drug loaded extrudates also have an amorphous halo with no distinct crystalline peaks. This indicates that the extrudate as produced is completely amorphous and only undergoes recrystallisation on heating (as per the DSC results).
Figure 5.11 – Powder X-Ray diffraction pattern of (a) isoniazid; (b) 20% SLP/IZD extrudate; (c) 30% SLP/IZD extrudate.

The PXRD patterns of Eudragit E-PO based extrudates and pure isoniazid are given in Figure 5.12. The diffraction patterns of the Eudragit E-PO based formulations are characterized by a ‘bowed’ baseline with many distinct crystalline peaks which can be attributed to the presence of crystalline isoniazid (in agreement with DSC results).

Figure 5.12 – Powder X-Ray diffraction pattern of (a) isoniazid; (b) 20% EPO/IZD extrudate; (c) 30% EPO/IZD extrudate.

5.4.4.2 Rifampicin Loaded Extrudates

As per the isoniazid loaded extrudates, the drug loading of the rifampicin loaded extrudates was first assessed to investigate whether rifampicin was being degraded during the extrusion
process. The results of these experiments are given in Table 5.9. It can be seen that, in all cases, the drug loading is 6-10% less than expected, indicating that some drug degradation is occurring during processing.

**Table 5.9 – Drug loading of rifampicin containing formulations. Drug loading is given as mean percentage ± standard deviation.**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Expected Drug Load (% w/w)</th>
<th>Actual Drug Load (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluplus</td>
<td>25</td>
<td>22.46 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>37.66 ± 1.11</td>
</tr>
<tr>
<td>Eudragit E-PO</td>
<td>25</td>
<td>23.17 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>37.98 ± 0.81</td>
</tr>
</tbody>
</table>

Thermal or mechanical degradation may occur during the hot melt extrusion process. TGA was used to investigate whether the degradation was as a result of thermal instability of the drug/polymer blends. The residence time of the drug/polymer blend in the hot melt extruder was measured as 12 minutes. To mimic this the samples were heated to the processing temperature used for each formulation and held at this temperature for 12 minutes.

The percentage mass loss due to degradation of each physical mixture is given in Table 5.10. It can be seen that in each case the degradation is extremely low (less than 0.25%), thus we can conclude that the degradation of rifampicin observed in the formulations is not due to thermal degradation. As noted in section 5.4.3.3, the torque values recorded by the machine when processing rifampicin containing mixtures are extremely high (~95%). It is likely that the extremely high pressure generated in the machine is causing mechanical degradation of the drug resulting in the lower than expected drug load for the extrudates.

**Table 5.10 – Water loss and degradation of rifampicin containing physical mixtures when held at processing temperature for 12 minutes to mimic residence time in hot melt extruder. Values are presented as percentage mass loss ± standard deviation.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Processing Temperature (°C)</th>
<th>Water Loss (%)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLP/RIF 25%</td>
<td>150</td>
<td>1.95 ± 0.22</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>SLP/RIF 40%</td>
<td>160</td>
<td>1.64 ± 0.18</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>EPO/RIF 25%</td>
<td>140</td>
<td>0.64 ± 0.02</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>EPO/RIF 40%</td>
<td>150</td>
<td>0.57 ± 0.08</td>
<td>0.23 ± 0.02</td>
</tr>
</tbody>
</table>
Figure 5.13 shows the TGA thermograms of each physical mixture when heated to their respective processing temperature and held at that temperature for 12 minutes.

(a)

(b)
It is likely that with the addition of an appropriate plasticizer it would be possible to produce rifampicin loaded extrudates. As these formulations are intended for paediatric patients, and thus it would be advantageous to keep excipients to a minimum, it was decided not to use plasticizers. On the basis of these results it was decided to take only the isoniazid containing extrudates forward for further analysis in this study, however, the extrusion of rifampicin and isoniazid together to produce a fixed dose combination formulation is investigated in Chapter 6.

Figure 5.13 – TGA curve of (a) SLP/RIF 25%; (b) SLP/RIF 40%; (c) EPO/RIF 25%; (d) EPO/RIF 40% heated to their respective hot melt extrusion processing temperatures and held to 12 minutes to mimic residence time in extruder.
5.4.5 Further Characterisation of Isoniazid Loaded Extrudates

5.4.5.1 In Vitro Drug Release

In vitro drug release studies were carried out to determine the rate of drug release from these formulations under GI conditions. Isoniazid is a borderline BCS Class I/III drug which means it has high solubility. It can be seen from Figure 5.14 that isoniazid dissolves extremely rapidly in 0.1N HCl, with 100% of the drug being dissolved within 5 minutes. As stated in Section 5.2, the aim of this study was to taste-mask the drug without adversely affecting the release profile. Therefore, it was hoped that a rapid dissolution profile, similar to that of isoniazid alone would be observed for the extruded formulations. Figure 5.14a shows the dissolution profile of the Soluplus based formulations compared to pure isoniazid. The 20% formulation demonstrates a drug release of 19.17% after 5 minutes while drug release from the 30% formulation at the same time point is significantly lower at just 2.00%. After 10 minutes the largest drug release is again observed from the 20% formulation which had released 62.14% of its drug load, compared to just 41.08% respectively from the 30% formulation. Complete drug release from both formulations is observed after 45 minutes. Soluplus, while freely soluble in water, appears to dissolve relatively slowly in 0.1N HCl, significantly retarding the release of the drug.

The dissolution profiles of the Eudragit EPO formulations compared to pure isoniazid are given in Figure 5.14b. A similar trend to that observed for the Soluplus based formulations was also observed for the Eudragit EPO formulations with the most rapid drug release being observed for the 20% drug loaded formulation which had 100% drug release after just 5 minutes. The 30% drug loaded formulation achieved 100% drug release after 10 minutes. Eudragit EPO, whilst insoluble above pH 5 is extremely soluble in acidic media, thus leading to rapid release of the drug under these conditions. It can also be seen that the 20% formulations have very similar release profile to that of the pure drug alone. The aim of the study was to produce taste-masked extrudates which did not adversely affect the release of the drug. On the basis of the dissolution results the Eudragit EPO extrudates are the most promising formulations, in particular the 20% w/w drug loaded formulation.
Figure 5.14 – Dissolution profile of (a) Soluplus/isoniazid extrudates and (b) Eudragit E-PO/isoniazid extrudates compared to the dissolution profile of pure isoniazid in 900 mL 0.1N HCl.
5.4.5.2 Taste Assessment

The taste of the formulations was assessed using two methods i.e. drug release under simulated oral conditions and the Insent electronic tongue.

5.4.5.2.1 Solubility Studies

The saturated solubility of isoniazid was measured in both distilled water and SSF (pH 7.4) at 37 ± 0.5°C. The saturated solubility of isoniazid in distilled water was found to be 1.92 ± 0.07 g/mL. The saturated solubility of isoniazid in SSF was found to be significantly higher at 2.94 ± 0.03 g/mL. The solubility of a drug is intrinsically linked to its taste, as only drug particles in solution can interact with the taste buds and produce a taste response.

5.4.5.2.2 Drug Release under Simulated Oral Conditions

Biorelevant dissolution testing was carried in SSF out to assess the amount of drug that would likely be released in the oral cavity in vivo. Typical dissolution experiments are carried out in 900 mL of liquid; however this is not representative of the conditions in the oral cavity. A more representative volume of fluid for the oral cavity would be 3-5 mL, however this small volume does not allow for a sufficient amount of liquid to be removed for analysis at each time point. In order to have a sufficient volume to allow sampling during the experiment the volume of SSF was increased tenfold to 50 mL and an amount of formulation equal to 10 unit doses of drug was added to keep the desired dilution factor of 1 dose unit in 5 mL.

The results are shown in Figure 5.15. It can be seen that extremely rapid drug release is observed for pure isoniazid with 100% of the drug being released after 30 seconds. The high solubility of isoniazid in both distilled water and SSF coupled with rapid dissolution of isoniazid under simulated oral conditions indicate that this will be a challenging drug to taste-mask as it can easily come into contact with the taste buds and produce a bitter taste response. The amount of drug released was compared to the IC$_{50}$ value obtained from BATA testing of 80.94 mM and EC$_{50}$ obtained from human testing of 259 mM (discussed in Chapter 3). The human EC$_{50}$ value is higher than the total possible drug release from the formulations, however, as previously discussed, paediatric patients are often more sensitive to the bitter taste of pharmaceuticals than adults. Therefore the rat IC$_{50}$ can be useful as a ‘worst case scenario’ measure of aversiveness.

The Eudragit E-PO extrudates showed very little drug release up to 5 minutes with drug release remaining well below the IC$_{50}$ value at all time points. This indicates that the taste of isoniazid is likely to be well masked by these formulations. The 20% Soluplus formulation significantly retards the release of the drug remaining below the IC$_{50}$ for up to 4 minutes,
while the 30% Soluplus formulation exceeds the IC50 value after just 1.5 minutes, indicating that this is likely to be the most aversive formulation.

Figure 5.15 – Dissolution profile of isoniazid and formulations in simulated salivary fluid (SSF) at pH 7.4 and 37°C. Dashed line indicates IC50 value of isoniazid obtained from BATA testing (80.94 mM).

5.4.5.2.3 Electronic Tongue
The taste of the formulations was also assessed using the Insent electronic tongue. As noted in section 5.3.2.10.4, the electronic tongue can only assess the taste of liquids, thus for solid dosage forms it is necessary to carry out a pre-dissolution step. The formulations were stirred in 10 mM potassium chloride solution for 1 minute before being filtered. The taste of this resultant liquid was then assessed using the electronic tongue. Principal component analysis was used to build a map from the sensor responses of the four sensors used for taste assessment (Figure 5.16). Solutions of pure isoniazid are located on the left hand side of the map. Euclidean distances were calculated to determine the differences between the pure drug, placebos, physical mixtures and extrudates, these are given in Table 5.11. The greater the Euclidean distance between the pure drug and the formulation, the greater the difference in taste.
Figure 5.16 – Principal Component Analysis (PCA) of sensor responses after 1 minute. The PCA map is built using output values of 3 bitter taste sensors (AC0, AN0, C00) and astringency sensor (AE1).

Table 5.11 – Euclidean distances from each formulation and placebo to pure isoniazid. Values are calculated from cluster centres. The greater the Euclidean distance between the pure drug and formulation the greater the difference in taste.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drug Loading (%w/w)</th>
<th>Placebo</th>
<th>Physical Mixture</th>
<th>Extrudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluplus</td>
<td>20</td>
<td>12.27</td>
<td>1.87</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>13.93</td>
<td>0.77</td>
<td>0.72</td>
</tr>
<tr>
<td>Eudragit E-PO</td>
<td>20</td>
<td>5.42</td>
<td>5.63</td>
<td>8.97</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.41</td>
<td>5.60</td>
<td>8.76</td>
</tr>
</tbody>
</table>

Based on the drug release from the formulations discussed in section 5.4.5.2.2 it would be expected that the Eudragit E-PO based extrudates would have a greater Euclidean distance from pure isoniazid than the Soluplus extrudates and this is indeed the case. Euclidean distances for the Soluplus based extrudates decrease from 4.51 for the 20% drug loaded extrudates to 0.72 for the 30% drug loaded extrudates in accordance with the increase in drug release from these formulations after 1 minute. The physical mixtures of Soluplus and isoniazid show smaller Euclidean distances than the extrudates, indicating that processing by HME improves the taste-masking efficiency of the polymer. As expected the greatest
Euclidean distance is observed for the placebo formulations. Soluplus is a water soluble polymer meaning it can readily dissolve in the oral cavity, allowing release of the drug. The DSC and PXRD analysis of the extrudates indicates that the Soluplus based formulations are amorphous. Amorphous solid dispersions have long been used to increase the dissolution rate of drugs. The amorphous nature of the drug, coupled with the high water solubility of Soluplus leads to a large amount of drug being released in the oral cavity and thus poor taste-masking compared to the Eudragit E-PO based formulations.

The Eudragit E-PO based formulations show a better taste-masking efficiency which is to be expected given that the drug release from these formulations under simulated oral conditions is significantly lower than that of Soluplus formulations. The greatest overall taste-masking efficiency is observed for the 20% drug loaded Eudragit E-PO extrudates which, again, is to be expected given that this is the formulation with the lowest amount of drug release after 1 minute. The physical mixtures showed significantly lower Euclidean distances than the corresponding extrudates, demonstrating the usefulness of HME as a technique for taste-masking.

5.5 DISCUSSION
In this study, hot melt extrusion was investigated as a processing technique for the production of taste-masked formulations of isoniazid and rifampicin. Two polymers with different physico-chemical properties, Soluplus and Eudragit E-PO were chosen as carriers for the drugs. Eudragit E-PO has already been widely used for taste-masking due to its selective release properties, while Soluplus has not been studied in this regard but provides a useful comparator of a polymer that should release the drug reasonably efficiently.

The theoretical miscibility of the drugs and polymers chosen was calculated using Hansen Solubility Parameters. The solubility parameter model suggests that compounds with similar δi values will be miscible. It is reported that compounds with a Δδi < 7MPa1/2 are likely to be miscible whereas compounds with Δδi > 10MPa1/2 are likely to be immiscible. This is due to the fact that the energy of mixing from intramolecular interactions is balanced with the energy of mixing from intermolecular interactions. Thus, solubility parameters provide a simple method for rational selection of polymeric carriers for solid dispersions. Isoniazid and rifampicin were both found to be theoretically miscible with the polymers chosen for this study.

As hot melt extrusion is a thermal technique, pre-formulation studies were carried out to assess the thermal properties of the drugs and polymeric carriers. Investigation of isoniazid
and rifampicin using DSC and TGA demonstrated that the T_m and T_d of the drugs were very similar, with degradation occurring almost immediately after melting. Typically hot melt extrusion is carried out above the melting point of the drug, however solid dispersions can be successfully formed by hot melt extrusion at temperatures below the melting point of the drug. Qi et al.\textsuperscript{175} produced solid dispersions of paracetamol with Eudragit E-PO using a maximum processing temperature of 140°C, which is well below the T_m of paracetamol form I (used in the study) of 170°C. Similarly, Chokshi et al.\textsuperscript{271} produced solid dispersions of indomethacin with a range of polymers including Eudragit E-PO and Poloxamer 188 at temperatures up to 100°C below the melting point of the drug. Thus it can be seen that solid dispersions can be formed below the melting point of the drug, though appropriate polymer selection is key.

Solid dispersions of isoniazid with both Soluplus and Eudragit E-PO were successfully formed at 140°C and 130°C respectively, well below the T_m of the drug (170.83 ± 0.03°C). Unfortunately when extruding rifampicin with either of these polymers solid dispersions could not be formed without degradation of the drug taking place. Investigation of the heat sensitivity of the physical mixtures of rifampicin and either Soluplus or Eudragit E-PO by TGA indicated that thermal degradation of the sample was not occurring. This, coupled with the extremely high torque values observed during the experiment indicates that the degradation is likely as a result of mechanical forces. Another possibility is that instead of being degraded the drug loss is as a result of the drug coating the inside of the extruder and getting stuck, however given the high torque value and the fact that rifampicin could be successfully extruded when combined with isoniazid (Chapter 6) it is most likely that the drug loss is as a result of degradation.

Sharma et al.\textsuperscript{272} have produced hot melt extruded formulations of rifampicin and Eudragit E-PO with 50% drug load using a processing temperature of 110°C and a screw speed of 40 rpm, however they pre-plasticised the polymer blend with 5% triethyl citrate. Triethyl citrate is generally recognised as safe (GRAS)\textsuperscript{183} however it is reported as having an aversive bitter taste\textsuperscript{273}, thus it was decided not to use it in this instance as it may adversely affect the taste of the final dosage form. It is also important to note that children can be more sensitive to excipient exposure than adults, thus when formulating paediatric medicines, keeping excipients to a minimum is key.\textsuperscript{1} On this basis it was decided to focus on only the isoniazid formulations for the remainder of this study. The extrusion of rifampicin was investigated again in Chapter 6 for the production of the final fixed dose combination formulation.
Analysis of the Soluplus based isoniazid extrudates using DSC and PXRD indicated that the drug was present in the amorphous form in the formulations. Typically, to produce amorphous solid dispersions the mixture is processed at temperatures higher than the $T_m$ of the drug which ensures complete conversion of the crystalline drug to the amorphous form.\textsuperscript{274} However, in this case conversion of the drug to the amorphous form is seen at temperatures over 30°C below the $T_m$ of the drug. This is most likely due to isoniazid being solubilised in the molten Soluplus during the extrusion process. For the 30% drug loaded extrudate a cold crystallisation is observed on heating. This may reflect the drug content exceeding the loading at which miscibility is stable, at least in terms of recrystallization induced by temperature ramping under the conditions used here.

Conversely, for the Eudragit E-PO formulations little or no interaction is observed between the drug and polymer with the drug remaining in the crystalline state in the formulation. As isoniazid is a borderline BCS Class I/III drug solubility is not an issue.\textsuperscript{231} Therefore the production of an amorphous solid dispersion is not essential to improve the bioavailability of this drug. In fact, having the drug in the crystalline state may be advantageous as it does not have the inherent instability associated with having the drug in the amorphous state.\textsuperscript{275}

The data obtained from \textit{in vitro} drug release studies demonstrates that, in the case of these formulations, having the drug in the amorphous form does not confer any improvement in dissolution behaviour. Rapid dissolution of pure isoniazid is seen in 0.1N HCl, with 100% of the drug being released within 5 minutes. For the Soluplus based formulations, complete drug release from both formulations is observed after 45 minutes. A lag phase is observed in the first five minutes of dissolution of these formulations. This may be due to hydrogens of the hydrazine group of isoniazid forming hydrogen bonds with the carbonyl groups of either the caprolactam or acetate group of Soluplus, retarding the initial release of the drug. Hydrophilic polymers such as Soluplus have been reported to form gel matrices which can retard the release of drug from the formulation\textsuperscript{276}, which may also be a contributory factor in this case.

The Eudragit E-PO formulations show much more rapid drug release, with the 20% and 30% drug loaded formulations exhibiting 100% drug release within 5 and 15 minutes respectively. Eudragit E-PO, whilst insoluble above pH 5 is extremely soluble in acidic media, thus leading to rapid release of the drug under these conditions. While Eudragit E-PO also contains carbonyl groups which could form hydrogen bonding interactions with isoniazid, they are
significantly more sterically hindered than those found in Soluplus, which may explain why no lag phase is observed for the formulations.

Isoniazid is highly soluble in both water and SSF. In order for a drug to interact with the taste buds it must be in solution, thus a highly soluble drug can be more challenging to taste-mask. Drug release under simulated oral conditions demonstrated that isoniazid undergoes extremely rapid dissolution in SSF, however the formulations manage to retard the release of the drug. The difference in drug release between the Soluplus and Eudragit E-PO based formulations can be attributed to the difference in water solubility of the polymers. As mentioned previously, Soluplus is a highly water soluble polymer which allows it to dissolve rapidly in the oral cavity and thus release a large amount of drug. The DSC and PXRD analysis of the extrudates indicates that the Soluplus based formulations are amorphous. The amorphous nature of the drug, coupled with the high water solubility of Soluplus leads to a large amount of drug being released in the oral cavity and thus poor taste-masking. Eudragit E-PO on the other hand is only soluble below pH 5 meaning it is poorly soluble in the oral cavity, leading to very little drug release from these formulations. It is important to remember however that Soluplus was chosen for this study as a comparator polymer which would release the drug reasonably efficiently in the stomach and in fact demonstrated a better taste-masking effect than expected.

As discussed in Chapter 4, the assessment of solid formulations using the electronic tongue can be challenging as a pre-dissolution step is required. One of the issues with the suggested method is that it uses one ‘dose unit’ of formulation in 100 mL, which is not representative of the amount of saliva that would be present in the mouth. In order to make the test more biorelevant in this study, an amount of formulation equivalent to one ‘dose unit’ in 5 mL was used. In addition, prior to assessing the formulations, an amount of isoniazid equivalent to the amount contained in the formulations was assessed to ensure that a sensor response significantly different to that elicited by pure water was observed.

Assessment of the formulations using the electronic tongue also found the Eudragit E-PO formulations to have the best taste-masking efficiency. The rank order of formulations in terms of aversiveness was found to be the same for both taste assessment methods, with the 20% Eudragit E-PO being the least aversive and 30% Soluplus being the most aversive. The mutual validation of the two methods is particularly encouraging as it demonstrates the utility of both for formulation development.
5.6 CONCLUSIONS
In summary, HME was successfully used to produce polymeric formulations of isoniazid with drug loadings of up to 30%. Unfortunately polymeric formulations of rifampicin could not be formed, however these will be further investigated in Chapter 6. Rapid *in vitro* release of isoniazid is observed from Eudragit E-PO formulations at pH 1.2, particularly the 20% drug loaded formulation which has a release profile similar to that of isoniazid alone. *In vitro* taste assessment of the formulations indicated that the bitter taste of isoniazid was most effectively masked by Eudragit E-PO due to the fact that Eudragit E-PO is insoluble above pH 5, thus preventing release of the drug in the mouth. Overall, the 20% drug loaded Eudragit E-PO formulation is the most promising as it successfully masks the bitter taste of isoniazid without adversely affecting the release profile and, by extension the bioavailability of the drug.
CHAPTER 6

Formulation of a Dispersible Tablet
Containing Isoniazid and Rifampicin
Suitable for Paediatric Administration
CHAPTER 6

6.1 INTRODUCTION
As discussed in Chapter 1, FDCs have been recommended by the WHO for the treatment of TB since 1994.\textsuperscript{61} There are a number of advantages to using FDCs for the treatment of paediatric TB, namely: (i) the tablet burden on the patient is reduced and dose calculations are simplified\textsuperscript{63}; (ii) monotherapy is prevented\textsuperscript{61} and (iii) the efficiency of the drug supply system is improved. However, uptake of FDCs has generally been quite poor due to a number of factors such as perceived inferiority of treatment, potential side effects, higher cost and, in the case of paediatric formulations, lack of dose flexibility.\textsuperscript{65}

In this chapter the production of a polymeric FDC containing isoniazid and rifampicin by HME was investigated. Eudragit E-PO was chosen as the polymeric carrier based on the promising results obtained in Chapter 5. The dose ratio of isoniazid to rifampicin was set at 1:1.5, as recommended for paediatric formulations.\textsuperscript{66}

Historically, impaired bioavailability of rifampicin from FDCs has been a common issue.\textsuperscript{278} A number of reasons for this have been postulated including poor quality of the formulations, adsorption of the drug by excipients, decomposition of the drug within the formulations and \textit{in situ} decomposition of the drug within the acid environment of the stomach in the presence of isoniazid.\textsuperscript{279} As of yet there is no definitive answer however the \textit{in situ} decomposition of rifampicin in the acidic environment of the stomach in the presence of isoniazid is suggested to be the most plausible. In this study the degradation of rifampicin in acidic media was investigated both alone and in the presence of isoniazid to ensure that the co-formulation of the drugs was not leading to increased degradation of rifampicin.

The production of child-friendly formulations of TB medicines is particularly challenging due to the high required dosages of these drugs. Conventional solid dosage forms such as tablets or capsules are unsuitable as the size of the final dosage form would be quite large and thus difficult or impossible for a child to swallow. In recent years a variety of novel solid dosage forms for paediatric administration have been developed such as multiparticulates, mini-tablets, chewable tablets and orodispersible tablets. Unfortunately the high doses, and by extension the large amount of extrudate, that would have to be incorporated into the formulation make these dosage forms unfeasible for these drugs. A dispersible tablet formulation was chosen as the most suitable for paediatric administration of these drugs as it allows for a large amount of drug to be incorporated into the formulation which can then be dispersed in water and easily swallowed by the child.
As discussed in Chapter 1, in 2016 two novel paediatric dispersible tablet FDCs were released by the Stop TB Alliance and their partners. The first contains isoniazid (50 mg), rifampicin (75 mg) and pyrazinamide (150 mg) and is intended for use in the intensive phase of treatment while the second contains isoniazid (50 mg) and rifampicin (75 mg) and is intended for use in the continuation phase of treatment. The exact components of these formulations have not been released however they are described as having a fruit flavour suggesting that a flavouring agent has been used.

This work aims to address the issue of taste masking these drugs by coating the drugs with a taste masking polymer rather than simply overwhelming the taste with a potent flavouring agent or sweetener. Therefore, in this study the feasibility of producing a taste-masked polymeric FDC containing isoniazid and rifampicin which could subsequently be incorporated into a dispersible tablet suitable for paediatric administration was investigated.

6.2 AIMS AND OBJECTIVES
The aim of the work described in this chapter was to produce a taste-masked polymeric formulation containing isoniazid and rifampicin and use this to produce a dispersible fixed dose combination tablet which would be suitable for paediatric administration.

6.3 MATERIALS AND METHODS

6.3.1 Materials
Isoniazid, potassium chloride, sodium chloride, potassium phosphate monobasic, sodium hydroxide, calcium chloride, potassium hydroxide, citric acid, sodium bicarbonate and tartaric acid were obtained from Sigma Aldrich (UK). Starch 1500 was obtained from Colorcon (Dartford, UK). Sodium stearyl fumarate was kindly donated by JRS Pharma (Rosenberg, Germany). Rifampicin was obtained from Fagron UK Ltd (Newcastle, UK). Soluplus was kindly donated by BASF (Ludwigshafen, Germany). Eudragit E-PO was obtained from Röhm GmbH & Co. (Sontheim/Brenz, Germany). Hydrochloric acid was obtained from Fisher Chemicals (Loughborough, UK). Distilled water was used for all experiments. All substances were used as received unless otherwise stated.

6.3.2 Methods
6.3.2.1 Hot Melt Extrusion
Hot-melt extrudates were prepared using a Thermo Scientific Process 11 co-rotating twin screw extruder (ThermoScientific, UK) fitted with a round die (die diameter: 2mm). Isoniazid, rifampicin and Eudragit E-PO were blended in a 20:30:50 ratio and extruded at 130°C and 40
rpm. Post extrusion the extrudates were cut into ~1 cm pieces using a Pharma 11 Varicut Pelletizer (Thermo Fisher Scientific, U.K.).

6.3.2.2 Determination of Drug Loading
A 0.1% w/v solution of each extrudate was prepared by dissolving 10 mg of ground extrudate in 10 mL methanol. The mixtures were sonicated to ensure complete dissolution of the extrudate. 4 mL of the resulting solution was added to 96 mL dibasic sodium phosphate solution. Drug content of samples was analysed by HPLC (section 6.3.2.11).

6.3.2.3 Differential Scanning Calorimetry
Standard DSC and modulated temperature DSC (MTDSC) thermograms were recorded using a TA Instruments Q2000 calorimeter (TA Instruments, New Castle, Delaware, USA). For analysis 4-6 mg of sample was accurately weighed and sealed in an aluminium pan (hermetic and pinhole pans used as appropriate). For standard DSC, samples were heated under nitrogen gas (flow rate 50 mL/min) at a rate of 10°C/min. For MTDSC, samples were heated under nitrogen gas (flow rate 50 mL/min) at a rate of 2°C/min, amplitude ± 0.212°C and a period of 40 seconds. Calibration was performed using n-octadecane, benzoic acid, indium, and tin. Samples were analysed in triplicate unless stated otherwise. Data analysis was carried out with TA Universal Analysis software.

6.3.2.4 Thermogravimetric Analysis
Thermogravimetric analysis (TGA) was carried out using a TA Instruments Hi-Res 2950 thermogravimetric analyser (TA Instruments, New Castle, Delaware, USA). Samples were analysed in open aluminium pans with a heating rate of 10°C/min. Data analysis was carried out with TA Universal Analysis software.

6.3.2.5 Powder X-Ray Diffraction
Powder X-Ray Diffraction (PXRD) was carried out using a Rigaku 600 Miniflex diffractometer (Rigaku, Tokyo, Japan), CuKα radiation, operating power: 40mV, 15mA. Patterns were recorded over the 2θ range 3° - 40° at a scan rate of 5°/min.

6.3.2.6 Hot Stage Microscopy
Hot stage microscopy was carried out using a system composed of a Mettler Toledo FP90 Central Processor, Mettler Toledo FP82HT Hot Stage and a Leica DM 2700M Microscope. Samples were heated at a rate of 2°C/min to mimic the conditions experienced during DSC testing.
6.3.2.7 In Vitro Dissolution Testing

In vitro drug release was tested using British Pharmacopoeia method 2.9.3 dissolution test for solid dosage forms with the aid of a Pharmatest PTWS 120D dissolution apparatus (Pharma Test Apparatebau AG, Hainburg, Germany). Samples were loaded into Size 4 gelatine capsules (Qualicaps Europe SA, Madrid) and placed into a basket. The basket was lowered into a dissolution bath containing 900 mL 0.1N HCl (pH 1.2 ± 0.2) at 37.0 ± 0.5°C with a rotation speed of 100 rpm. At predetermined time intervals, a 10 mL sample was withdrawn from each vessel and replaced with the same amount of fresh media to ensure constant volume of medium within the vessel. Samples were diluted 1:25 with dibasic sodium phosphate solution. The concentration of isoniazid in the dissolution medium was measured by HPLC (section 6.3.2.11), while the concentration of rifampicin was measured by determining UV-Vis absorbance at 475 nm. Experiments were carried out in triplicate and dissolution profiles are plotted as mean percentage drug release ± standard deviation.

6.3.2.8 Determination of Rifampicin Degradation in Acidic Media

The degradation of both rifampicin and rifampicin from the FDC formulation on dissolution in acidic media was determined with the aid of a Pharmatest PTWS 120D dissolution apparatus (Pharma Test Apparatebau AG, Hainburg, Germany). Samples were loaded into Size 4 gelatine capsules (Qualicaps Europe SA, Madrid) and placed into a basket. The basket was lowered into a dissolution bath containing 900 mL 0.1N HCl (pH 1.2 ± 0.2) at 37 ± 0.5°C with a rotation speed of 100 rpm. At predetermined time intervals, a 10 mL sample was withdrawn from each vessel and replaced with the same amount of fresh media to ensure constant volume of medium within the vessel. The concentration of rifampicin was measured by determining UV-Vis absorbance at 475 nm.

6.3.2.9 Solubility Studies

The saturated solubility of rifampicin was assessed in both distilled water and simulated salivary fluid (SSF) at pH 7.4 adapted from Hughes et al.260 (Table 6.1). An excess amount of pure drug was added to 30 mL distilled water or SSF at 37 ± 0.5°C and shaken for 72h until equilibrium was reached. The samples were then filtered using 0.22µm filters (Merck-Millipore, Cork, Ireland) and the absorbance of the samples recorded at 475 nm using a Jenway 6305 UV-Vis Spectrophotometer (Bibby Scientific, Staffordshire, UK).
Table 6.1 – Composition of Simulated Salivary Fluid.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium phosphate monobasic</td>
<td>12 mM</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>40 mM</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>to pH 7.4</td>
</tr>
<tr>
<td>Distilled water</td>
<td>to 1 L</td>
</tr>
</tbody>
</table>

6.3.2.10 Simulation of Drug Release in Oral Cavity

Dissolution testing was carried out to simulate the dissolution of pure drugs and the polymeric formulations in the oral cavity. Exposure of the drug/formulations to oral cavity conditions was mimicked by 5 minutes drug contact with 5 mL of SSF at pH 7.4. The unit dose of the fixed dose combination was set at 50 mg isoniazid and 75 mg rifampicin (to match the currently available paediatric FDC formulation), however to have sufficient volume to allow sampling during the experiment an amount of formulation equal to 10 unit doses of drug was added to 50 mL SSF. 3 mL samples were manually withdrawn at 0.5, 1, 1.5, 2, 2.5, 3 and 5 minutes and replaced with fresh media. A temperature of 37 ± 0.5°C and a rotational speed of 50 rpm were maintained during the experiment. Samples were filtered through 0.22 µm filters (Merck-Millipore, Cork, Ireland) and analysed by HPLC (section 6.3.2.11). Drug release was compared to the human EC50 and rodent IC50 values for isoniazid and rifampicin to determine whether the formulations were likely to be aversive or not.

6.3.2.11 High Performance Liquid Chromatography (HPLC)

6.3.2.11.1 Equipment

Samples were analysed using a HPLC system comprised of 1200 series G1322A degasser, G1311A quaternary pump, G1329A ASL autosampler, G1316A diode array detector and ChemStation for LC and LC/MS systems software (all Agilent Technologies, Santa Clara, California, USA). A Zorbax Eclipse XDB-C18, 4.6 x 250mm, 5µm column (Agilent Technologies, Santa Clara, California, USA) was used for all separations.

6.3.2.11.2 Chromatographic Conditions

Separation was carried out according to USP Method for rifampicin and isoniazid capsules. The method parameters are given in Table 6.2 and the gradient program employed is given in Table 6.3.
Table 6.2 – HPLC parameters for separation of isoniazid and rifampicin as per USP method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>4.6 x 250mm containing 5µm base deactivated packing L1</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Buffer solution: 1.4 g of dibasic sodium phosphate in 1 litre water (adjusted to pH 6.8 with phosphoric acid). Solution A: Buffer:ACN (96:4) Solution B: Buffer:ACN (45:55)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>238nm</td>
</tr>
<tr>
<td>Column temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20µL</td>
</tr>
</tbody>
</table>

Table 6.3 – Gradient program as per USP method.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% Solution A</th>
<th>% Solution B</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>Equilibration</td>
</tr>
<tr>
<td>0 – 5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5 – 6</td>
<td>100</td>
<td>0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>6 – 15</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

6.3.2.12 Production of Dispersible Fixed Dose Combination Tablets

6.3.2.12.1 Powder Blend Production
The FDC extrudate was milled to a powder using a Fritsch Planetary Mill Pulverisette 5 (Fritsch GmbH, Idar – Oberstein, Germany). Extrudates were milled at a speed of 200 rpm for 10 minutes. The resulting milled extrudate was combined with 15% w/w Starch 1500 and 2% sodium stearyl fumarate before being mixed for 10 minutes with the aid of rolling blender (Pascall Engineering, Sussex, UK).

6.3.2.12.2 Angle of Repose
The angle of repose was measured using the fixed height cone method, i.e. the powder was poured into a plugged funnel held at a fixed height above a flat base. The plug was removed and the powder blend allowed to flow through. The diameter of the powder cone was measured and the angle of repose calculated using the following equation:
The angle of repose was used to classify the flow properties of the powder based on Carr’s classification (Table 6.4).\textsuperscript{281}

**Table 6.4 – Angle of repose as an indicator of powder flow properties.\textsuperscript{281}**

<table>
<thead>
<tr>
<th>Angle of repose (degrees)</th>
<th>Type of flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 – 30</td>
<td>Excellent</td>
</tr>
<tr>
<td>31 – 35</td>
<td>Good</td>
</tr>
<tr>
<td>36 – 40</td>
<td>Fair (flow aid not needed)</td>
</tr>
<tr>
<td>41 – 45</td>
<td>Passable (flow aid may be needed)</td>
</tr>
<tr>
<td>46 – 55</td>
<td>Poor (agitation or vibration needed)</td>
</tr>
<tr>
<td>56 – 65</td>
<td>Very poor</td>
</tr>
<tr>
<td>&gt;66</td>
<td>Very, very poor</td>
</tr>
</tbody>
</table>

**6.3.2.12.3 Tapped Bulk Density**

The tapped bulk density of the powder blend was measured using a Tapped Density Volumeter (Copley Scientific Ltd., Nottingham, UK). A known mass of the powder blend was placed in the Tapped Density Volumeter. The powder blend was tapped until no further change in mass was observed. The compressibility index and Hausner ratio of the blend were calculated according to the following equations:

\[
\text{Compressibility Index} = 100 \times \frac{V_0 - V_f}{V_0} \quad \text{Equation 6.2}
\]

\[
\text{Hausner ratio} = \frac{V_0}{V_f} \quad \text{Equation 6.3}
\]

where \(V_0\) refers to initial volume of the powder blend and \(V_f\) refers to the final volume of the powder blend. The relationship between compressibility index and Hausner ratio is given in Table 6.5.
Table 6.5 – Relationship between compressibility index, Hausner ratio and powder flowability.

<table>
<thead>
<tr>
<th>Compressibility Index (%)</th>
<th>Type of Flow</th>
<th>Hausner Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 10</td>
<td>Excellent</td>
<td>1.0 – 1.11</td>
</tr>
<tr>
<td>11 – 15</td>
<td>Good</td>
<td>1.12 – 1.18</td>
</tr>
<tr>
<td>16 – 20</td>
<td>Fair (flow aid not needed)</td>
<td>1.19 – 1.25</td>
</tr>
<tr>
<td>21 – 25</td>
<td>Passable (flow aid may be needed)</td>
<td>1.26 – 1.34</td>
</tr>
<tr>
<td>26 – 31</td>
<td>Poor (agitation or vibration needed)</td>
<td>1.35 – 1.45</td>
</tr>
<tr>
<td>32 – 37</td>
<td>Very poor</td>
<td>1.46 – 1.59</td>
</tr>
<tr>
<td>&gt;38</td>
<td>Very, very poor</td>
<td>&gt;1.60</td>
</tr>
</tbody>
</table>

6.3.2.12.4 Tablet Production

Tablets were produced with the aid of a Manesty Type F3 Tablet Press (Manesty, Liverpool, UK) fitted with 10 mm diameter round, flat faced punches.

6.3.2.13 Tablet Characterisation

6.2.3.13.1 Weight Uniformity

The weight uniformity of the tablets was assessed by individually weighing twenty tablets using an XS205 Dual Range Analytical Balance (Mettler Toledo, Leicester, United Kingdom). The weight of each tablet was compared to the tablet mean weight to ensure that no more than two tablets had a deviation greater ± 5% of the mean weight as per Ph. Eur. 2.9.5.

6.3.2.13.2 Hardness Testing

The hardness of ten individual tablets was assessed by measuring the force required to break each tablet with the aid of a Tablet Hardness Tester (Copley Scientific Ltd., Nottingham, UK).

6.2.2.13.3 Thickness Testing

The thickness of ten individual tablets was assessed using a digital calliper (Mitutoyo, Kawasaki, Japan). The thickness of each tablet was compared to the tablet mean thickness to ensure that there was no more than ± 5% deviation from the mean.

6.3.2.13.4 Disintegration Testing

Disintegration testing was carried out with the aid of ZT-34 disintegration testing apparatus (Copley Scientific Ltd., Nottingham, UK). Six tablets were placed in the apparatus and immersed in a beaker containing 900 mL purified water at 22°C. Samples were moved up and
down at a rate of 30 cycles per minute until complete disintegration of the tablets was observed (Ph. Eur. 2.9.1).

6.3.2.13.5 Content Uniformity
The content uniformity of ten individual tablets was measured by dissolving each tablet in 40 mL methanol. 4 mL of the resulting solution was added to 96 mL dibasic sodium phosphate solution. Drug content of samples was analysed by HPLC (section 6.3.2.11).

6.3.2.13.6 Dissolution Testing
In vitro drug release was tested using British Pharmacopoeia method 2.9.3 dissolution test for solid dosage forms with the aid of Pharmatest PTWS 120D dissolution apparatus (Pharma Test Apparatebau AG, Hainburg, Germany). One tablet was placed into a basket. The basket was lowered in a dissolution bath containing 900 mL 0.1N HCl (pH 1.2 ± 0.2) at 37.0 ± 0.5°C with a rotation speed of 100 rpm. At predetermined time intervals, a 10 mL sample was withdrawn from each vessel and replaced with the same amount of fresh media to ensure constant volume of medium within the vessel. Samples were diluted 1:25 with dibasic sodium phosphate solution. The concentration of isoniazid in the dissolution medium was measured by HPLC (section 6.3.2.11), while the concentration of rifampicin was measured by determining UV-Vis absorbance at 475 nm.

6.3.2.13.7 Taste Assessment
The taste-masking efficiency of the tablets were assessed by mimicking the manner in which they would be administered and measuring the drug release from the tablet under these conditions. As they are intended to be dispersible tablets, it is envisaged that the tablets will be dispersed in an amount of water which the patient will then drink. One tablet was placed in 10 mL deionised water at room temperature and gently shaken. 0.5 mL samples were manually withdrawn at 0.5, 1, 1.5, 2, 2.5, 3 and 5 minutes and replaced with fresh media. Samples were filtered through 0.22 µm filters (Merck-Millipore, Cork, Ireland) and analysed by HPLC (section 6.3.2.11). Drug release was compared to the human EC$_{50}$ and rodent IC$_{50}$ values for isoniazid and rifampicin to determine whether the formulations were likely to be aversive or not.

6.4 RESULTS

6.4.1 Hot Melt Extrusion
Eudragit E-PO was chosen as the polymeric carrier for the FDC due to the excellent extrusion and taste-masking properties it demonstrated for isoniazid (Chapter 5). Extrusion was initially attempted at 130°C and a screw speed of 50 rpm however the torque was too high
for extrusion to occur. Increasing the temperature to 140°C and decreasing the screw speed to 40 rpm allowed for successful extrusion of the mixture. Dark red (due to the red colour of rifampicin) extrudates were formed which had a uniform diameter and flowed well from the extruder.

6.4.2 Physicochemical Characterisation

The drug loading of the FDC extrudate was assessed to ensure that the drug was not being degraded during the extrusion process (Table 6.6). It can be seen that the drug loading is within the expected range for FDC formulation, demonstrating that neither of the drugs are being degraded during the extrusion process.

Table 6.6 – Drug loading of fixed dose combination formulation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Expected Drug Load (% w/w)</th>
<th>Actual Drug Load (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>20</td>
<td>20.07 ± 0.43</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>30</td>
<td>30.70 ± 0.74</td>
</tr>
</tbody>
</table>

The DSC thermogram of the FDC formulation is given in Figure 6.1. A $T_g$ related to the glass transition of Eudragit E-PO is observed at 47.5 ± 0.5°C. This is lower than the $T_g$ of Eudragit E-PO alone (55.3°C ± 0.5°C) indicating that the polymer is being plasticised by the drug mixture. A melting peak is also observed at 165.0 ± 0.2°C. This is significantly lower than the melting point of either isoniazid or rifampicin which were found to have melting points of 173.1 ± 0.2°C and 181.2 ± 2.9°C respectively.
Figure 6.1 – Modulated temperature DSC (2°C/min, 0.212°C/40s) of fixed dose combination formulation (pinhole pans).

The PXRD patterns of FDC formulation, pure isoniazid and pure rifampicin are given in Figure 6.2. Similar to the formulations containing only isoniazid, the pattern is characterised by a 'bowed' baseline with many sharp crystalline peaks. The peaks in the PXRD pattern of the FDC formulation were compared to those of the pure drugs to attempt to determine whether both drugs or just one drug was present in the crystalline form. The characteristic peaks of isoniazid occur at 15.6°, 16.8°, 19.6° and 25.2°. Isoniazid is not reported to have any known polymorphs.283 Rifampicin is reported to have two crystal forms, Form I and II, and an amorphous form.265 Form I and Form II can be differentiated in PXRD by characteristic peaks at 13.65° and 14.35° for Form I and 9.93° and 11.10° for Form II.266 As discussed in Chapter 5, the rifampicin used in these formulations is predominantly composed of Form II but also contains small amounts of Form I and amorphous rifampicin.
(a) 13.65°, 25.2°

(b)
In terms of peaks in the FDC diffraction pattern which could be attributed to crystalline isoniazid, the only one is that at 25.2°. It is possible that some isoniazid is present in the amorphous form which would lead to a reduction in peak intensity. It is also important to remember that isoniazid w/w is the smallest component of the formulation and thus, due to its lower concentration some of its peaks may be masked.

They characteristic peaks of Form II rifampicin at 9.93° and 11.10° are not observed in the diffraction pattern of the FDC extrudate, however there is a peak at 13.65° indicating that Form I rifampicin is present in the sample. The high shear force and temperatures used in HME processing are capable of inducing polymorphic change, thus it is likely that rifampicin has been converted from predominantly Form II to Form I during processing. It is also possible that some rifampicin has also been converted to the amorphous form. Polymorphic changes can often lead to a change in a drug’s dissolution rate, however it has been shown that the polymorphic form of rifampicin does not significantly affect the dissolution rate. The key factor affecting dissolution of both rifampicin Form I and Form II is particle size.\(^{284}\)

Overall, based on the results obtained from PXRD, the physical state of the drugs contained within the FDC extrudate cannot be conclusively determined. It is likely that a combination of crystalline and amorphous isoniazid and rifampicin is present, with the crystalline rifampicin being mostly in Form I.

Figure 6.2 – Powder X-Ray diffraction pattern of (a) fixed dose combination formulation and (b) isoniazid and (c) rifampicin.
As discussed above, the $T_m$ of the FDC is significantly lower than that of either rifampicin or isoniazid. DSC experiments were carried out to determine the melting point depression of various ratios of isoniazid and rifampicin. The DSC thermograms of the isoniazid – rifampicin physical mixtures are given in Figure 6.3. It can be seen that a single melt is observed rather than two melting peaks, suggesting that, the drugs are interacting in such a way as to influence their respective melting behaviour.

*Figure 6.3 – DSC thermograms (2°C/min) of isoniazid – rifampicin physical mixtures (pinhole pans).*

In an attempt to understand what is happening during the melting process, hot stage microscopy was carried out. Isoniazid and rifampicin were first assessed separately to determine their individual crystal shapes. Isoniazid crystals are relatively large, between 250 µm to 1 mm in length, with a cylindrical shape (Figure 6.4a). Rifampicin crystals on the other hand are much smaller, approximately 25 – 30 µm in length with a less defined morphology and distinct red colour (Figure 6.4b).
A physical mixture containing 40% isoniazid and 60% rifampicin, the ratio of drug contained within FDC formulation and the ratio which had the greatest melting point depression, was assessed using the hot stage microscope. Figure 6.5 shows what happens as the sample is heated. At the beginning of the heating process (Figure 6.5a) a large isoniazid crystal is observed, surrounded by smaller crystals of rifampicin. As the sample is heated, rifampicin begins to melt at approximately 150°C (Figure 6.5b). Smaller crystals of rifampicin located on the surface of the isoniazid crystal begin to melt and erode away the surface of the crystal (Figure 6.5c). The sample then continues to melt, with complete melting of the isoniazid
crystal being observed at approximately 164.4°C, similar to the $T_m$ obtained from the DSC experiments (Figure 6.5e).
Figure 6.5 – Hot stage microscopy images of the heating of isoniazid – rifampicin 40:60 physical mixture (2°C/min).

The results of these experiments show that, on heating, rifampicin melts first and isoniazid then melts into it. This explains why a single melting point is observed for the FDC extrudate in DSC experiments.

6.4.3 In Vitro Dissolution Testing

In vitro drug release studies were carried out to determine the rate of drug release from these formulations under GI conditions. Figure 6.6 shows the dissolution profile of the FDC formulation compared to that of the pure drugs. As discussed in Chapter 5, rapid dissolution of pure isoniazid is seen in 0.1N HCl, with 100% of the drug being released within 5 minutes. Rifampicin on the other hand, while soluble in 0.1N HCl, has a significantly slower dissolution rate. Initial experiments were carried out using USP Apparatus Type II (paddle) with metal sinkers however it was observed that, on dissolution of the capsule, rifampicin floated to the surface of the media and formed clumps. This indicates that rifampicin has poor wetting behaviour which is likely as a result of its hydrophobic nature (logP = 3.719). To keep the rifampicin submerged for the duration of the experiment USP Apparatus Type I (basket) was used. In spite of this significant dissolution of rifampicin is not observed until after 10 minutes, suggesting that the rifampicin is clumping within the basket, however due to the small mesh size of the basket this is not directly observable.
Figure 6.6 – Dissolution of isoniazid and rifampicin from FDC compared to pure isoniazid and rifampicin in 900 mL 0.1N HCl at 37°C.

Isoniazid is rapidly released from the formulation, with 100% dissolution within 10 minutes. Rifampicin is released more slowly from the formulation, with complete release occurring within 25 minutes. Rifampicin exhibits a biphasic release profile. 53.51 ± 3.71% of the rifampicin is released within the first 10 minutes of dissolution. The rate then slows significantly with approximately 4% being released in the following 5 minutes. The remaining amount of drug is then released over the course of 10 minutes. As mentioned in section 6.4.2 the dissolution rate of both forms of rifampicin is predominantly governed by the particle size of the drug. Thus it is likely that smaller particles of rifampicin contained within the polymer matrix dissolve more quickly followed by the larger particles leading to the biphasic release profile observed here.

It has been reported in the literature that rifampicin undergoes degradation in acidic media, and that this degradation may be increased by the presence of isoniazid.\textsuperscript{285} To determine if this was happening in this case dissolution testing was carried out in which the FDC and pure rifampicin were dissolved in 900 mL 0.1N HCl and monitored for 2 hours to determine if degradation was occurring.

Figure 6.7 shows that while up to 10% of the rifampicin does degrade over time, the amount of degradation of rifampicin from the FDC is not statistically significantly different to that of
rifampicin alone. This indicates that degradation is only occurring as a result of the acidic media and not due to the presence of isoniazid.

Figure 6.7 – Percentage degradation over time of pure rifampicin compared to rifampicin from FDC formulation in acidic media (900 mL 0.1N HCl at 37°C).

6.4.4 Taste Assessment

6.4.4.1 Solubility Studies

The saturated solubility of rifampicin was measured in both distilled water and SSF (pH 7.4) at 37 ± 0.5°C. The saturated solubility of rifampicin in distilled water was found to be 2.4 ± 0.07 mg/mL. The saturated solubility of rifampicin in SSF was found to be significantly higher at 3.71 ± 0.27 mg/mL. The saturated solubility of isoniazid in distilled water and SSF was determined in Chapter 5 and found to be 1.92 ± 0.07 g/mL and 2.94 ± 0.03 g/mL respectively. It can be seen that isoniazid is much more water soluble than rifampicin which suggests it may be more challenging to taste-mask, however it is important to remember that rifampicin has much lower EC50 and IC50 values (Chapter 3) than isoniazid. Thus, in spite of its low solubility rifampicin may prove more difficult to mask due to its more intense bitterness.

6.4.4.2 Drug Release Under Simulated Oral Conditions

Biorelevant dissolution testing was carried in SSF out to assess the amount of drug that would likely be released in the oral cavity in vivo. The release profile of isoniazid from the FDC
compared to pure isoniazid is given in Figure 6.8a. The release profile of rifampicin from the FDC compared to pure rifampicin in given in Figure 6.8b.

It can be seen from Figure 6.8a that isoniazid is extremely soluble in SSF with 100% of the pure drug dissolving within 30 seconds. The human EC$_{50}$ and rat IC$_{50}$ for isoniazid are 259 mM and 80.94 mM. As discussed in Chapter 3, this is a relatively high EC$_{50}$ meaning that the drug is mildly bitter. The EC$_{50}$ is high in relation to the total amount of drug which could potentially be released from the formulation and thus in this experiment the EC$_{50}$ was not reached. The FDC formulation significantly retards the release of the drug with only 4.04 ± 0.95 % being released within 5 minutes remaining well below the IC$_{50}$ and of course the EC$_{50}$.

Rifampicin is a much more bitter drug than isoniazid as evidenced by its much lower IC$_{50}$ and EC$_{50}$ values, which are 1.31 mM and 3.6 mM respectively. It is also significantly less soluble in SSF than isoniazid with only 3.44 ± 0.17% being dissolved within 5 minutes. Due to the low water solubility of rifampicin it does not exceed either the IC$_{50}$ and EC$_{50}$ during the experiment. The formulation significantly retards the release of rifampicin with a maximum drug release of 0.21 ± 0.01% after 5 minutes. These results indicate that the taste of both isoniazid and rifampicin are well masked in this formulation.

Please note that, due to the adsorption of rifampicin onto the electronic tongue sensors as discussed in Chapter 4, these formulations were not assessed using the electronic tongue.
Figure 6.8 – Dissolution profile of (a) pure isoniazid and isoniazid from FDC formulation and (b) pure rifampicin and rifampicin from FDC formulation in simulated salivary fluid (SSF) at pH 7.4 and 37°C.

6.4.5 Tablet Production

6.4.5.1 Powder Blend Characterisation

The flowability of the powder blend was assessed via two methods, namely by measuring the angle of repose and the tapped density. The angle of repose was found to be 43.2°. The initial volume $V_0$ of the powder blend was 55 cm$^3$ and the final volume $V_f$ was 44 cm$^3$. The
Hausner ratio was found to be 1.27 while the compressibility index was found to be 21%. These findings indicate that the powder blend has ‘passable’ flow properties.

6.4.5.2 Tablet Production

FDC dispersible tablets were produced using 10mm flat faced punches. The final composition of the tablets is given in Table 6.7. Tablets of uniform shape were produced and were easily expelled from the die without sticking. The total amount of drug loaded extrudate used was 83% w/w per tablet. The drug loading of the extrudate was 20% w/w isoniazid and 30% w/w rifampicin which is equivalent to an overall drug loading of 16.6% isoniazid and 24.9% rifampicin.

![Fixed dose combination dispersible tablets produced by direct compression.](image)

**Figure 6.9 – Fixed dose combination dispersible tablets produced by direct compression.**

**Table 6.7 – Composition of fixed dose combination dispersible tablet.**

<table>
<thead>
<tr>
<th>Component</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug loaded extrudate</td>
<td>83</td>
</tr>
<tr>
<td>Starch 1500</td>
<td>15</td>
</tr>
<tr>
<td>Sodium stearyl fumarate</td>
<td>2</td>
</tr>
</tbody>
</table>

The weight uniformity of the tablets was assessed by individually weighing twenty tablets and comparing the weight of each tablet to the tablet mean weight to ensure that no more
than two tablets had a deviation greater ±5% of the mean weight as per *Ph. Eur. 2.9.5*. The mean weight of the tablets was found to be 291.12 ± 4.20 mg. The weight of each tablet and the percentage deviation from the mean are given in Table 6.8. It can be seen that none of the tablets deviate from the mean weight by more than ±5%, with the greatest deviation being -2.19%.

**Table 6.8 – Weight of each tablet assessed and percentage deviation from mean tablet weight (291.12 ± 4.20 mg).**

<table>
<thead>
<tr>
<th>Tablet Number</th>
<th>Tablet Weight (mg)</th>
<th>Deviation from Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>292.66</td>
<td>0.53</td>
</tr>
<tr>
<td>2</td>
<td>290.35</td>
<td>-0.26</td>
</tr>
<tr>
<td>3</td>
<td>294.72</td>
<td>1.24</td>
</tr>
<tr>
<td>4</td>
<td>292.25</td>
<td>0.39</td>
</tr>
<tr>
<td>5</td>
<td>296.57</td>
<td>1.87</td>
</tr>
<tr>
<td>6</td>
<td>295.75</td>
<td>1.59</td>
</tr>
<tr>
<td>7</td>
<td>285.39</td>
<td>-1.97</td>
</tr>
<tr>
<td>8</td>
<td>295.24</td>
<td>1.42</td>
</tr>
<tr>
<td>9</td>
<td>293.92</td>
<td>0.96</td>
</tr>
<tr>
<td>10</td>
<td>291.03</td>
<td>-0.03</td>
</tr>
<tr>
<td>11</td>
<td>296.26</td>
<td>1.77</td>
</tr>
<tr>
<td>12</td>
<td>284.75</td>
<td>-2.19</td>
</tr>
<tr>
<td>13</td>
<td>289.23</td>
<td>-0.65</td>
</tr>
<tr>
<td>14</td>
<td>286.50</td>
<td>-1.59</td>
</tr>
<tr>
<td>15</td>
<td>285.98</td>
<td>-1.77</td>
</tr>
<tr>
<td>16</td>
<td>292.45</td>
<td>0.46</td>
</tr>
<tr>
<td>17</td>
<td>293.12</td>
<td>0.69</td>
</tr>
<tr>
<td>18</td>
<td>285.47</td>
<td>-1.94</td>
</tr>
<tr>
<td>19</td>
<td>295.50</td>
<td>1.51</td>
</tr>
<tr>
<td>20</td>
<td>285.23</td>
<td>-2.02</td>
</tr>
</tbody>
</table>

The thickness of ten individual tablets was assessed and compared to the tablet mean thickness to ensure no tablets deviated by more than ±5%. The mean tablet thickness was...
3.88 ± 0.03 mm. It can be seen from Table 6.9 that none of the tablets deviate by more than ± 5%, with the greatest deviation being + 1.46%.

**Table 6.9 – Thickness of each tablet assessed and deviation from mean tablet thickness (3.88 ± 0.03 mm)**

<table>
<thead>
<tr>
<th>Tablet Number</th>
<th>Tablet Thickness (mm)</th>
<th>Deviation from Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.85</td>
<td>-0.85</td>
</tr>
<tr>
<td>2</td>
<td>3.89</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>3.86</td>
<td>-0.59</td>
</tr>
<tr>
<td>4</td>
<td>3.89</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>3.89</td>
<td>0.18</td>
</tr>
<tr>
<td>6</td>
<td>3.94</td>
<td>1.46</td>
</tr>
<tr>
<td>7</td>
<td>3.90</td>
<td>0.44</td>
</tr>
<tr>
<td>8</td>
<td>3.86</td>
<td>-0.59</td>
</tr>
<tr>
<td>9</td>
<td>3.87</td>
<td>-0.33</td>
</tr>
<tr>
<td>10</td>
<td>3.88</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

The hardness of ten individual tablets was assessed by measuring the force required to break each tablet with the aid of a Tablet Hardness Tester. Oral tablets should typically have a hardness in excess of 40 N. The mean tablet hardness was found to be 44.9 ± 1.63 N. In addition to hardness testing, tablets are usually subjected to friability testing to assess the tendency for a tablet to chip, crumble or break post compression. For tablets which have a unit weight of less than 650 mg an amount of tablets equal to 6.5 g should be assessed. Unfortunately there was an insufficient number of tablets produced to carry out this test while still having enough to carry out disintegration testing, content uniformity, dissolution and taste assessment experiments.

Disintegration testing was carried out in deionised water to ensure that the tablet disintegrated within an appropriate time limit. The British Pharmacopoeia states that dispersible tablets must fully disintegrate within 3 minutes. The FDC tablets were found to completely disintegrate within 1 minute 10 seconds, well within the specified time limits.

The content uniformity of the tablets was assessed by fully dissolving ten tablets in methanol and determining the concentration of drug in the resultant solution by HPLC. The average content was found to be 49.75 mg isoniazid and 71.34 mg rifampicin. The British
Pharmacopoeia states that a preparation complies if the individual content of not more than one tablet falls outside the limits of 85 – 115% of the average content, and none is out the limits of 75 – 125% of the average content. The results of this experiment are given in Table 6.10. All of the tablets tested fall well within the limits of 85 – 115%, thus the content uniformity of the tablets is acceptable.

Table 6.10 – Content uniformity of fixed dose combination tablets.

<table>
<thead>
<tr>
<th>Tablet Number</th>
<th>Isoniazid</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected Mass (mg)</td>
<td>Actual Mass (mg)</td>
</tr>
<tr>
<td>1</td>
<td>48.01</td>
<td>49.87</td>
</tr>
<tr>
<td>2</td>
<td>48.47</td>
<td>50.27</td>
</tr>
<tr>
<td>3</td>
<td>47.34</td>
<td>50.78</td>
</tr>
<tr>
<td>4</td>
<td>47.91</td>
<td>48.48</td>
</tr>
<tr>
<td>5</td>
<td>47.36</td>
<td>49.28</td>
</tr>
<tr>
<td>6</td>
<td>48.22</td>
<td>49.59</td>
</tr>
<tr>
<td>7</td>
<td>48.60</td>
<td>50.03</td>
</tr>
<tr>
<td>8</td>
<td>47.34</td>
<td>48.86</td>
</tr>
<tr>
<td>9</td>
<td>48.19</td>
<td>49.65</td>
</tr>
<tr>
<td>10</td>
<td>47.51</td>
<td>50.72</td>
</tr>
</tbody>
</table>

Overall, the tablets meet the specifications for solid dosage forms, falling well within the accepted range for each test.

6.4.5.3 Dissolution Testing

Dissolution testing was carried out to assess the rate of drug release from the tablets and to determine the differences in dissolution profile between the FDC extrudates and the final dosage form. When carrying out dissolution testing of the FDC extrudates the extrudates were cut into pellets of approximately 1 cm length, however for tabletting the extrudates were milled into a powder with particle size of approximately 200 – 250 µm, thus it would be expected that the dissolution rate would increase in line with the increase in surface area.

The results of this experiment are given in Figure 6.10. Similar to what is observed for the FDC extrudates (Figure 6.6) we see rapid dissolution of isoniazid with 100% release within 10
minutes. The biphasic release profile observed for rifampicin from the extrudate is not observed for the dispersible tablet. This can be attributed to the more uniform particle size of the milled rifampicin contained in the dispersible tablet compared to the FDC extrudate. The overall release rate of rifampicin is not improved with complete release again being observed within 25 minutes. The release rate of rifampicin from the dispersible tablet is improved compared to pure rifampicin. This is encouraging as studies have suggested that the variable dissolution rate of rifampicin may contribute to the poor bioavailability observed for some rifampicin containing formulations. Therefore the increase in the release rate coupled with the more uniform release profile observed for rifampicin from the dispersible tablet suggests that good bioavailability of rifampicin should be observed. However, bioequivalence testing would need to be carried out to confirm this.

Figure 6.10 – Dissolution of isoniazid and rifampicin from dispersible tablet compared to pure isoniazid and rifampicin in 900 mL 0.1N HCl at 37°C.

6.4.5.4 Taste Assessment
The taste-masking efficiency of the dispersible tablet formulation was assessed by determining the amount of drug released when the tablet is dispersed in water for administration. The EMA recommends that dispersible tablets for paediatric use should be dispersed in 5 mL water for patients under 5 years old and 10 mL water for patients 5 years and older. In this experiment one tablet was dispersed in 10 mL of water to form a red suspension (Figure 6.11).
Figure 6.11 – Appearance of dispersible tablet after dispersion in 10 mL water.

The amount of drug released into the water over 5 minutes was determined by HPLC. 5 minutes was chosen as the ‘worst case scenario’ time point but it is envisaged that patient would typically ingest the solution between 30 seconds to 1 minute after dispersion. The results are given in Figure 6.12.

Isoniazid is extremely water soluble with 100% of the pure drug dissolving within 30 seconds. One dispersible tablet contains 48.32 ± 0.93 mg isoniazid which if fully dissolved in the 10 mL of water used would give a concentration of approximately 34.72 mM. As discussed in section 6.4.4.2 the human EC$_{50}$ and the rodent IC$_{50}$ of isoniazid are 259 mM and 80.94 mM respectively, thus these values cannot be reached in the experiment. After 1 minute approximately 40% of the drug is released from the formulation, with 100% release occurring within 3 minutes. In terms of taste-masking it would of course be preferable if very little drug was released however, in the case of isoniazid, having a large amount of drug release may not be detrimental to the overall taste given the relatively high EC$_{50}$ of the drug. Compared to the release of isoniazid from the pelletised FDC extrudate in SSF we see a huge increase in drug release from 4.04 ± 0.95 % to 100% after 5 minutes. This is due to the increased surface area of the milled extrudate in the tablet compared to the pelletised extrudate coupled with the high water solubility of isoniazid.
Rifampicin is significantly less water soluble than isoniazid as evidenced by the fact that only $3.31 \pm 0.78\%$ of the pure drug is dissolved in water after 5 minutes. After 1 minute $7.26 \pm 0.51\%$ of rifampicin is released from the tablet. The maximum amount of drug released after 5 minutes is $22.17 \pm 5.12\%$. This increase in dissolution compared to pure rifampicin can again be attributed to the increased surface area of the milled FDC extrudate contained in
the tablet. Despite the relatively high amount of drug release from the formulation the EC$_{50}$ is never exceeded and the IC$_{50}$ is only exceeded after 5 minutes suggesting that the bitter taste of rifampicin may still be reasonably well masked by the formulation, especially if the solution is ingested rapidly after dispersion.

6.5 DISCUSSION

In this study, hot melt extrusion was used to produce a polymeric taste-masked fixed dose combination containing isoniazid and rifampicin. Based on the extremely encouraging results obtained previously with Eudragit E-PO for isoniazid, it was decided to use this polymer for the fixed dose combination. It should be noted that when extrusion of rifampicin alone with Eudragit E-PO was attempted, extrudates could not successfully be formed and it appeared that rifampicin was being mechanically degraded during the extrusion process (Chapter 5).

Initially it was decided to investigate whether the combination of isoniazid, rifampicin and Eudragit E-PO would extrude more easily with the option to add plasticizers if it would not. Isoniazid, rifampicin and Eudragit E-PO were combined in 20:30:50 ratio and extruded at 130°C and 40 rpm. The combination of isoniazid and rifampicin with Eudragit E-PO extruded much more easily than rifampicin alone with Eudragit E-PO and smooth, uniform extrudates were successfully formed. Drug loading experiments demonstrated that neither of the drugs were being degraded during the extrusion process.

Physicochemical characterisation of the FDC extrudates was carried out using DSC and PXRD. When analysed by DSC the FDC extrudate exhibits a $T_g$ at 47.5 ± 0.5°C, which can be attributed to the glass transition of Eudragit E-PO, and a single $T_m$ at 165.0 ± 0.2°C which is lower than that of either isoniazid (173.1 ± 0.2°C) or rifampicin (181.2 ± 2.9°C). The PXRD of the FDC extrudate was compared to the patterns of pure isoniazid and rifampicin. The presence of peaks at 13.65° and 25.2° indicate the presence of Form I rifampicin and isoniazid respectively. The lack of peaks which could be attributed to Form II rifampicin suggests that the drug undergoes a polymorphic change on processing.

Dissolution testing of pure rifampicin in 0.1N HCl was first attempted using USP Apparatus Type II (paddle). It was observed that on dissolution of the gelatine capsule, rifampicin floated to the surface of the media and formed clumps. Changing the dissolution apparatus to Type I ensured that the rifampicin remained submerged for the duration of the experiment, however a lag phase was still observed which is attributed to the poor wetting behaviour and clumping of the drug in the media. Agrawal et al. encountered a similar issue when investigating the dissolution rate of various commercial samples of rifampicin. This
issue was overcome by pre-mixing the rifampicin for dissolution with glass beads in the dissolution vessel and dispersing in a small amount (5 mL) of dissolution medium. The remaining dissolution medium was then slowly added to avoid formation of the aggregate. Using this technique a lag phase was not observed. This technique, while useful in the mentioned study as it was directly comparing a number of powders, was not used in the present study as it would not allow for results to be directly compared to dissolution results for the FDC extrudates or dispersible tablets given that they would not need to be dispersed with glass beads or dispersed in dissolution media before the experiment.

A biphasic release profile is observed for rifampicin from the FDC extrudate which can be attributed to the different dissolution rates of larger and smaller rifampicin particles. When the extrudate is milled and incorporated into the dispersible tablet the release rate is more uniform, likely as a result of the more uniform particle size distribution of rifampicin in the milled powder.

Historically impaired bioavailability of rifampicin from FDCs has been a common issue. A number of reasons for this have been postulated including adsorption of the drug by excipients, decomposition of the drug within the formulations and in situ decomposition of the drug within the stomach in the presence of isoniazid. As of yet there is no definitive answer however the in situ decomposition of rifampicin in the stomach in the presence of isoniazid is suggested to be the most plausible. Rifampicin is also known to undergo degradation in acidic conditions without the presence of isoniazid.

To determine whether the presence of isoniazid in the FDC extrudate was causing enhanced degradation of rifampicin, dissolution testing was carried out in which the FDC and pure rifampicin was dissolved in 900 mL 0.1N HCl and monitored for 2 hours. The degradation of rifampicin from the FDC was shown to not be significantly different to the degradation of rifampicin alone indicating that, in this case, degradation was only occurring as a result of the acidic media and not the presence of isoniazid.

The taste-masking efficiency of the FDC extrudate was assessed under simulated oral conditions. Isoniazid is readily soluble in SSF with 100% dissolution occurring within 30 seconds while rifampicin is significantly less soluble with approximately 3.5% dissolution after 5 minutes. The formulation significantly retards the release of both isoniazid and rifampicin suggesting that the taste of the drug is well masked by the extrudate.
To assess the taste-masking efficiency of the dispersible tablet the way in which the tablet would be administered was mimicked. The tablet was dispersed in 10 mL water and the amount of both drugs released was determined. The release of both isoniazid and rifampicin from the dispersible tablet was higher than that released by the FDC extrudate as a result of the higher surface area of the milled extrudate. In particular, the release of rifampicin from the dispersible tablet is higher than the dissolution of rifampicin alone. As discussed previously, the dissolution rate of rifampicin is predominantly governed by its particle size. Thus, the smaller particle size of the milled extruded rifampicin contained in the dispersible tablet leads to much more rapid dissolution of the drug compared to the original powder. However, given the dilution factor the human EC50 values for both drugs are not reached suggesting that the dispersion is unlikely to be aversive. A flavouring or sweetening agent could be added to the tablet to improve the taste of the overall dispersion; however this would need to be carefully chosen. For example, reducing sugars such as lactose, galactose, glucose or maltose could not be used as they have been shown to form hydrazones with isoniazid which are poorly absorbed, leading to reduced bioavailability of isoniazid.

The Stop TB Alliance FDC dispersible tablet containing isoniazid and rifampicin was released in early 2016. Contact was made with the Stop TB Alliance to try and obtain information regarding the composition of this formulation however this was unsuccessful. It is described as having a ‘fruit flavour that is palatable for children’ suggesting that a flavouring agent has been used. No studies have been published regarding the palatability of these formulations. It would be interesting to compare the palatability of these formulations with that of the formulations produced in this study using the BATA model and a human taste panel to determine which formulation technique i.e. addition of flavours or HME was more effective.

6.6 CONCLUSIONS

In summary, HME was successfully used to produce a polymeric FDC extrudate containing 50% w/w isoniazid and rifampicin in a dose ratio of 1:1.5. Analysis of the in vitro dissolution rate of the FDC extrudate demonstrated that both drugs were rapidly released from the FDC formulation with complete drug release observed after 10 minutes for isoniazid and 25 minutes for rifampicin. The taste-masking efficiency of the formulation was determined by assessing the amount of drug released under simulated oral conditions. The extrudate effectively masked the taste of both drugs with the release of each remaining well below the human EC50 at all times.
The extrudate was then milled and blended with starch 1500 and sodium stearyl fumarate to produce dispersible tablets. The weight uniformity, thickness, hardness, disintegration time and content uniformity of the tablets were investigated and found to conform to the specifications for solid dosage forms, falling well within the accepted ranges for each test. The overall dissolution rate of the drugs was not increased from the dispersible tablet. However the taste-masking efficiency was found to be less than that of the FDC extrudate, due to the greater surface area of the milled powder contained in the tablet compared to the extrudate. Overall this study has demonstrated the utility of hot melt extrusion for taste-masking, however further work will need to be done to determine how to use extruded formulations to make child-friendly dosage forms without adversely affecting the taste-masking efficiency.
CHAPTER 7

Conclusions and Recommendations for
Future Work
CHAPTER 7

7.1 INTRODUCTION

As discussed in Chapter 1, the overall aim of the research detailed in this thesis was to investigate the feasibility of using hot melt extrusion to mask the bitter taste of anti-tuberculosis drugs and develop a prototype paediatric-appropriate, taste-masked FDC formulation containing isoniazid and rifampicin. To achieve this, the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was assessed using a human taste panel to determine EC\textsubscript{50} values for each drug. In addition to this the ability of two non-human models, i.e. the rodent BATA model and Insent electronic tongue, to detect and assess the taste of these drugs, was investigated. HME was used to a produce polymeric taste masked extrudate of isoniazid and a fixed dose combination extrudate containing isoniazid and rifampicin. Finally, the feasibility of incorporating this extrudate into dispersible tablet suitable for paediatric administration was assessed. In the following sections, the results obtained will be summarised and generally discussed. Key findings along with any potential future work will also be highlighted.

7.2 IN VIVO TASTE ASSESSMENT OF ISONIAZID, RIFAMPCIN, PYRAZINAMIDE AND ETHAMBUTOL

7.2.1 Summary of Results

As mentioned above, one of the key aims of this study was to produce a taste masked formulation suitable for the treatment of paediatric TB. However, in order to successfully mask the taste of a drug, the taste profile of the drug must first be understood. An extensive literature search was carried out to try and ascertain the bitterness levels of these drugs. It is reported in the literature that isoniazid, rifampicin, pyrazinamide and ethambutol, have aversive tastes. For example, a study by the Hong Kong Chest Service assessing the acceptability of isoniazid, rifampicin and pyrazinamide FDCs compared to individual tablets states ‘difficulty in swallowing and can lead to nausea or even vomiting, especially when the pyrazinamide is prescribed as uncoated, bitter-tasting tablets’.\textsuperscript{206} While Rutherford \textit{et al.}\textsuperscript{208}, when investigating adherence to isoniazid preventative therapy in Indonesian children, found that all survey respondents mentioned difficulty in the administration of isoniazid due to its bitter taste. However, despite this, there is no quantitative data available as to the bitterness levels of these drugs.

To address this lack of quantitative data, a human taste panel was carried out to assess the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride. 20 participants assessed the taste of four concentrations of each drug using the ‘swirl and spit’ technique described in Chapter 3. The EC\textsubscript{50} values for isoniazid, rifampicin, pyrazinamide and
ethambutol were found to be 259 mM, 3.6 mM, 158 mM and 27 mM respectively. Quinine, a standard bitter reference compound, has an EC₅₀ of 0.257 mM. Therefore, compared to quinine we can say that these four drugs are mildly bitter. However it is important to remember that, when considering EC₅₀ values, the dose of a drug must also be taken into consideration. Like many antibiotics the dose required for each of these drugs is quite high, therefore it is still possible that the EC₅₀ will be reached within the mouth and thus taste masking will be required. In addition to this, children have been found to be more sensitive to the bitter taste of drugs than adults, therefore a drug that is considered mildly bitter by adults may be considered more aversive by a child.

Six concentrations of each of these drugs (the four concentrations used in human testing plus two extra) were assessed using the rodent BATA model. Rats were found to be more sensitive to the taste of isoniazid, rifampicin and ethambutol dihydrochloride than humans with IC₅₀ values obtained of 80.94 mM, 1.31 mM and 13.63 mM respectively. However the calculated IC₅₀ and EC₅₀ values were found to be within one half-log unit of molar concentration of each other, which is in line with previous studies. Rats were found to be relatively insensitive to the taste of pyrazinamide compared to human subjects with only the highest concentration of drug eliciting a response significantly different to that of water. As such, an IC₅₀ value could not be calculated for this drug. The reasons for this lack of sensitivity are unclear due the complex nature of taste perception, however it is most likely due to genetic differences between rats and humans. The greater sensitivity of rats to isoniazid, rifampicin and ethambutol may prove to be particularly useful for the assessment of paediatric formulations containing these drugs as it has been shown that children are more sensitive to bitter tastes than adults are.

Overall this research has determined human EC₅₀ values for the four most commonly used anti-tuberculosis drugs and has shown that the rodent BATA model may be useful for taste assessment of formulations containing isoniazid, rifampicin and/or ethambutol and, in particular, paediatric formulations due to the greater sensitivity of rats to the bitter taste of these drugs.

7.2.2 Recommendations for Future Work

In the present study participants were not screened on the basis of their ethnicities and details of their ethnicities were not recorded or analysed. However, as discussed in Chapter 3 ethnic differences in taste perception do exist. For example incidence rates of taste blindness to PTC/PROP vary around the world ranging from ~3% in western Africa to 40% in India, while in the adult Caucasian population of North America roughly 30% are taste blind.
Chapter 7

It would be interesting to assess whether there were ethnic differences in taste perception of these drugs although this would require a much larger sample size.

With regard to the testing of these drugs using the BATA model, it would be interesting to assess higher concentrations of pyrazinamide to see if an IC\textsubscript{50} could be obtained. As the rats responded to the highest concentration of pyrazinamide it is likely that increasing the concentration further would allow construction of a dose response curve from which an IC\textsubscript{50} could be calculated. The rat oral LD50 of pyrazinamide is high at 3 g/kg.\textsuperscript{291} The highest concentration of pyrazinamide used in the current study is equivalent to 14 mg/mL, thus it is likely that this value could be increased significantly without causing undue harm to the rats. Although at higher concentrations solubility will become an issue which may necessitate the use of solubilising agents. Recently, work has been carried out within the group to establish whether solubilising agents such as glycol, propylene glycol, PEG-400 and ethanol can be detected by both rats and humans and if so, at what concentrations. The results of this work have yet to be published but it is possible that one of these solubilising agents may be suitable for use with pyrazinamide to allow greater concentrations of the drug to be tested and thus further investigate the rat taste response to pyrazinamide.

This thesis focused on the development of a fixed dose combination formulation, meaning that the drugs will be administered simultaneously, however in each of the taste assessment experiments the drugs were assessed individually. Assessment of combinations of these drugs in therapeutically relevant ratios would be useful as you could have a combined EC\textsubscript{50} or IC\textsubscript{50} value which could be used as a reference when assessing drug release from formulations.

There is significant debate about the use of IC\textsubscript{50} and EC\textsubscript{50} values as measures of aversiveness with the suggestion that a drug/formulation may be aversive at concentrations below the IC\textsubscript{50}/EC\textsubscript{50} for example the EC\textsubscript{75} or EC\textsubscript{90}. It may also be appropriate to choose the first concentration to elicit a significantly different response from water as the ‘aversiveness limit’. Further investigation is required to fully understand which is the most appropriate measure to use.

7.3 INVESTIGATION OF THE INSENT E-TONGUE FOR TASTE ASSESSMENT OF ISONIAZID, RIFAMPICIN, PYRAZINAMIDE AND ETHAMBUTOL

7.3.1 Summary of Results

The use of \textit{in vitro} tools for taste assessment offers many advantages over human/animal models such as lower cost, no ethical issues and potential higher throughput. However, in
order to be useful they must first be validated and the correlation between human responses and those of the in vitro tool determined. In Chapter 4 of this thesis the ability of the Insent TS-5000Z electronic tongue to detect and assess the taste of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride was investigated.

It was found that for isoniazid, rifampicin and pyrazinamide a non-linear response was obtained from the sensors and certain concentrations of each drug could not be differentiated from water. For isoniazid and pyrazinamide this erratic response can be attributed to the fact that isoniazid and pyrazinamide are not ionised under the experimental conditions and thus it is more challenging for these molecules to interact with the lipid membrane of the sensors. For rifampicin, higher concentrations of drug were found to elicit erratic responses from the electronic tongue. This may be for a number of reasons such as the threshold concentration for rifampicin being reached (i.e. the concentration above which the sensor becomes saturated can no longer accurately differentiate between solutions of drug), or that rifampicin, as a lipophilic molecule, partitions into the lipid membrane of the sensors. Conversely, ethambutol dihydrochloride was found to elicit a linear response from all sensors. This can be attributed to the fact that, as it is a hydrochloride salt, it will be fully ionised in aqueous solution, thus allowing the molecule to easily interact with the lipid membrane of the taste sensors.

The correlation between the responses obtained from the human taste panel and the electronic tongue was investigated for each drug by choosing the individual sensor for which the most linear response was observed and comparing these values to human taste panel scores for each concentration of drug. The correlation between human and electronic tongue responses for isoniazid, rifampicin and pyrazinamide were found to be 0.4368, 0.02291 and 0.79785 respectively. This suggests that the electronic tongue may not be ideal for assessment of formulations containing these drugs. An excellent correlation however was observed for ethambutol dihydrochloride with each sensor having a correlation coefficient in excess of 0.99, indicating that the electronic tongue will be useful for assessing formulations containing these drugs.

Overall this study demonstrated the limitations of using the electronic tongue for the assessment of these tuberculosis drugs and suggests that it cannot be used to replace in vivo methods of taste assessment for these drugs.
7.3.2 Recommendations for Future Work

As discussed in Chapter 4 one of the major issues affecting the detection of a drug by the electronic tongue is whether the drug is ionised in solution or not. It has been shown that altering the pH of solution to ensure a drug is ionised can improve the results obtained. In the present study this was not assessed as the conditions of the human/rodent experiments were being replicated. It would be interesting however to assess whether the detection of isoniazid could be improved by altering the pH of the test solution to ensure that the drug would be ionised. For pyrazinamide, given that it is a very weak base with a reported \( pK_a \) of 0.5, even at pH 1 only \(~24\%\) of the drug would be ionised, thus it is unlikely that changing the pH of the test solution would help. Also, the electronic tongue sensors have an operating range of pH 3 – 8, making it unfeasible to test pyrazinamide at pH 1.

As discussed above, it was found that rifampicin adsorbed onto the lipid membrane of the sensors. This is concerning as it suggests that this is something that can happen for all lipophilic drugs. The adsorption of rifampicin is easy to detect due to the highly coloured nature of the drug, however with other non-coloured drugs this may go undetected and lead to erroneous results. It would be useful to assess other known lipophilic compounds using the electronic tongue to determine whether this adsorption was a common occurrence. This could be done by assessing various concentrations of lipophilic drugs and then immediately carrying out a calibration test to determine whether the sensor responses were still within the accepted limits.

7.4 Production of Taste Masked Formulations of Isoniazid and Rifampicin by Hot Melt Extrusion

7.4.1 Summary of Results

The latter half of this thesis focused on investigating the utility of HME as a taste masking technique. In Chapter 5 the extrusion of rifampicin and isoniazid with two polymers i.e. Soluplus and Eudragit E-PO was investigated. The aim of this work was to produce taste masked polymeric formulations of these drugs which would undergo rapid dissolution in the stomach. The combinations of isoniazid/Soluplus and isoniazid/Eudragit E-PO extruded well and formulations with drug loadings of 20% and 30% w/w were produced. Conversely the combinations of rifampicin/Soluplus and rifampicin/Eudragit E-PO did not extrude well and mechanical degradation of the drug was observed during the extrusion process. The extrusion of rifampicin was investigated again in Chapter 6.
The physicochemical properties of the isoniazid loaded extrudates were investigated using PXRD and DSC. Physicochemical characterisation of the isoniazid loaded extrudates showed that, in the Soluplus formulations, isoniazid was present in the amorphous form, while in the Eudragit E-PO formulations the drug was present in the crystalline form. Isoniazid was found to exert a plasticising effect on Soluplus, as evidenced by the decrease in \( T_g \) of the polymeric formulations compared to Soluplus alone. Conversely, little or no interaction was observed between isoniazid and Eudragit E-PO.

The in vitro release of isoniazid from the polymeric formulations was investigated at pH 1.2. As mentioned above, the aim of this work was to produce formulations which would undergo rapid dissolution in the stomach. Isoniazid alone dissolves extremely rapidly at pH 1.2, with 100% of the drug being dissolved within 5 minutes. Soluplus was found to significantly retard the release of isoniazid, with complete dissolution of the drug taking 45 minutes to occur. Eudragit E-PO on the other hand did not significantly retard the release of the drug. Complete drug release was achieved from the 20% and 30% w/w formulations within 5 minutes and 10 minutes respectively. The 20% w/w formulation was particularly promising as it had a release profile very similar to that of isoniazid alone.

In vitro taste assessment of the formulations was carried out by assessing the amount of drug released from the formulations under simulated oral conditions and by using the electronic tongue. Both of these methods indicated that the that the bitter taste of isoniazid was most effectively masked by Eudragit E-PO. This is as a result of the different water solubilities of the polymers. Soluplus is a highly water soluble polymer\(^{170} \) whereas Eudragit E-PO is only soluble below pH 5.\(^{277} \) Again, it is important note however that Soluplus was chosen for this study as a comparator polymer which would release the drug reasonably efficiently in the stomach and in fact demonstrated a better taste masking effect than expected.

Overall, this study demonstrated the utility of HME as a taste masking technique. Of the formulations produced, the 20% drug loaded Eudragit E-PO formulation was the most promising as it successfully masked the bitter taste of isoniazid without adversely affecting the release profile and, by extension the bioavailability of the drug.

### 7.4.2 Recommendations for Future Work

The extrusion of rifampicin with Soluplus and Eudragit E-PO was unsuccessful as rifampicin was found to undergo mechanical degradation during the extrusion process. Plasticisers may be added to extrusion blends to improve the extrusion properties of the mix. Hot melt extruded formulations of rifampicin and Eudragit E-PO with 50% drug load have been
produced by pre-plasticising the polymer blend with 5% triethyl citrate.\textsuperscript{272} Triethyl citrate is reported as having an aversive bitter taste\textsuperscript{273}, therefore it would not be suitable for a taste masked formulation. Future work in this area could focus on investigating other plasticizers which may be more suitable and allow for successful extrusion of rifampicin with Soluplus or Eudragit E-PO. Any plasticizer used however must not have an aversive taste and must also be safe for use in paediatric formulations.

Extrusion of lipids has also been investigated for taste masking. For example, praziquantel was extruded with glyceryl tristearate and PEG 6000 to form taste masked extrudates with drug loadings of 50 – 70\%.\textsuperscript{292} Lipids are generally easily digested and have GRAS status making them particularly advantageous for use in paediatric formulations. It would be interesting to investigate the extrusion of isoniazid and rifampicin with various lipids to see if taste masked lipid based formulations of these drugs could be produced.

Additional work in this area could also investigate the extrusion of the other first line anti-tuberculosis drugs pyrazinamide and ethambutol dihydrochloride with either taste masking polymers or lipids.

The taste masking efficiency of formulations was assessed by determining the drug release under simulated oral conditions. For this an amount of formulation equivalent to one dose unit was exposed to 5mL SSF for up to 5 minutes. While this is a useful screening test, it has many limitations as it does not accurately model the conditions a formulation will experience in the oral cavity. Recently Teo \textit{et al.} have developed a dissolution method which more accurately represents the conditions in the oral cavity (Figure 7.1).\textsuperscript{293} In this technique the dosage form is placed into a specially adapted column of length 5 cm and inner diameter 5 mm. Meshes of 50 µm and 100 µm are placed at each end of the column to keep the sample in place. The column is then attached to a peristaltic pump which pumps simulated salivary fluid (SSF) through the column at a rate of 1 ml/min. The SSF is then collected at set time points and analysed for drug content by HPLC.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{oral_dissolution_setup.png}
\caption{Simulated oral dissolution apparatus developed by Teo \textit{et al.}}\textsuperscript{293}
\end{figure}
This technique more accurately represents the conditions in the oral cavity in terms of volume of saliva, saliva flow rate and hydrodynamics. The method has currently only been investigated for multiparticulate formulations. Future work could investigate the feasibility of using this technique for the assessment of larger extruded formulations and, if successful the isoniazid based extrudates could be assessed using this technique and the results compared to those detailed in Chapter 5 of this thesis.

7.5 Formulation of a Dispersible Tablet Containing Isoniazid and Rifampicin Suitable for Paediatric Administration

7.5.1 Summary of Results

In the final chapter of this thesis the extrusion of a fixed dose combination containing isoniazid and rifampicin was investigated. The ratio of isoniazid to rifampicin was set at 1:1.5 which is the ratio of these drugs used to treat paediatric TB. A final drug loading of 50% w/w was achieved which corresponded to 20% w/w isoniazid and 30% w/w rifampicin.

The physicochemical properties of the FDC extrudate were analysed using PXRD and DSC. PXRD analysis indicated the presence of both crystalline and amorphous isoniazid and rifampicin in the sample. DSC analysis of the FDC extrudate showed a $T_g$ at $47.5 \pm 0.5^\circ C$, lower than that of Eudragit E-PO alone ($55.3^\circ C \pm 0.5^\circ C$) indicating that the polymer was being plasticised by the drug mixture. A single sharp melting peak was also observed at $165.0 \pm 0.2^\circ C$, significantly lower than melting point of either isoniazid ($T_m = 173.1 \pm 0.2^\circ C$) or rifampicin ($T_m = 181.2 \pm 2.9^\circ C$) alone. PXRD analysis indicated the presence of both crystalline and amorphous isoniazid and rifampicin in the sample. Hot stage microscopy was carried out to try and understand what was happening during the melting process. It was observed that, on heating, rifampicin begins to melt at approximately $150^\circ C$. The molten rifampicin interacts with isoniazid causing it to melt, resulting in the melting point depression and single melting peak observed in DSC.

The in vitro dissolution rate of the formulation was measured at pH 1.2. Both drugs were rapidly released from the FDC formulation with complete drug release observed after 10 minutes for isoniazid and 25 minutes for rifampicin. Rifampicin was released from the FDC in a biphasic manner which is likely due to their being different particle sizes of rifampicin contained within the extrudate. Smaller particles dissolve more rapidly than their larger counterparts which results in a biphasic release pattern. It has been reported in the literature that rifampicin undergoes degradation in acidic media, and that this degradation may be increased by the presence of isoniazid. To determine whether this would be an issue with
the FDC extrudate, dissolution testing was carried out in which the FDC and pure rifampicin were dissolved in 900mL 0.1N HCl and monitored for 2 hours to determine if degradation was occurring. No statistically significant difference was observed between the degradation of rifampicin alone or rifampicin from the extrudate, suggesting that any degradation was only occurring as a result of the acidic media and not due to the presence of isoniazid.

The taste masking efficiency of the formulation was determined by assessing the amount of drug released under simulated oral conditions. The extrudate was found to effectively mask the taste of both drugs, with the release of each remaining well below the human EC$_{50}$ values for isoniazid and rifampicin at all times. These formulations were not analysed using the electronic tongue as rifampicin was found to adsorb onto the lipid membrane of the sensor which may lead to erroneous readings.

The hot melt extrudates as produced are not suitable for paediatric administration and as such efforts were made to determine a suitable final dosage form that they could be incorporated into. Conventional solid dosage forms such as a tablet or capsule were ruled out as, given the high dosages required of these drugs, the size of the final dosage form would be quite large and therefore difficult or impossible for a child to swallow. Novel solid paediatric dosage forms such as multiparticulates, mini-tablets, chewable tablets and orodispersible tablets were considered, however these were also ruled out as unsuitable given the high dosages required of these drugs. A dispersible tablet was chosen as the final dosage form as it would allow a large amount of extrudate to be incorporated while still being easy for the child to swallow as it would be dispersed in water before administration.

The extrudate was milled and blended with starch 1500 (disintigrant) and sodium stearyl fumarate (lubricant). This powder blend was used to produce dispersible tablets with a diameter of 10 cm and a mean weight of $291.12 \pm 4.20$ mg. The weight uniformity, thickness, hardness, disintegration time and content uniformity of the tablets were investigated and found to conform to the specifications for solid dosage forms, falling well within the accepted ranges for each test.

The in vitro dissolution rate and taste masking efficiency of the dispersible tablets were investigated and compared to the FDC extrudate. The overall dissolution rate of the drugs was not increased from the dispersible tablet compared to the extrudate, however, the biphasic release profile of rifampicin was not observed for the dispersible tablet. The taste masking efficiency of the dispersible tablet was found to be less than that of the FDC extrudate, with a significantly larger quantity of drug released due to the greater surface area
of the milled powder contained in the tablet compared to the extrudate. However, the drug release still remains below the EC$_{50}$ values for each drug suggesting that the formulation may still have an acceptable taste.

In summary, the work described in this chapter demonstrated the utility of hot melt extrusion for taste masking, however future work will need to be done to determine how to use extruded formulations to make child-friendly dosage forms without adversely affecting the taste masking efficiency.

**7.5.2 Recommendations for Future Work**

One of the challenges faced in this work was that the exact physical form of the drugs contained within the FDC extrudate could not be definitively elucidated. One option to further investigate this would be to use solid state NMR (SSNMR). Amorphous and crystalline drug can be distinguished in SSNMR by the fact that crystalline drug will have sharp, defined peaks while amorphous drug will have less defined, broader peaks. It would also allow rifampicin Form I and Form II to be distinguished as they have different characteristic peaks. For example, the C36 methyl group of Form I rifampicin displays a singlet in SSNMR, while in Form II the C36 methyl group displays as doublet, most likely as a result of long range dipolar coupling to nitrogen when in the Form II conformation.$^{266}$

The extrusion work described in this chapter could be built upon by investigating the extrudability of a 3 drug FDC containing isoniazid, rifampicin and pyrazinamide and a four drug FDC containing isoniazid, rifampicin, pyrazinamide and ethambutol. Eudragit E-PO could be used as a polymeric carrier for these formulations and other taste masking polymers or lipids could also be investigated.

Milling of the extrudate increased the particle size and thus the taste masking efficiency of the final formulation was compromised. In order to improve the taste of the final dosage form a sweetener or flavouring agent could be added. It is important that reducing sugars such as lactose, galactose, glucose or maltose are not used as they have been shown to form hydrazones with isoniazid which are poorly absorbed, leading to reduced bioavailability of isoniazid.$^{269}$ Future work to improve the palatability of the FDC formulation could be to identify suitable sweetening or flavouring agents to be added to the powder blend for tabletting. Any agent used would have to be GRAS and also approved for use in paediatric medicines.
As discussed in Chapter 6, the Stop TB Alliance FDC dispersible tablet containing isoniazid and rifampicin was released in early 2016. Contact was made with the Stop TB Alliance to try and obtain information regarding the composition of this formulation however this was unsuccessful. If possible, it would be very interesting to obtain samples of these formulations to compare the taste to the formulations prepared during the course of this thesis.

If a viable prototype formulation was developed, the taste could be assessed using the rodent BATA model. The taste of the Stop TB Alliance dispersible tablet could also be assessed using the BATA model for comparison. If BATA testing indicated that the prototype formulation exhibited good taste masking and was not aversive then the formulation could be assessed using a human taste panel, although it is important to note that formulations used for a human taste panel would need to be produced under GMP conditions.

7.6 CONCLUSIONS
Taste masking and taste assessment are very important to ensure that medicine will be acceptable to patients, particularly paediatric patients. This is especially true for diseases like TB which require very long treatment regimens. This thesis has described the production of a taste masked dosage form from initial palatability testing of the pure drug through to the development of a final taste masked prototype formulation.

Human taste panels remain the ‘gold-standard’ method for taste assessment, and using this technique the bitterness of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride has been quantified. However human taste panels can be challenging when developing paediatric dosage forms due to safety and ethical concerns. The potential utility of the BATA model as an alternative in vivo technique for taste evaluation of isoniazid, rifampicin and ethambutol containing medicines has been demonstrated. The electronic tongue was investigated as an in vitro technique which could be utilised as a screening tool for TB formulations, however its utility is limited to assessing formulations containing ethambutol dihydrochloride.

HME has been demonstrated to be a useful taste masking technique, achieving excellent taste masking results when an appropriate polymeric carrier is used. Further work will need to be done to establish ways in which extrudates produced by HME can be incorporated into age-appropriate final dosage forms, without adversely affecting the taste masking properties of the extrudate.


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## Appendix A – UCL Research Ethics Committee Approval (Project ID 4612/009)

### UCL Research Ethics Committee

**Important:** All fields must be completed. The form should be completed in plain English understandable to lay committee members. See notes in status bar for advice on completing each field. You should read the ethics application guidelines and have them available as you complete this form.

### Application Form

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<tr>
<th>Section A</th>
<th>Application Details</th>
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<tr>
<td><strong>A1</strong></td>
<td>Project Title: Bitterness Assessment of Commonly Used Anti-Tuberculosis Drugs.</td>
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<tr>
<td>Date of Submission: 16/10/2015</td>
<td>Proposed Start Date: 23/11/2015</td>
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<tr>
<td>UCL Ethics Project ID Number: 4612/009</td>
<td>Proposed End Date: 21/11/2016</td>
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<tr>
<td>If this is an application for classroom research as distinct from independent study courses, please provide the following additional details:</td>
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**A2**

**Principal Researcher**

Please note that a student – undergraduate, postgraduate or research postgraduate cannot be the Principal Researcher for Ethics purposes.

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**Declaration To be Signed by the Principal Researcher**

- I have met with and advised the student on the ethical aspects of this project design (applicable only if the Principal Researcher is not also the Applicant).
- I understand that it is a UCL requirement for both students & staff researchers to undergo Disclosure and Barring Service (DBS) Checks when working in controlled or regulated activity with children, young people or vulnerable adults. The required DBS Check Disclosure Number(s) is: N/A
- I have obtained approval from the UCL Data Protection Officer stating that the research project is compliant with the Data Protection Act 1998. My Data Protection Registration Number is: 23684/10/6/2015/10/21
- I am satisfied that the research complies with current professional, departmental and university guidelines including UCL’s Risk Assessment Procedures and insurance arrangements.
- I undertake to complete and submit the ‘Continuing Review Approval Form’ on an annual basis to the UCL Research Ethics Committee.
- I will ensure that changes in approved research protocols are reported promptly and are not initiated without approval by the UCL Research Ethics Committee, except when necessary to eliminate apparent immediate hazards to the participant.
- I will ensure that all adverse or unforeseen problems arising from the research project are reported in a timely fashion to the UCL Research Ethics Committee.
- I will undertake to provide notification when the study is complete and if it fails to start or is abandoned.

**Signature:**

**Date:** 15/10/2015

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### Sponsor/ Other Organisations Involved and Funding

- **Sponsor:** UCL  
- Other institution

If your project is sponsored by an institution other than UCL, please provide details.

- **b) Other Organisations:** If your study involves another organisation, please provide details. Evidence that the relevant authority has given permission should be attached or confirmation provided that this will be available upon request.

- **c) Funding:** What are the sources of funding for this study and will the study result in financial payment or payment in kind to the department or College? If study is funded solely by UCL this should be stated; the section should not be left blank. Funded solely by UCL.

### Signature of Head of Department or Chair of the Departmental Ethics Committee

*(This must not be the same signature as the Principal Researcher)*
I have discussed this project with the principal researcher who is suitably qualified to carry out this research and I approve it. The project is registered with the UCL Data Protection Officer, a formal signed risk assessment form has been completed, and appropriate insurance arrangements are in place. Links to details of UCL’s policies on data protection, risk assessment, and insurance arrangements can be found at: http://ethics.grad.ucl.ac.uk/procedures.php

UCL is required by law to ensure that researchers undergo a Disclosure and Barring Service (DBS) Check if their research project puts them in a position of trust with children under 18 or vulnerable adults.

"HEAD OF DEPARTMENT TO DELETE BELOW AS APPLICABLE"
I am satisfied that checks:  
(1) have been satisfactorily completed  
(2) have been initiated  
(3) are not required

If checks are not required please clarify why below.

Chair’s Action Recommended: ☐ Yes ☐ No
A recommendation for Chair’s action can be based only on the criteria of minimal risk as defined in the Terms of Reference of the UCL Research Ethics Committee.

| PRINT NAME: |  |
| SIGNATURE: |  |
| DATE: | 15/10/2015 |

### SECTION B

**DETAILS OF THE PROJECT**

<table>
<thead>
<tr>
<th>B1</th>
<th>Please provide a brief summary of the project in simple prose outlining the intended value of the project, giving necessary scientific background (max 500 words).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palatability of medicines is crucial for patient acceptability and compliance, particularly in the paediatric population. Non-acceptance of medicines due to unpleasant taste can result in poor treatment outcomes if the medicine is partially taken or not taken at all. This is a persistent issue in the treatment of paediatric tuberculosis where the poor palatability of the drugs, coupled with long treatment regimens (minimum 6 months) leads to very low treatment compliance rates and thus poor treatment outcomes. There are four drugs which are the first line of treatment for tuberculosis: isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride. While anecdotal evidence exists regarding the bitter taste of these medicines, to the best of the researcher’s knowledge, no human taste panels have been published with details of palatability assessment of these drugs.</td>
</tr>
<tr>
<td></td>
<td>The four drugs used in this study (isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride) have already been assessed using the rat brief-access taste aversion (BATA) model. The BATA model is an in vivo taste assessment method where mildly water-deprived rats are presented with several concentrations of a compound for a few seconds; the taste is assessed by the number of licks recorded with a “lickometer”.</td>
</tr>
<tr>
<td></td>
<td>The aim of this study is to conduct an in vivo sensory analysis using human taste panels to obtain human data regarding the taste of these drugs and to validate the data obtained with the rat BATA model for these 4 drugs. This data in turn can be further used to assess the taste of innovative non-GMP fixed dose combinations formulated with taste masking technologies e.g. Hot melt co-extrudates. The widely used “swirl and spit” method will be used, in which participants swirl a small volume of the test solution in their mouth for some seconds before spitting it out. The quality and intensity of the test stimuli is assessed using scales, and reliable feedback regarding taste can be provided.</td>
</tr>
</tbody>
</table>
Appendix A

B2

Briefly characterise in simple prose the research protocol, type of procedure and/or research methodology (e.g. observational, survey research, experimental). Give details of any samples or measurements to be taken (max 500 words).

Type: Single blind, cross over, single centre study (Annex 1 – flow diagram)

Duration: 1-2 hours per day for 5 sessions (with up to 72 hours of washout period between sessions) to reduce the burden on the participants.

Food: Breakfast or neutral lunch (not spiced, lightly salted) should be taken at least 30 minutes before investigation.

Inclusion criteria: Healthy male or female adults, able to understand and speak English. We will attempt to recruit an equal number of male and female subjects.

Exclusion criteria: Antecedent deterioration of taste or smell, smoker, recent dental care, medicinal treatment (excluding contraceptives) up to 15 days before the tests and any known drug allergies. Sensory disorders affecting the mouth or local anaesthetics into the mouth within 24 hours of the study.

Research methodology: sensory (palatability) evaluation of isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride.

Preparation of test samples: According to the BP general monograph, the production of unlicensed medicines should be undertaken by competent staff and prepared extemporaneously under the supervision of a registered UK pharmacist (GPhC registration: Dr. Catherine Tuleu 2052921; Fatima de Gomes Pina 2081805). Samples will be prepared under strict quality measures in a dedicated area according to Standard Operating Procedures that have been approved by the PI, departmental safety officer and head of department (Annex 2).

Tasting protocol: Swirl and Spit method (~2h per session - washout period min 72h) = 10ml samples of solution (numbered with a random three-digit code) will be presented in a randomised order. Participants will rinse their mouths with the solution for 5 seconds to cover all oral surfaces, and then spit the sample into a receptacle provided. Immediately upon expectoration, they will rate the taste using a computerised questionnaire with continuous and categorical scales (annex 3 - data collection form). Before and after each sample, participants will rinse their mouth with water and have lightly salted crackers to neutralise their taste. There will be an interval of up to 10 minutes between samples, until the previous sample cannot be perceived. Participants will be allowed to re-taste immediately each sample once if needed.

All participants will taste per session a total of 12 samples (n=4: concentrations of one drug x 3 times/day) plus the positive and negative controls. The formulations will be coded with random three-digit codes and presented in a random order.

In order to calibrate panelists on each day they will first taste two controls – a sample of bottled water (indicating “no taste”) and the highest concentration of the drug being assessed (indicating “worst taste”).

Immediately upon expectoration, subjects will rate the taste intensity on a bipolar scale. The rated taste intensities are measured using the software Qualtrics.

Data processing and analysis: multisample difference test; rating approach – evaluation by ANOVA with a statistical significance p<0.05 and post-hoc analysis [Tukey’s honestly significant difference (HSD) test].

Attach any questionnaires, psychological tests, etc. (a standardised questionnaire does not need to be attached, but please provide the name and details of the questionnaire together with a published reference to its prior usage).

B3

Where will the study take place (please provide name of institution/department)?
If the study is to be carried out overseas, what steps have been taken to secure research and ethical permission in the study country?
Is the research compliant with Data Protection legislation in the country concerned or is it compliant with the UK Data Protection Act 1998?

UCL School of pharmacy - in the Pharmacy Practice Dispensary. Participants will be seated at individual computer stations and screened off from other volunteers.
Environment: Calm, daylight, aired and odourless (to avoid any influence on the sensory part of the test).

Have collaborating departments whose resources will be needed been informed and agreed to participate?  
Attach any relevant correspondence.  
N/A

How will the results be disseminated, including communication of results with research participants?  
Once the participants have been recruited, they will be assigned a code known only to the researchers. The results will be available directly to the participants so that individuals may know how they performed after the study if they wish to. Once completed, the results of the study may be anonymously reported and disseminated in peer reviewed scientific journals, internal reports and conference presentations.

A statement is included in the patient information sheet inviting participants to contact the research team should they wish to know the results of the study. In such cases, an abstract of the overall outcomes will be shared with participants. As the results are collected anonymously, participant confidentiality will be maintained when these are disseminated. Further, as the outcomes of the research do not lead to direct benefit or value to the participants involved, collecting and storing personal contact information has not been deemed justifiable, simply for the purposes of disseminating the end results. Instead participants will be able to request and obtain the results if they wish to do so.

Please outline any ethical issues that might arise from the proposed study and how they are to be addressed. Please note that all research projects have some ethical considerations so do not leave this section blank.

Confidentiality: Minimum personal details will be recorded (contact details, date of birth and gender). Confidentiality will not be breached as once participants are recruited they will be assigned a code, known only to the researchers. The results will be available directly to the participants so that individuals may know how the performed after the study if they wish so. On completion of the study, the results may be anonymously reported and disseminated in peer reviewed scientific journals, internal reports and conference presentations. If the data produced is published confidentially and anonymity will be maintained and it will not be possible to identify participants from the publications.

Participant burden: The study will require each participant to attend ‘swirl & spit’ method sessions for 2h on four separate days. A 72-hour washout period will be allowed in between assessment days to reduce burden on participants and minimise taste fatigue. Contact with the test samples will be kept to a minimum.

There is a potential for participants to suffer from temporary oral discomfort if the taste or aftertaste of the solutions is bitter. Some sensitive participants may gag in response to the bitter solutions, and some may vomit (though this is rare and has never occurred in our experience of undertaking these studies). If any participant shows high discomfort to bitterness, the assessment will be immediately stopped.

Adverse effects: The time of contact has been minimised (5 seconds of ‘swirl’ then ‘spit’) which decreases the potential for adverse effects, risks or hazards. There is potential for buccal absorption of the drug or accidental swallowing of the formulations tested. The inclusion criteria requires understanding of English, and the researcher will ensure that participants fully understand that samples should not be swallowed at each session. Participants taking any concurrent medications will also be excluded.

Concentrations for isoniazid: 2.5, 5.0, 10.0 and 20.0 mg/ml

Isoniazid is a well known drug which is the cornerstone of modern anti-tuberculosis regimens. In the
Appendix A

extremely unlikely event that accidental ingestion of a single sample occurs, the maximum amount of drug that participants could be exposed to is 200mg which is well below the maximum dosage (for both adults and children) of 300mg/day or 900mg every 3 days for intermittent supervised treatment. If participants were exposed to the full dose of all samples (including repeats) the cumulative dose would be 1.25g/day. However, this will never happen as the study will be immediately stopped if any single sample is swallowed. Side effects of isoniazid include nausea, vomiting, constipation and dry mouth.

Concentrations for rifampicin: 0.8, 1.2, 1.6 and 2.0 mg/ml

Rifampicin, like isoniazid is a key component of all anti-tuberculosis regimens. It can also be used to treat leprosy, legionella and meningooccal disease. The recommended dosages for adults are 450mg/day for patients under 50kg and 600mg/day for patients over 50kg. In the extremely unlikely event that accidental ingestion occurs the maximum amount of drug that participants could be exposed to is 20mg, which is well below the maximum dosages for both body weight categories. If patients were exposed to the full dose of all samples (including repeats) the cumulative dose would be 160mg/day. However, this will never happen as the study will be immediately stopped if any single sample is swallowed. Side effects include nausea, vomiting, diarrhoea, loss of appetite and headache.

Concentrations for pyrazinamide: 0.875, 1.75, 3.5 and 7.0 mg/ml

Pyrazinamide is a bactericidal drug used in the initial intensive phase of treatment for tuberculosis (2-3 months). The maximum dose for treatment of both adults and children is 1.5g/day for patients under 50kg and 2.0g/day for patients above 50kg. In the extremely unlikely event that accidental ingestion occurs the maximum amount of drug that participants could be exposed to is 70mg. If patients were exposed to the full dose of all samples (including repeats), the cumulative dose received would be a maximum of 395mg/day. However, this will never happen as the study will be immediately stopped if any single sample is swallowed. Side effects include skin rash, fever, vomiting and loss of appetite.

Concentrations for Ethambutol dihydrochloride: 3.125, 6.25, 12.5 and 25 mg/ml

Ethambutol dihydrochloride is included in the initial intensive phase of treatment if isoniazid resistance is suspected. The recommended dosage for adults is 15mg/kg/day while the recommended dosage for children is 20mg/kg/day. Based on the average male weight of 68kg (as stated in the BNF) and average female weight of 58kg (as stated in the BNF) this equates to an average dosage of 1.02g/day and 0.70g/day for men and women respectively. In the extremely unlikely event that accidental ingestion of a single sample occurs the maximum amount that participants could be exposed to is 250mg. If participants were exposed to the full dose of all samples (including repeats), the cumulative dose received would be a maximum of 1.400g/day. However, this will never happen as the study will be immediately stopped if any single sample is swallowed. Side effects include headache, loss of appetite, nausea and vomiting.

Nevertheless, participants will not swallow the solutions presented and will rinse their mouth after each sample is presented to further minimise exposure. More importantly, if any single accidental ingestion occurs, the study will be stopped and the participant will be excluded from the study. In the case of any adverse events, first aid will be sought as necessary and the study will be stopped. Dr Tuleu is a First aider at work (St John’s Ambulance) and we can seek for medical help on the study premises (Dr Kirsten Harvey, MD and Dr Yucheng Sheng, MD). If someone complains, the supervisor or a third party (not directly involved in research team) Ms Joanna O’Brien (Institute Manager) can be contacted for further advice.

The research team has previous experience in taste assessment studies using the “swirl and spit” method. The principal investigator has considerable experience of conducting and supervising such studies using volunteer panels including the following published/presented research:


SECTION C

DETAILS OF PARTICIPANTS

C1
Participants to be studied

C1a. Number of volunteers: 20

<table>
<thead>
<tr>
<th>Upper age limit</th>
<th>40</th>
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<tbody>
<tr>
<td>Lower age limit</td>
<td>18</td>
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</table>

C1b. Please justify the age range and sample size:
Any healthy adults (staff and students) working at UCL. We will attempt to recruit an equal number of male and female subjects. It is expected that we might recruit in the younger age range.

Sample Size and Justification: This is within the range of volunteers used in previous human taste panel palatability studies and large enough to apply appropriate statistical analysis.

C2
If you are using data or information held by a third party, please explain how you will obtain this. You should confirm that the information has been obtained in accordance with the UK Data Protection Act 1998.

N/A

C3
Will the research include children or vulnerable adults such as individuals with a learning disability or cognitive impairment or individuals in a dependent or unequal relationship? □ Yes □ No

How will you ensure that participants in these groups are competent to give consent to take part in this study? If you have relevant correspondence, please attach it.

C4
Will payment or any other incentive, such as gift service or free services, be made to any research participant?

□ Yes □ No

If yes, please specify the level of payment to be made and/or the source of the funds/gift/free service to be used.

Each participant will receive £7.50 per session attended (total of £30 if the participant completes all four sessions).
This will be paid from the GL account of the PI. Participants will have the option to withdraw from the study both before and during any part of the study if they wish.

Please justify the payment/other incentive you intend to offer.
This is a thank you gesture for time committed to the study.

C5
Recruitment

(i) Describe how potential participants will be identified:
Potential participants will be students and staff at UCL (healthy adults aged 18 to 40 years), and these will be the only persons invited to participate in this study.

(ii) Describe how potential participants will be approached:
An email (annex 4) will be circulated using UCL announce to advertise the study. If needed a printed advertisement will also be placed around the School of Pharmacy. Potential participants interested in taking
Appendix A

C9 Will you provide a full debriefing at the end of the data collection phase?  
☐ Yes  ☐ No
If 'No', please explain why below.

C10 Information Sheets And Consent Forms
A poorly written Information Sheet(s) and Consent Form(s) that lack clarity and simplicity frequently delay ethics approval of research projects. The wording and content of the Information Sheet and Consent Form must be appropriate to the age and educational level of the research participants and clearly state in simple non-technical language what the participant is agreeing to. Use the active voice e.g. “we will book” rather than “bookings will be made”. Refer to participants as “you” and yourself as “I” or “we”. An appropriate translation of the Forms should be provided where the first language of the participants is not English. If you have different participant groups you should provide Information Sheets and Consent Forms as appropriate (e.g., one for children and one for parents/guardians) using the templates below. Where children are of a reading age, a written Information Sheet should be provided. When participants cannot read or the use of forms would be inappropriate, a description of the verbal information to be provided should be given. Please ensure that you trial the forms on an age-appropriate person before you submit your application.

UCL SCHOOL OF PHARMACY
BRUNSWICK SQUARE

Bitterness Assessment of Commonly Used Anti-Tuberculosis Drugs

Participant Information Sheet
You will be provided with a copy of this information sheet.

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 4612/000

Principal Investigator:  
Dr Catherine Tuleu  
Department of Pharmaceutics  
UCL School of Pharmacy  
29/39 Brunswick Square  
London, WC1N 1AX  
Tel: 020 7765 5857  
Email: c.tuleu@ucl.ac.uk

We would like to invite you to participate in this research project. Taking part is voluntary; it is up to you to decide whether or not to take part, and choosing not to will not disadvantage you in any way. If you do decide to take part, you will still be free to withdraw at any time without the need to give a reason.

Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Please feel free to ask us if there is anything that is not clear or you would like more information.
Appendix A

Details of the Study

What is the purpose and aim of this project?

Palatability of medicines is crucial for patient acceptability and compliance, particularly in the paediatric population. Non-acceptance of medicines due to unpleasant taste contributes to poor treatment outcomes if the medicine is partially taken or not taken at all. This is a particular problem in the treatment of paediatric tuberculosis where the bitter taste of medicines, coupled with long treatment regimens (minimum 6 months) leads to very low treatment compliance rates and thus poor treatment outcomes. While anecdotal evidence exists regarding the bitter taste of the medicines used to treat tuberculosis in both adults and children to the best of the researcher’s knowledge, no human taste panels have been published with details of palatability assessment of these drugs.

The four drugs used in this study (isoniazid, rifampicin, pyrazinamide and ethambutol dihydrochloride) have already been assessed using the rat brief-access taste aversion (BATA) model. The BATA model is an in vivo taste assessment method where mildly water-deprived rats are presented with several concentrations of a compound for a few seconds; the taste is assessed by the number of licks recorded with a “lickometer”.

The aim of this study is to conduct an in vivo sensory analysis using human taste panels to obtain human data regarding the taste of these drugs and to validate the data obtained with the rat BATA model. The widely used “swirl and spit” method will be used, in which participants swirl a small volume of the test solution in their mouth for some seconds before spitting it out. The quality and intensity of the test stimuli is assessed using scales, and reliable feedback regarding taste can be provided.

Who can take part in this study?

We are looking for healthy adults aged between 18 and 40 years to take part.

If you have problems with the sense of taste or smell, any drug allergies, or if you have had any dental care or medicinal treatment (except contraceptives) during the 15 days before the tests, then unfortunately you will be unable to take part.

What will happen if I agree to take part?

If you decide to take part, you will be asked to taste, but not swallow, various solutions of the drugs cited above. We will ask you to swirl 10ml of solution in the mouth for 5 seconds and then spit it out. We will ask you to rate the taste each time on a scale. You will be asked to rinse your mouth out before and after each test, and plain crackers will also be available to help clean the mouth after.

The study will take place in the consultation rooms of the pharmacy practice dispensary at UCL School of Pharmacy during 4 sessions over a three week period. You will be asked to commit 8 hours in total.

Are there any risks involved?

The solutions being tested will not be swallowed at any point during the study. If a sample is accidentally swallowed, you must alert a member of the team and we will stop the study immediately. The researchers are trained in First Aid and medical doctors will be present to assess the situation. If necessary, we will also contact emergency services.

If the samples you taste have a poor taste, there is potential to suffer from temporary oral discomfort. Some sensitive participants may gag in response to the samples and vomit, however this is rare. Side effects of isoniazid can include nausea, vomiting, constipation and dry mouth. Side effects of rifampicin include nausea, vomiting, diarrhoea, loss of appetite and headache. Side effects of pyrazinamide include skin rash, fever, vomiting and loss of appetite. Side effects of ethambutol include headache, loss of appetite, nausea and vomiting. Nevertheless, the time of swilling has been kept to the minimum of 5 seconds, which minimises the potential for adverse
effects, risks or hazards; further a delay of 10 minutes will be respected between each tested solution.

**Who will know that I took part, and what happens after?**

Only members of the research team will know that you took part and have access to the results; confidentiality will be maintained during the study and after it has finished. If the study is published or presented to a wider audience, your anonymity will be respected through anonymisation procedures. All data will be collected and stored in accordance with the Data Protection Act 1998.

If you would like to know the results of the study once it has finished, please feel free to contact us using the details overleaf, as we’d be happy to share these with you.

**Who can I contact for more information?**

Please contact the research team, using the details overleaf, if you would like to take part, or have any questions about the study.

If you would like to discuss it with someone outside of the research team, please contact

Ms Joanna O’Brien
Institute Manager
UCL School of Pharmacy
29/39 Brunswick Square
London, WC1N 1AX
Tel: 020 7753 5814
Email: joanna.o'brien@ucl.ac.uk

Thank you for taking the time to read this information sheet
Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Project: **Bitterness Assessment of Commonly Used Anti-Tuberculosis Drugs**

This study has been approved by the UCL Research Ethics Committee (Project ID Number: 4612/009)

Thank you for your interest in taking part in this research. Before you agree to take part, the person organising the research must explain the project to you.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

**Participant’s Statement**

I

- have read the notes written above and the Information Sheet, and understand what the study involves.
- understand that I should not take part if I have had any dental care or medicinal treatment (except contraceptives) during the 15 days before the tests.
- understand that if I decide at any time that I no longer wish to take part in this project, I can notify the researchers involved and withdraw immediately without penalty.
- consent to the processing of my personal information for the purposes of this research study.
- understand that the information I have submitted will be published as a report and I can request a copy by contacting the researchers. Confidentiality and anonymity will be maintained and it will not be possible to identify me from any publications.
- understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.
- agree that the research project named above has been explained to me to my satisfaction and I agree to take part in this study.

Signed: ___________________________ Date: ___________________________
### SECTION D  DETAILS OF RISKS AND BENEFITS TO THE RESEARCHER AND THE RESEARCHED

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<tbody>
<tr>
<td>D1</td>
<td>Have UCL’s Risk Assessment Procedures been followed?</td>
<td>☐ Yes ☐ No</td>
<td></td>
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</tr>
</tbody>
</table>

If No, please explain.

There is no significant risk - see annex 5 for UCL risk assessment form as well as UCL School of Pharmacy General Risk Assessment and COSHH forms

| D2 | Does UCL’s insurer need to be notified about your project before insurance cover can be provided? | ☐ Yes ☐ No |

The insurance for all UCL studies is provided by a commercial insurer. For the majority of studies the cover is automatic. However, for a minority of studies, in certain categories, the insurer requires prior notification of the project before cover can be provided.

If Yes, please provide confirmation that the appropriate insurance cover has been agreed. Please attach your UCL insurance registration form and any related correspondence.

| D3 | Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research participants). |

This project holds little risk to the researchers that has been risk assessed (see annex 5). Standard operating procedures as well as working sheets reviewed by the School safety officer are also in annex 2.

| D4 | Will these participants participate in any activities that may be potentially stressful or harmful in connection with this research? | ☐ Yes ☐ No |

If Yes, please describe the nature of the risk or stress and how you will minimise and monitor it.

The procedures may cause temporary physical discomfort (exposure to unpleasant taste) during tasting if the test samples taste or aftertaste is bitter. The potential discomfort is minimal and not greater than that ordinarily encountered in daily life. In order to minimise the discomfort, a delay of up to 10 minutes will be respected between each tested sample. Before and after each test sample, subjects will rinse their mouth with water until they can no longer perceive the previous sample and have one lightly salted cracker to neutralize the mouth taste. The participants will be provided with necessary instruction on properly testing the samples. The level of discomfort may evolve during the study. Risk will be continuously monitored by asking participants how they feel between sessions. If participant report any distress, they will be excluded from the study. Stopping rules are included in the study protocol. Some sensitive participants may gag in response to the bitter solutions and vomit. If some participants show high discomfort to bitterness, the tasting will be immediately stopped.
Appendix A

D5 Will group or individual interviews/questionnaires raise any topics or issues that might be sensitive, embarrassing or upsetting for participants?

If Yes, please explain how you will deal with this.
N/A

D6 Please describe any expected benefits to the participant.

Indirect benefit through contribution by obtaining thorough palatability assessment of these drugs and validation of rat BATA model. This information in turn can be used to assess novel formulations, age appropriate formulations, the development of which may lead to improvement in patient care and treatment outcome for tuberculosis.

D7 Specify whether the following procedures are involved:

Any invasive procedure(s) □ Yes □ No
Physical contact □ Yes □ No
Any procedure(s) that may cause mental distress □ Yes □ No

Please state briefly any precautions being taken to protect the health and safety of the research participants.

D8 Does the research involve the use of drugs? □ Yes □ No

If Yes, please name the drug/product and its intended use in the research and then complete Appendix 1

Following the MHRA algorithm “Is it a clinical trial of a medicinal product?” it can be concluded that palatability studies are not clinical trials on a IMP. http://www.mhra.gov.uk/home/groups/l-unit1/documents/websiteresources/con003634.pdf

Does the project involve the use of genetically modified materials? □ Yes □ No

If Yes, has approval from the Genetic Modification Safety Committee been obtained for work? □ Yes □ No

If Yes, please quote the Genetic Modification Reference Number:
Appendix A

D9 Will any non-ionising radiation be used on the research participant(s)? □ Yes □ No
If Yes, please complete Appendix III.

D10 Are you using a medical device in the UK that is CE-marked and is being used within its product indication? □ Yes □ No
If Yes, please complete Appendix III.

CHECKLIST

Please submit either 12 copies (1 original + 11 double sided photocopies) of your completed application form for full committee review or 3 copies (1 original + 2 double sided copies) for chair’s action, together with the appropriate supporting documentation from the list below to the UCL Research Ethics Committee Administrator. You should also submit your application form electronically to the Administrator at: ethics@ucl.ac.uk.

Documents to be Attached to Application Form (if applicable)

<table>
<thead>
<tr>
<th>Section B: Details of the Project</th>
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<tbody>
<tr>
<td>• Questionnaire(s) / Psychological Tests □☐</td>
</tr>
<tr>
<td>• Relevant correspondence relating to involvement of collaborating department(s) and agreed participation in the research. □☐</td>
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</table>

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<tr>
<th>Section C: Details of Participants</th>
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<tbody>
<tr>
<td>• Parental/guardian consent form for research involving participants under 18 □☐</td>
</tr>
<tr>
<td>• Participant’s information sheet □☐</td>
</tr>
<tr>
<td>• Participant’s consent form/s □☐</td>
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<tr>
<td>• Advertisement □☐</td>
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<thead>
<tr>
<th>Section D: Details of Risks and Benefits to the Researcher and the Researched</th>
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<tbody>
<tr>
<td>• Insurance registration form and related correspondence □☐</td>
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<tr>
<th>Appendix I: Research Involving the Use of Drugs</th>
</tr>
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<tbody>
<tr>
<td>• Relevant correspondence relating to agreed arrangements for dispensing with the pharmacy □☐</td>
</tr>
<tr>
<td>• Written confirmation from the manufacturer that the drug/substance has been manufactured to GMP □☐</td>
</tr>
<tr>
<td>• Proposed volunteer contract □☐</td>
</tr>
<tr>
<td>• Full declaration of financial or direct interest □☐</td>
</tr>
<tr>
<td>• Copies of certificates: CTA etc… □☐</td>
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</table>

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<tr>
<th>Appendix II: Use of Non-Ionising Radiation</th>
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Appendix III: Use Medical Devices
Appendix B

Appendix B - Preparation of Standard Calibration Solutions for Insent E-Tongue

B.1 Materials
Tartaric acid, potassium chloride, potassium hydroxide, monosodium glutamate, tannic acid and quinine hydrochloride were obtained from Sigma-Aldrich (UK). Iso-alpha acid was obtained from Insent (Atsugi-shi, Japan). Hydrochloric acid was obtained from Fisher Chemicals (Loughborough, UK). All substances were used as received.

B.2 Methods

B.2.1 Standard Reference Solution
0.045 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL of deionised water. The solution was stirred thoroughly before being made up to 1 L with deionised water.

B.2.2 Negatively Charged Washing Solution
150 mL ethanol was added to 250 mL deionised water and mixed thoroughly. 50 mL 1M hydrochloric acid solution was added and the solution was made up to 500 mL using deionised water.

B.2.3 Positively Charged Washing Solution
3.73 g potassium chloride was added to 250 mL deionised water and stirred thoroughly. 150 mL ethanol and 5 mL 1 M potassium hydroxide solution were added and thoroughly mixed before making up to 500 mL with deionised water.

B.2.4 Salty Reference Solution
0.045 g tartaric acid and 22.25 g potassium chloride were dissolved in about 900 mL deionised water a mixed thoroughly before being made up to 1 L with deionised water.

B.2.5 Umami Reference Solution
0.045 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL deionised water. 1.87 g of monosodium glutamate was then added a mixed thoroughly before making up to 1 L with deionised water.

B.2.6 Astringent Reference Solution
0.045 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL deionised water. 0.50 g tannic acid was added and mixed thoroughly before making up to 1 L with deionised water.
B.2.7 Bitter (-) Reference Solution
0.045 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL deionised water. 100 μL iso-alpha acid was added and mixed thoroughly before being made up to 1 L with deionised water.

B.2.8 Bitter (+) Reference Solution
0.045 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL deionised water. 0.04 g quinine hydrochloride was added and mixed thoroughly before being made up to 1 L with deionised water.

B.2.9 Sour Reference Solution
0.45 g tartaric acid and 2.25 g potassium chloride were dissolved in about 900 mL deionised water and mixed thoroughly before being made up to 1 L with deionised water.