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Solid State Characterisation and Taste Masking Efficiency Evaluation of Polymer Based Extrudates of Isoniazid for Paediatric Administration

Alison V. Keating[1], Jessica Soto[1,2], Catherine Tuleu[1], Claire Forbes[3], Min Zhao[4], Duncan Q.M. Craig[1]*

[1] UCL School of Pharmacy, 29-39 Brunswick Square, London, WC1N 1AX, UK.

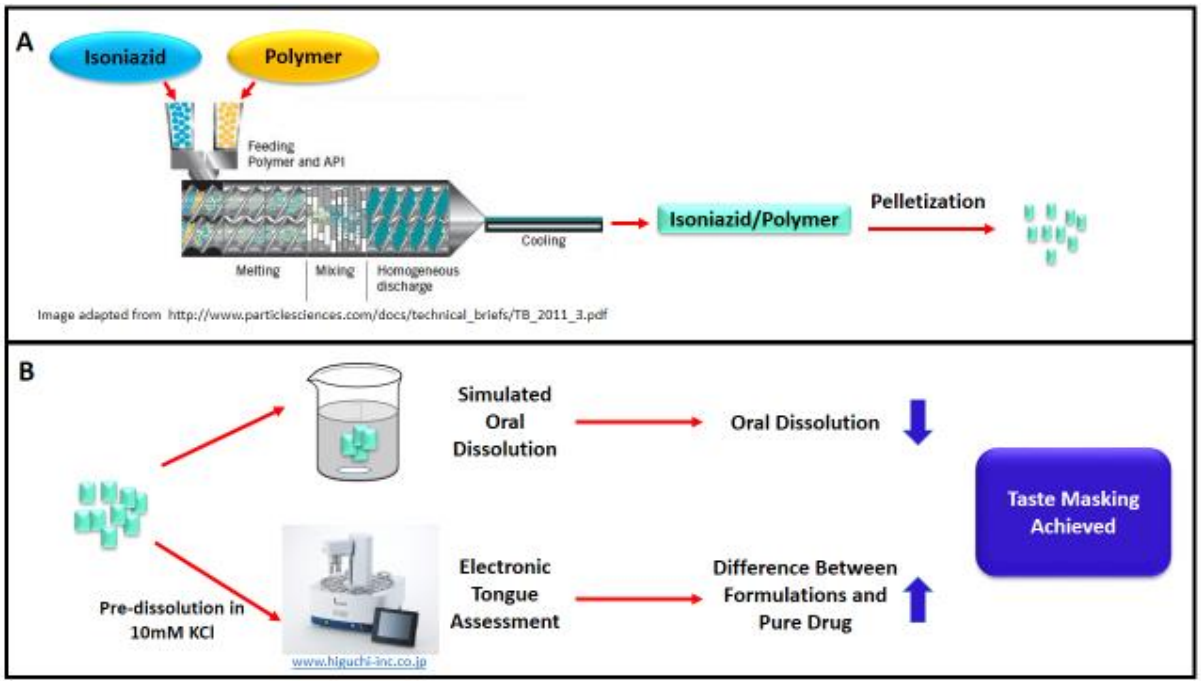
[2] Novartis Pharma AG, Basel, Switzerland.

[3] Pfizer UK Ltd., Ramsgate Road, Sandwich, Kent, CT13 9ND, UK.

[4] School of Pharmacy, Queen's University Belfast, Lisburn Road, Belfast, BT9 7BL, UK.

*corresponding author – duncan.craig@ucl.ac.uk , Tel: +44 (0) 207 753 5819, Fax: +44 (0) 207 753 5560

Graphical abstract



In this study hot melt extrusion was investigated as a processing method to mask the bitter taste of isoniazid. Hot melt extrusion masks the taste by applying a polymeric coating around the API, preventing it from coming into contact with the taste buds and eliciting a bitter taste response. Briefly, the hot melt extrusion process works as follows: the API and polymer are fed into the extruder which consists of a heated barrel and two screws. The screws convey they drug and polymer through the heated barrel which causes the material to melt and be mixed together to form a homogenous extrudate which is shaped by forcing through a die. This extrudate is then cooled and can be sent for downstream processing such as pelleting or milling.

HME has many advantages over traditional taste masking processes. It is quick process, it can be run as a continuous manufacturing process. It is easy to scale up and, perhaps most importantly for paediatric formulations, it is solvent free therefore you don't have to worry about removal of residual solvents from your formulations.

Abstract

Hot melt extrusion has gained considerable attention as a novel technique for taste masking of bitter APIs. The aim of this study was to investigate whether hot melt extrusion could be used to develop taste masked formulations of isoniazid and also to evaluate and correlate different taste assessment

methods. Two polymers with different physico-chemical properties, Soluplus and Eudragit E-PO were chosen as carriers for the drug. 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. Polymeric formulations of isoniazid were produced with drug loadings of 20% and 30% w/w. The solid state characteristics of the formulations were assessed by differential scanning calorimetry and powder X-ray diffraction. The taste of isoniazid was assessed using the rodent Brief Access Taste Aversion (BATA) model, while formulations were assessed using the electronic tongue and dissolution under simulated oral conditions. Investigation into the drug loading effect with these two polymers showed that all Soluplus based extrudates with drug loading up to 30% w/w were fully amorphous while Eudragit E-PO based extrudates contained crystalline drug as demonstrated by both DSC and PXRD, dependent on loading. BATA testing of isoniazid gave an IC_{50} value, i.e. the dose of drug which inhibits 50% of licks, of 11.1mg/mL. Taste assessment of the formulations using both simulated oral drug release and the electronic tongue demonstrated that Eudragit E-PO based formulations had a better taste masking efficiency than Soluplus. This is due to the fact that significantly less isoniazid is released from the Eudragit E-PO based formulations under oral conditions.

Keywords: hot melt extrusion, taste masking, paediatric formulations, tuberculosis, taste assessment.

1. Introduction

A significant 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 formulating 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 (Albertini et al., 2004; de Aguiar et al., 2010; Fini et al., 2008; Harada et al., 2010), lipophilic vehicles (Nitanai et al., 2012; Qi et al., 2008a; Vaassen et al., 2012), salt formation (Aitipamula et al., 2014; Rahman et al., 2012), solid dispersions (Du et al., 2013; Kulkarni and Amin, 2008; Tan et al., 2013), salting out layers (Yoshida et al., 2009, 2008), ion exchange resins (Agresti et al., 2008; Bhise et al., 2008; Yewale et al., 2013; Yu et al., 2012), complexation with cyclodextrins (Arima et al., 2012; Nieddu et al., 2014; Orlu-Gul et al., 2013; Preis et al., 2014), or film coating (Joshi and Petereit, 2013).

In recent years hot melt extrusion (HME) has emerged as a novel processing method for taste masking of bitter drugs (Maniruzzaman et al., 2014). HME 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 (Wilson et al., 2012). There are many advantages of using this approach 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 who can be more sensitive to the toxic effect of solvents than adults (Walsh et al., 2014). Hot melt extrusion 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 relatively simple to scale up (Maniruzzaman et al., 2014), and can be adapted to meet the goals of the FDA process analytical technology scheme for quality by design in pharmaceutical products (Saerens et al., 2014).

The taste of medicines can be assessed using *in vivo* or *in vitro* methods (Mohamed-Ahmed et al., 2016). *In vivo* taste assessment methods involve using either humans or animals to assess the taste of substances. Human taste panel studies involve evaluating the taste of medicines or dosage forms by estimating the gustatory sensation responses in healthy adult human volunteers (Schiffman et al., 2000). 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 (Devantier et al., 2008; Rudnitskaya et al., 2013). In this animal taste model, rodents, most often mice or rats (Soto et al., 2015), are mildly water-deprived and then put in an apparatus known as 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 using very few animals.

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. When a molecule interacts with an electronic tongue 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 (Woertz et al., 2011).

Isoniazid is a highly bactericidal drug which is used to treat tuberculosis in both paediatric and adult populations (Donald and Schaaf, 2007). It is a borderline BCS Class I/III drug (Becker et al., 2007), with an aqueous solubility of approximately 125 mg/mL (Nair et al., 2011), which has been found to be

close to 100% bioavailable under most circumstances (Isoniazid, 2008). Isoniazid is used in both the intensive and continuation phases of TB treatment, meaning that patients typically have to take the drug for a minimum of six months. It is a bitter tasting drug which, coupled with long treatment regimens, tends to lead to poor patient adherence particularly in paediatric populations (Rutherford et al., 2012). It is hoped that by developing an age appropriate, taste masked formulation of this drug that patient adherence and thus treatment outcome will be improved.

As mentioned previously, isoniazid has been found to have close to 100% bioavailablility, thus it is essential that any taste masking strategy used does not adversely affect absorption, hence the formulations must 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 (Qi et al., 2008b). It has been extensively used previously in the pharmaceutical industry as a taste-masking excipient (Thakral et al., 2013). Soluplus is a polyvinyl caprolactam - polyvinyl acetate - polyethylene glycol graft copolymer which is freely water soluble (Aitipamula et al., 2014; Fule et al., 2013; Fule and Amin, 2014; Shah et al., 2013). It has not yet been widely used for taste masking applications, but also provides a means of comparing the efficacy of Eudragit E-PO from a comparable HME formulation. 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.

2. Materials and Methods

2.1 Materials

Isoniazid, potassium chloride, potassium hydroxide, tartaric acid, potassium phosphate monobasic, sodium chloride, calcium chloride and sodium hydroxide were obtained from Sigma Aldrich (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 stated otherwise.

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 1. Post extrusion the extrudates were cut into ~1 mm pieces using a Pharma 11 Varicut Pelletizer (Thermo Fisher Scientific, U.K.).

2.3 Determination of Drug Loading

A 0.1% w/v solution of each extrudate was prepared by dissolving 10mg of ground extrudate in 10mL ethanol. The mixtures were sonicated (XUBA3 ultrasonic bath, Grant Instruments Ltd., Cambridgeshire, UK) for 30 minutes to ensure complete dissolution of the extrudate. Each solution was diluted 1 in 10 before the UV absorbance was recorded at 263nm using a Jenway 6305 UV-Vis Spectrophotometer (Bibby Scientific, Staffordshire, UK) where the polymers showed no absorbance.

2.4 Differential Scanning Calorimetry (DSC)

Modulated temperature DSC thermograms were recorded using a TA Instruments Q2000 calorimeter (TA Instruments, New Castle, Delaware, USA). For analysis 4-6mg of sample was accurately weighed and sealed in an aluminium pan (hermetic and pinholed 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. Samples were analysed in triplicate unless stated otherwise. Data analysis was carried out with TA Universal Analysis software.

2.5 Powder X-Ray Diffraction (PXRD)

Powder X-Ray Diffraction 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.

2.6 *In Vitro* Drug Release Test

In vitro drug release was tested using the British Pharmacopoeia method 2.9.3 dissolution test for solid dosage forms with the aid of a Caleva 8ST dissolution apparatus (G.B. Caleva Ltd., UK). Samples equivalent to 150mg of isoniazid were loaded into Size 0 gelatine capsules (Qualicaps Europe SA, Madrid) and placed into a metallic sinker. The sinker was placed in a dissolution bath containing 900mL 0.1N HCl (pH: 1.2 ± 0.2) at 37.0 ± 0.5°C and a paddle speed of 50rpm. At predetermined time intervals, a 10mL 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 263nm. No interference from the polymers used or gelatine capsules was observed at this wavelength. Experiments were carried out in triplicate and dissolution profiles are plotted as mean percentage drug release ± standard deviation.

2.7 Rodent Brief Access Taste Aversion (BATA) Testing

A total of ten adult male Sprague–Dawley rats (Charles-River, Kent, UK) were used for these experiments. They were housed in pairs in a room maintained at 21 ± 2°C with 55 ± 10% relative humidity and with a 12:12 h light/dark cycle. Animals had free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a 22h water-restriction schedule occurred (section 2.7.2). Throughout the experiments, daily food and water consumption were monitored. As a safety and welfare measure, each rat was weighed daily to ensure that their weight did not drop below 85% of their free-feeding weight. All the procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668).

2.7.1 Taste Solutions

In order to establish an aversiveness – concentration standard curve, six solutions of isoniazid in deionised water ranging from 1.25 mg/mL to 40 mg/mL were prepared. They were prepared on the day of the study and presented to the rats at room temperature.

2.7.2 BATA Experiment

Each mildly water deprived rat was placed individually in the lickometer ‘‘Davis MS-160” (DiLog Instruments, Tallahassee, Florida, USA) for a maximum of 40 min (session-length). After each session, the rats received tap water for rehydration in their home cage. The rats received a first 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 to sixteen moving tubes containing deionised water, with the shutter opening for 8 s and closing. The training sessions were followed by two testing sessions during which each rat was presented for 8 seconds with either deionised water or one of the six concentrations of drug in a randomised order. Each sample was intercepted by a water rinse to minimise carry over effects.

2.7.3 Data Analysis

The normality of the data was checked with the Shapiro-Wilk test. As the data were not normally distributed, the Kruskal-Wallis test was performed to check if there were significant differences in the number of licks between the different concentrations tested. The same test was done to check which concentrations elicited a number of licks significantly different compared to deionised water. When significant, post-hoc analysis was carried out with Gao *et al.* non-parametric multiple test (Gao et al., 2008). An IC_{50} value which corresponds to the concentration of the drug that inhibits 50% of the maximum number of licks compared to the reference, deionised water, was calculated with an E_{max} model (Sheng et al., 2016; Soto et al., 2015).

2.8 Solubility Studies

The solubility of isoniazid was assessed in both distilled water and simulated salivary fluid (SSF) at pH 7.4 adapted from Hughes *et al*.(Hughes and Gehris, 2003) (Table 2). An excess amount of pure isoniazid was added to 30mL 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 were recorded at 263nm using a Jenway 6305 UV-Vis Spectrophotometer (Bibby Scientific, Staffordshire, UK).

2.9 Simulation of Drug Release in the Oral Cavity

Dissolution testing was carried out to simulate the dissolution of pure isoniazid and the polymeric formulations in the oral cavity. Exposure of the drug/formulations to oral cavity conditions was mimicked by 5 min drug contact with 5mL of SSF at pH 7.4. Samples were manually withdrawn at 0.5, 1, 1.5, 2, 2.5, 3 and 5 min and replaced with fresh media. A temperature of 37 ± 0.5°C and a rotational speed of 50 rpm (magnetic stirring) were maintained during the experiment. Samples were filtered through 0.22µm filters (Merck-Millipore, Cork, Ireland) and analysed by UV-Vis Spectrometry (Jenway 6305 Spectrophotometer; Bibby Scientific, Staffordshire, UK) at 263nm.

2.9.1 Data Analysis

Drug release was compared to the IC_{50} value for isoniazid obtained from BATA testing to determine whether the formulations were likely to be aversive or not.

2.10 Electronic Tongue Assessment

The TS-5000Z (Insent Inc., Atsugi-shi, Japan) was equipped with four lipid membrane sensors and two corresponding reference electrodes (PPM Instruments, West Sussex). 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). *Reference solution* used for cleaning and as a reference solution was prepared by dissolving 30mmol/L potassium chloride and 0.3mmol/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 100mmol/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 100mmol/L potassium chloride and 10mmol/L potassium hydroxide. 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). The sensor output for taste (relative value, R value) was calculated relative to the preliminary sensor response to the reference solution (V_r).

$$R = V_s - V_r \qquad \text{(Equation 1)}$$

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

2.10.1 Sample Preparation for Electronic Tongue

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 use 'taste extracted water'. The dose unit of isoniazid was set at 150mg. For taste evaluation 20 dose units (equivalent to 3g isoniazid) were added to 100mL of 10mmol potassium chloride solution at 37°C and gently stirred for 1 minute. This represents a concentration of one dose in 5ml which is suitable for taste assessment as there is only slight dilution of the sample (Preis et al., 2012). The mixture was then filtered through 0.22 µm filters (Merck-Millipore, Cork, Ireland), removing any suspended particles. This 'taste extracted liquid' was then assessed using the measurement procedure described in section 2.10.2.

2.10.2 Data Analysis

The sensor signal results were evaluated using Principal Component Analysis (PCA). 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.(Abdi and Williams, 2010) 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 (2):

$$\boldsymbol{d(p,q)} = \sqrt{\sum_{i=1}^{n}(\boldsymbol{p_i} - \boldsymbol{q_i})^2} \qquad \text{(Equation 2)}$$

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

3. Results and Discussion

3.1 Hot Melt Extrusion

Hot melt extrusion processing of all Soluplus based formulations was carried out at 140°C and 50rpm. Opaque extrudates were formed at both 20% w/w and 30% w/w drug loading. Increasing the drug loading did not appear to have any effect on the processability of the mixture. All Eudragit E-PO based formulations were processed at 130°C and 50rpm. At both 20% w/w and 30% w/w drug loading uniform, opaque off-white extrudates were formed. As found for the Soluplus based systems, increasing the drug loading did not appear to have any effect on the processability of the mixture.

3.2 Physicochemical Characterisation of Extrudates

The drug loading of the formulations was assessed to ensure that the drug was not being degraded *in situ* during the extrusion process. The results of these experiments are given in Table 3. The drug loading was found to be within the expected range for all formulations, indicating that degradation was not occurring.

Figure 1 shows the DSC thermograms of the Soluplus based extrudates. The T_g of each of these formulation was lower than the T_g of Soluplus alone (70.4 ± 2.2 °C) 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. This is likely to be due to the drug exerting a plasticization effect on the polymer. Soluplus is a hygroscopic polymer, however thermogravimetric analysis of both the pure polymer and extrudates (data not shown) demonstrated that water content of the samples was negligible, thus the decrease in T_g is solely due to the plasticization effect of the drug.

For the 20% extrudates a single T_g was observed for indicating that the drug is in a fully amorphous form. Typically, to produce amorphous solid dispersions the mixture processed at temperatures higher than the T_m of the drug which ensures complete conversion of the crystalline drug to the amorphous form (Liu et al., 2013). 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.

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 143.4 ± 0.2 and peak at 156.1 ± 0.4°C. 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. The glass transition is most easily envisaged in the reversing heat flow (Figure 1b).

The PXRD patterns of pure isoniazid and the Soluplus based extrudates are displayed in Figure 2. The diffraction pattern of the 20% w/w drug loaded extrudates show an 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 shows that the extrudate as produced is completely amorphous and only undergoes recrystallisation on heating (as per the DSC results).

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 (Figure 3). 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 170.83 ± 0.03°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.

The diffraction patterns of Eudragit E-PO based extrudates and pure isoniazid are given in Figure 4. 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 at both concentrations (in agreement with DSC results).

3.3 *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. Rapid dissolution of pure isoniazid is seen in 0.1N HCl, with 100% of the drug being released within 5 minutes. As stated previously, the aim of this study was to taste mask the drug without altering the release profile of the drug. Therefore it was intended that a rapid dissolution profile would be observed for the extruded formulations.

Figure 5a shows the dissolution profile of the Soluplus based formulations compared to pure isoniazid. The 20% formulation demonstrates a drug release of 19.2% after 5 minutes while drug release from the 30% formulation at the same time point is significantly lower at just 2.0%. After 10 minutes the most rapid drug release is again observed from the 20% formulation which had released 62.1% of its drug load, compared to just 41.1% respectively from the 30% formulation. 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.

The dissolution profiles of the Eudragit E-PO formulations compared to pure isoniazid are given in Fig 5b. A similar trend to that observed for the Soluplus based formulations was also observed for the Eudragit E-PO formulations with the most rapid drug release being observed for the 20% drug loaded formulation with 100% drug release within 5 minutes. The 30% drug loaded formulation achieves 100% drug release within 15 minutes. 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. It can also be seen that the 20% formulations have very similar release profile to that of the pure drug alone. While Eudragit E-PO also contains carbonyl groups with 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.

3.4 BATA Testing of Pure Isoniazid

The aversiveness of pure isoniazid was assessed using the rodent BATA model and a dose-aversiveness response curve was established (Figure 6). For isoniazid, all except the two lowest concentrations (1.25mg/mL and 2.5mg/mL) were statistically different from water ($p=0.53$ and $p=0.41$). Isoniazid was found to have a clear aversive taste that could be quantified by a calculated IC_{50} value using the E_{max} model(Soto et al., 2015) which was found to be 11.1 mg/mL. Comparing this to a highly bitter compound such as quinine hydrochloride which has an IC_{50} value of 0.019 mg/mL (Soto et al., 2015), isoniazid can be classified as a weakly bitter compound.

3.5 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 ± 0.07g/mL. The saturated solubility of isoniazid in SSF was found to be significantly higher at 2.94 ± 0.03g/mL. The solubility of a drug is intrinsically linked to its taste, as only drug in solution can interact with the taste buds and produce a taste response.

3.6 Simulation of Drug Release in the Oral Cavity

Biorelevant dissolution testing was carried in SSF out to assess the amount of drug that would likely be released under in the oral cavity *in vivo*. The results are shown in Figure 7. 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 for isoniazid obtained from BATA testing of 11.1mg/mL.

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. The total drug release from the formulations was 1.33 ± 0.40 mg/mL and 1.55 ± 0.57 mg/mL for the 20% and 30% drug loaded formulations respectively. This indicates that the taste of isoniazid is likely to be adequately 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 IC_{50} value after just 1.5 mins, indicating that this is likely to be the most aversive formulation. 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. Soluplus is a highly water soluble polymer(BASF Group, 2010) which allows it to dissolve rapidly in the oral cavity and thus release a large amount of drug. Eudragit E-PO on the other hand is only soluble below pH 5 (Evonik Industries, 2014) meaning it is poorly soluble in the oral cavity, leading to very little drug release from these formulations.

Previous work on the correlation between the rat BATA model and human responses have suggested than an offset of one half log unit of concentration may be observed between the IC_{50} value obtained from BATA testing and the EC_{50} value obtained from human testing (Devantier et al., 2008; Rudnitskaya et al., 2013). The EC_{50} is the concentration of drug which elicits half the maximum taste

response in humans and may be directly compared to IC_{50} values. Taking into account the potential 'worst case scenario' in which the human EC_{50} is a half log unit of concentration below the obtained IC_{50} this would result in an EC_{50} value of 3.52 mg/mL. For the Soluplus based formulations this would mean that the drug release from the formulations would exceed the EC_{50} in less than 30 seconds, thus they would not effectively mask the taste of the drug. Conversely, the Eudragit E-PO based formulations would still remain well below the estimated EC_{50} at all times as the maximum drug release from the formulations was 1.33 ± 0.40 mg/mL and 1.55 ± 0.57 mg/mL for the 20% and 30% drug loaded extrudates respectively. This indicates that, even in this worst case scenario, the bitter taste of the drug would still be well masked by the Eudragit E-PO based formulations.

3.7 Electronic Tongue

The taste of the formulations was also assessed using the Insent electronic tongue. As noted in section 2.10.2, the electronic tongue can only assess the taste of liquids, thus for solid dosage forms it is necessary to use 'taste extracted water' i.e. the samples were stirred in 10mmol potassium chloride solution for 1 minute and the taste of the resulting solution was assessed. Principal component analysis was used to build a map from the sensor responses of the four sensors used for taste assessment (Figure 8). Solutions of pure isoniazid are located on the upper left hand side of the map. Euclidean distances were calculated to determine the differences between the pure drug, physical mixtures, placebo and extrudates, these are given in Table 4. The greater the Euclidean distance between the pure drug and the formulation, the greater the difference in taste.

Based on the drug release from the formulations discussed in section 3.6 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 1.32 for the 30% drug loaded extrudates in accordance with the increase in drug release from these formulations after 1 min. The

physical mixtures of Soluplus and isoniazid show smaller Euclidean distances than the extrudates, indicating that processing by hot melt extrusion improves the taste masking efficiency of the polymer.

Soluplus is a water soluble polymer (BASF Group, 2010) 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 (Vasconcelos et al., 2007). 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.

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 with a Euclidean distance of 8.97. This is to be expected given that this is the formulation with the lowest amount of drug release after 1 minute. The 30% Eudragit E-PO extrudate also has good taste masking efficiency with a Euclidean distance of 8.76. Again, the physical mixtures showed significantly lower Euclidean distances than the corresponding extrudates, demonstrating the usefulness of hot melt extrusion as a technique for taste masking. Ranking the formulations in order of taste masking efficiency it was found that;

20% Eudragit E-PO > 30% Eudragit E-PO > 20% Soluplus > 30% Soluplus.

The observed difference in taste masking efficiency between the two polymeric carriers can be attributed to their different water solubilities. As discussed in section 3.3, Soluplus is highly water soluble (BASF Group, 2010), while Eudragit E-PO is only soluble below pH 5 (Evonik Industries, 2014). The pH of the oral cavity is generally between 6.8 – 7.4 (Aframian et al., 2006). Eudragit E-PO is insoluble under these conditions, although it can swell, releasing a small amount of drug. Soluplus is

freely soluble at the pH values found in the oral cavity, thus allowing a large amount of drug (in excess of threshold bitterness value) to be released.

The taste masking efficiency was evaluated using two different methods, i.e. simulated oral dissolution and electronic tongue. The rank order of formulations in terms of aversiveness was found to be the same for both 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.

4. Conclusions

In summary, HME was successfully used to produce polymeric formulations of isoniazid with drug loadings of up to 30%. Rapid *in vitro* release of isoniazid is observed from Eudragit E-PO formulation at pH 1.2, particularly the 20% drug loaded formulation which has a release profile similar to that of isoniazid alone. BATA testing was used to determine an IC_{50} value for isonaizid of 11.1 mg/mL which was used as a threshold bitterness value for taste assessment of the formulations. 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 insolublle below pH 5, thus preventing release of the drug in the mouth. Overall, the 20% drug loaded Eudragit E-PO formulation is the most promising to mask the bitter taste of isoniazid without signifcantly affecting dissolution performance and, by extension, the bioavailability of the drug.

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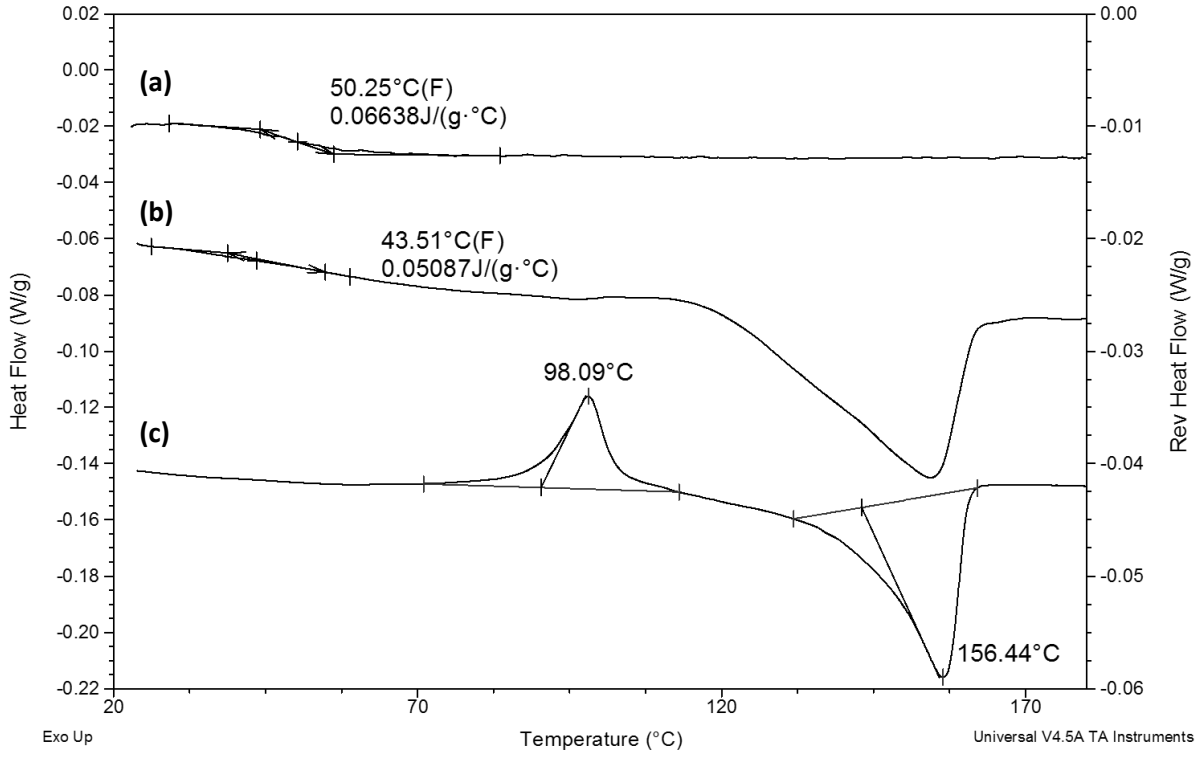


Figure 1 – DSC thermograms of Soluplus based extrudates (a) 20% w/w drug loading (reversing heat flow); (b) 30% w/w drug loading (reversing heat flow); (c) 30% w/w drug loading (total heat flow); processed at 140°C and 50 rpm (pinholed pans).

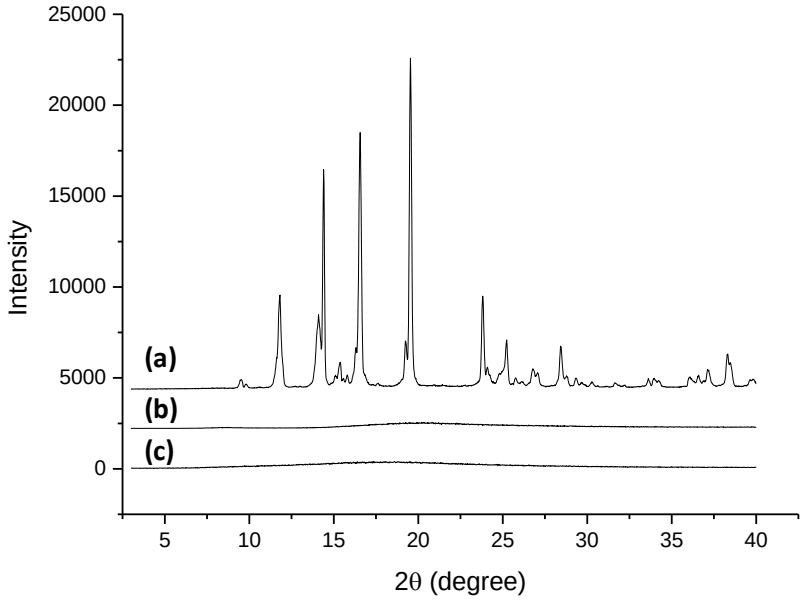


Figure 2 – PXRD of (a) pure Isoniazid (b) 20% w/w drug loaded extrudate; (c) 30% w/w drug loaded extrudate.

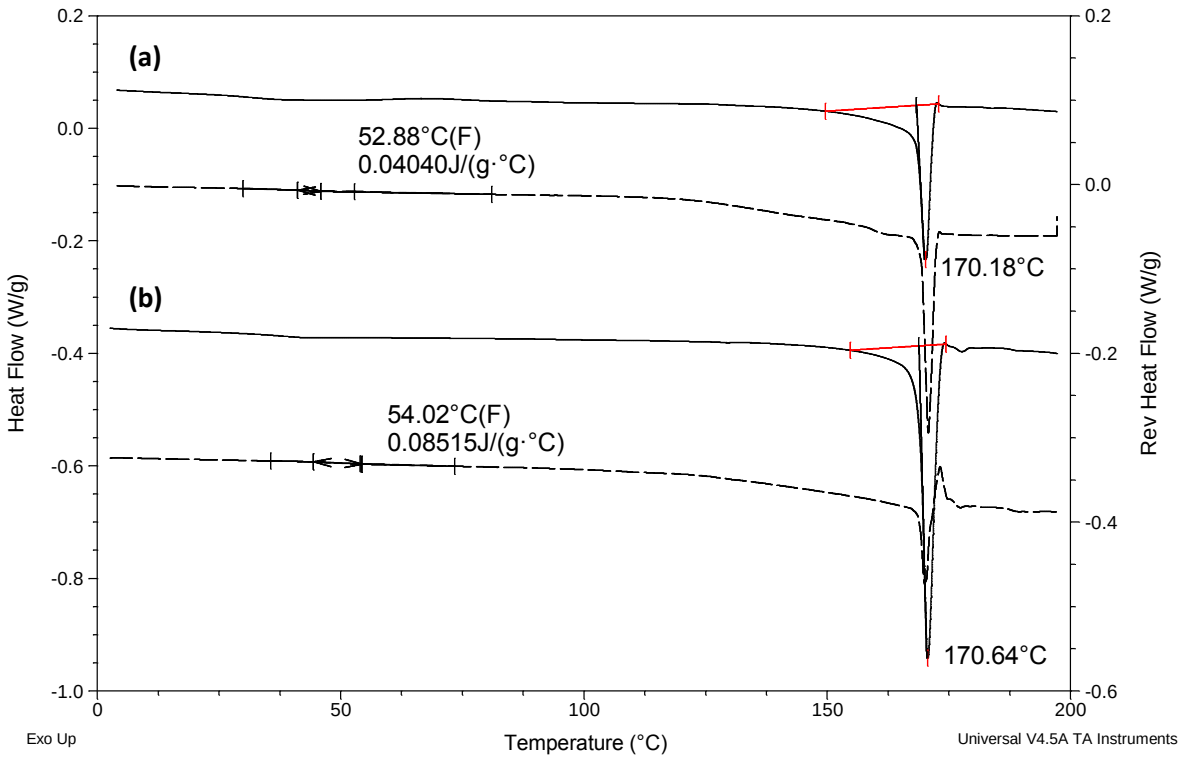


Figure 3- DSC thermograms showing total heat flow (solid line) and reversing heat flow (dashed line) of (a) 20% w/w EPO/Isoniazid extrudates; (b) 30% w/w EPO/Isoniazid extrudates processed at 130°C and 50 rpm (pinhole pans).

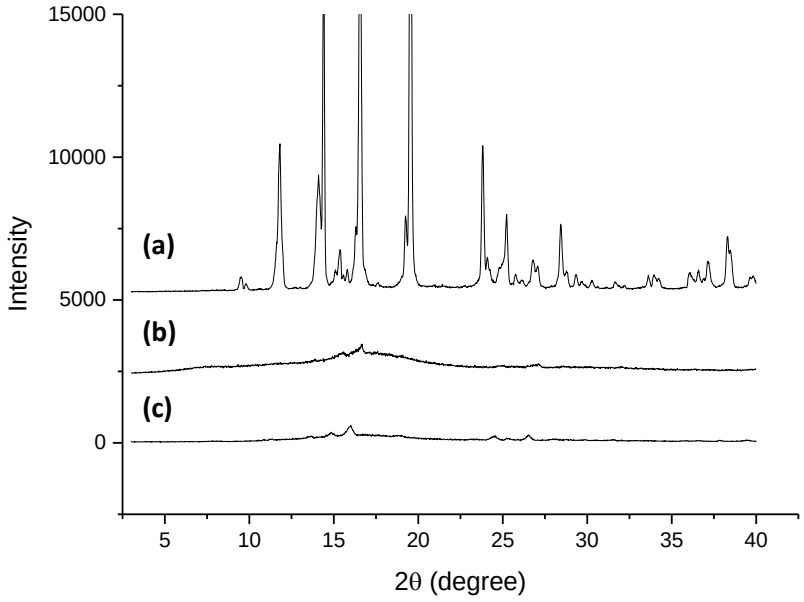
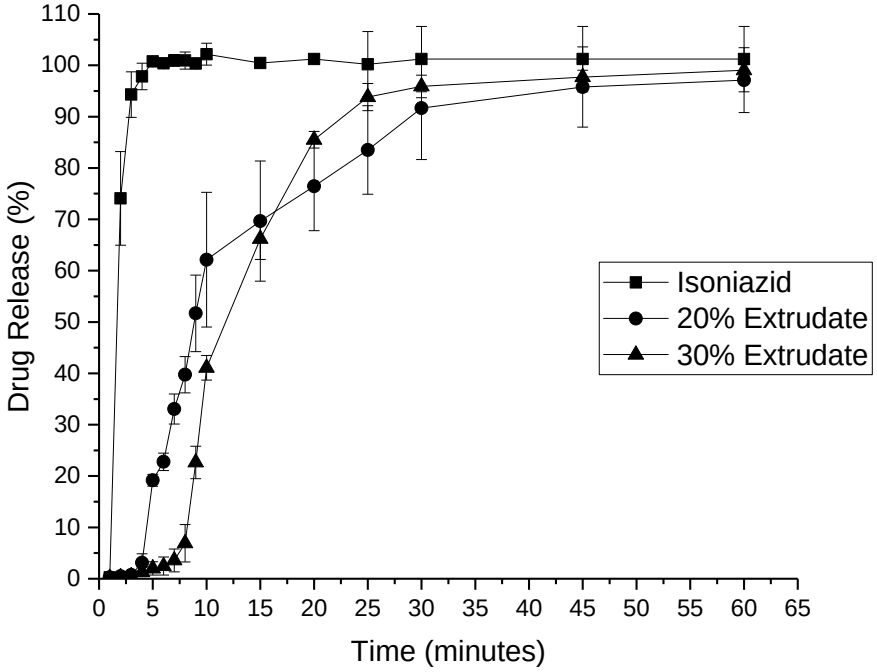


Figure 4 - PXRD of (a) pure isoniazid; (b) 20% w/w Eudragit E-PO/isoniazid extrudate; (c) 30% w/w Eudragit E-PO/isoniazid extrudate.

(a)



(b)

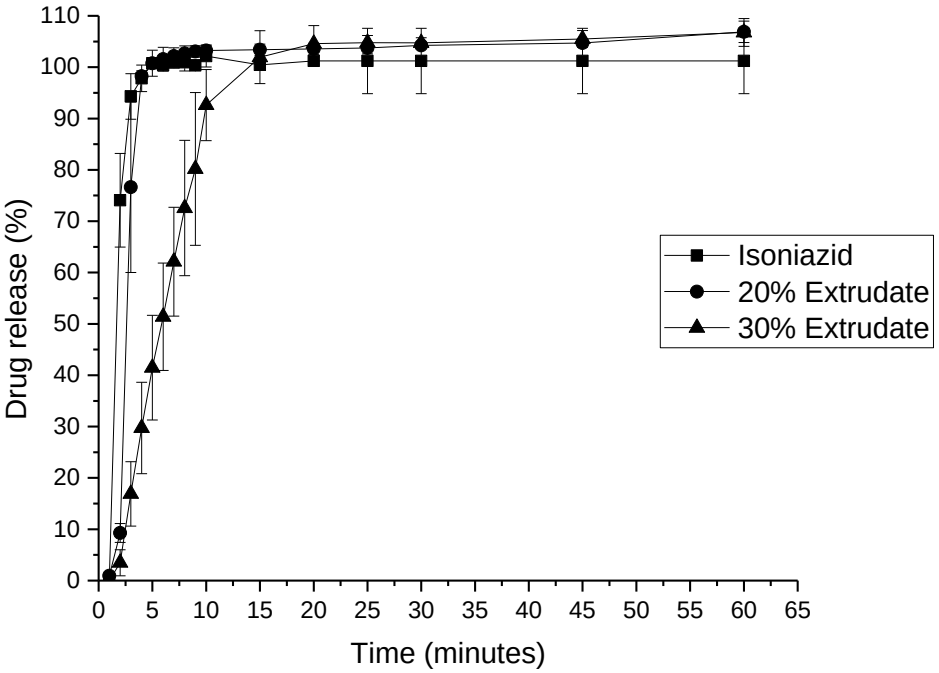


Figure 5– Dissolution profile of (a) Soluplus/isoniazid extrudates and (b) Eudragit E-PO/isoniazid extrudates compared to the dissolution profile of pure isoniazid.

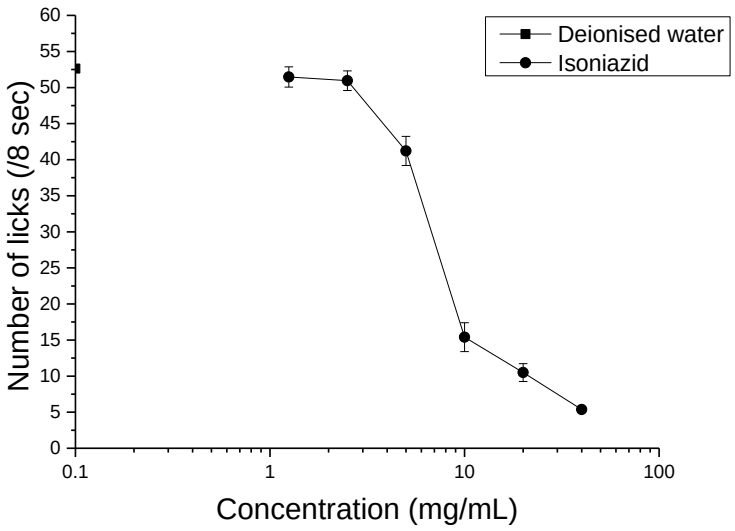


Figure 6 – Average number of licks recorded in 8 sec (± SEM) as a function of concentration (mg/mL) for isoniazid (n=10 rats).

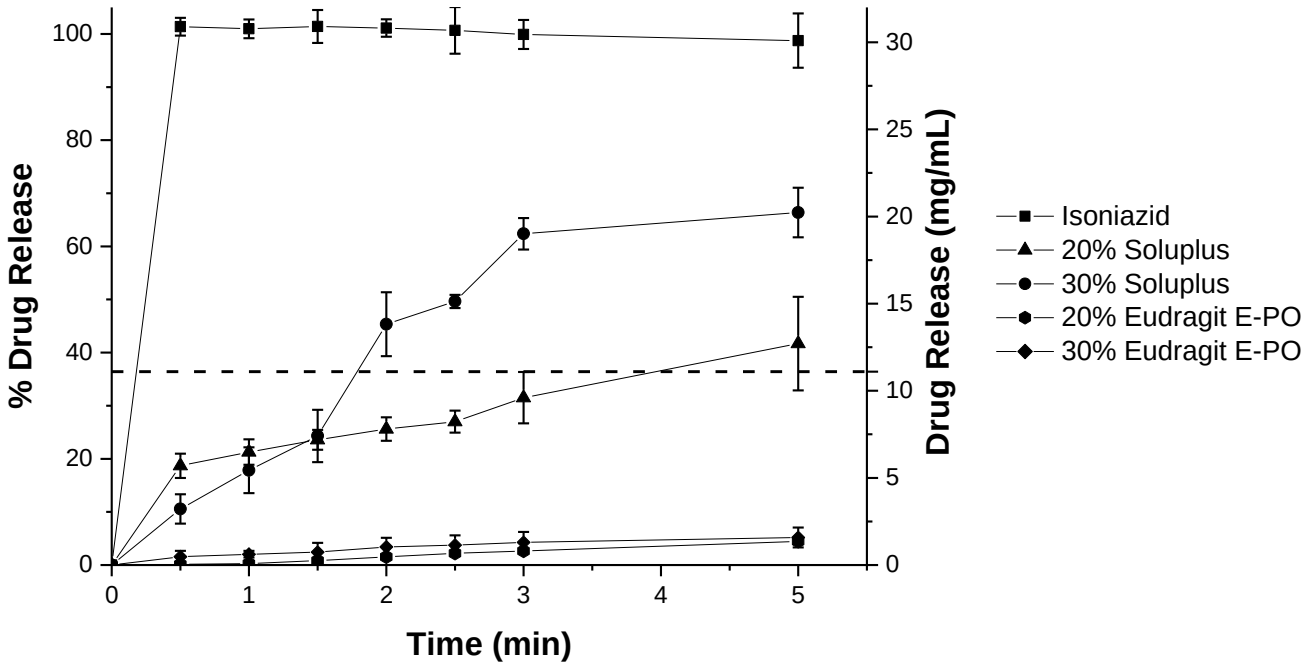


Figure 7 – Dissolution profile of isoniazid and formulations in simulated salivary fluid (SSF) at pH 7.4 and 37°C. Dashed line indicates IC_{50} value of isoniazid obtained from BATA testing (11.1 mg/mL)

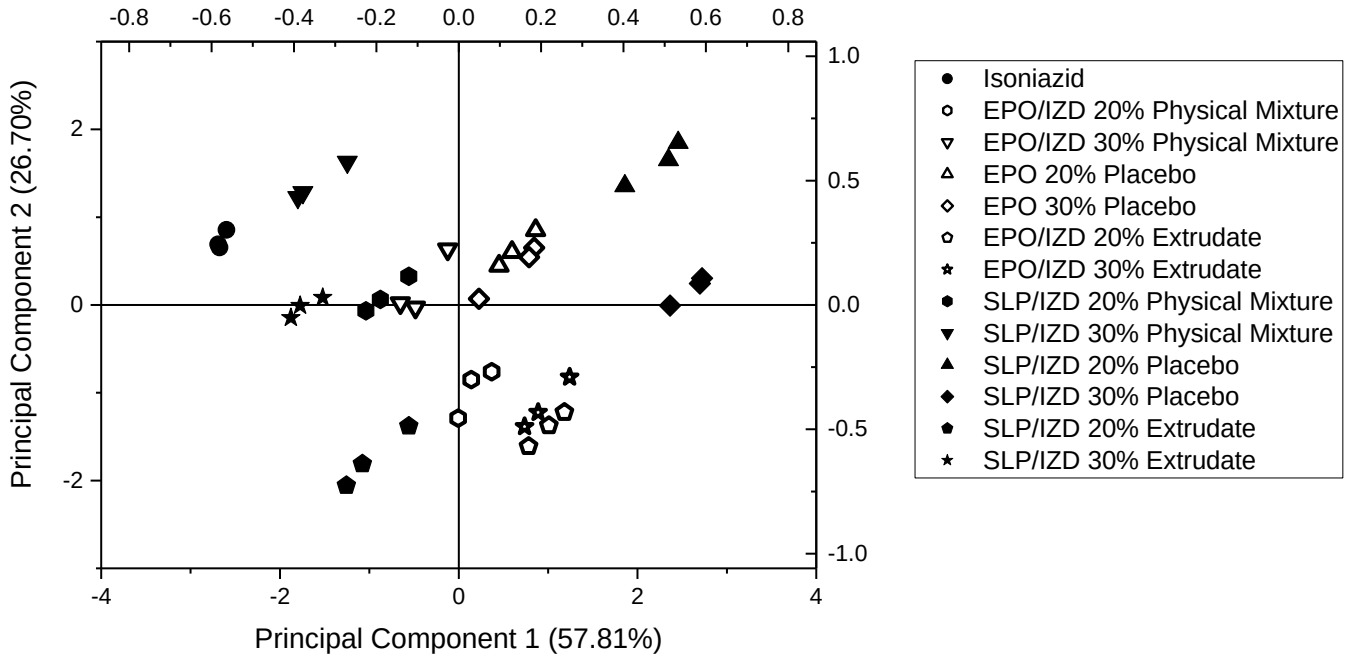


Figure 8 - Principal Component Analysis (PCA) of sensor responses after 1 minute of all extrudates compared with their physical mixtures and isoniazid alone. The PCA map is built using output values of 3 bitter taste sensors (AC0, AN0, C00) and astringency sensor (AE1).

Table 1 - Formulations and extrusion parameters used for hot melt extrusion.

Isoniazid (% w/w)	Soluplus (% w/w)	Eudragit EPO (% w/w)	Temperature (°C)	Speed (rpm)
20	80	-	140	50
30	70	-	140	50
20	-	80	130	50
30	-	70	130	50

Table 2 – Composition of Simulated Salivary Fluid.[47]

Compound	Concentration
Potassium phosphate monobasic	12 mM
Sodium chloride	40 mM
Calcium chloride	1.5 mM
Sodium hydroxide	to pH 7.4
Distilled water	to 1 L

Table 3 - Results of drug loading experiments for isoniazid containing formulations. Drug loading is given as mean percentage ± standard deviation

Polymer	Expected Drug Load (% w/w)	Actual Drug Load (% w/w)
Soluplus	20	20.38 ± 0.71
	30	29.47 ± 1.58
Eudragit E-PO	20	20.13 ± 0.48
	30	29.72 ± 0.32

Table 4 – Euclidean distances from each formulation, placebo and physical mixture 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.

		Euclidean Distance to Pure Isoniazid		
Polymer	**Drug Loading (%w/w)**	**Placebo**	**Physical Mixture**	**Extrudate**
Soluplus	20	12.27	1.87	4.51
	30	13.93	0.77	0.72
Eudragit E-PO	20	5.42	5.63	8.97
	30	5.41	5.60	8.76