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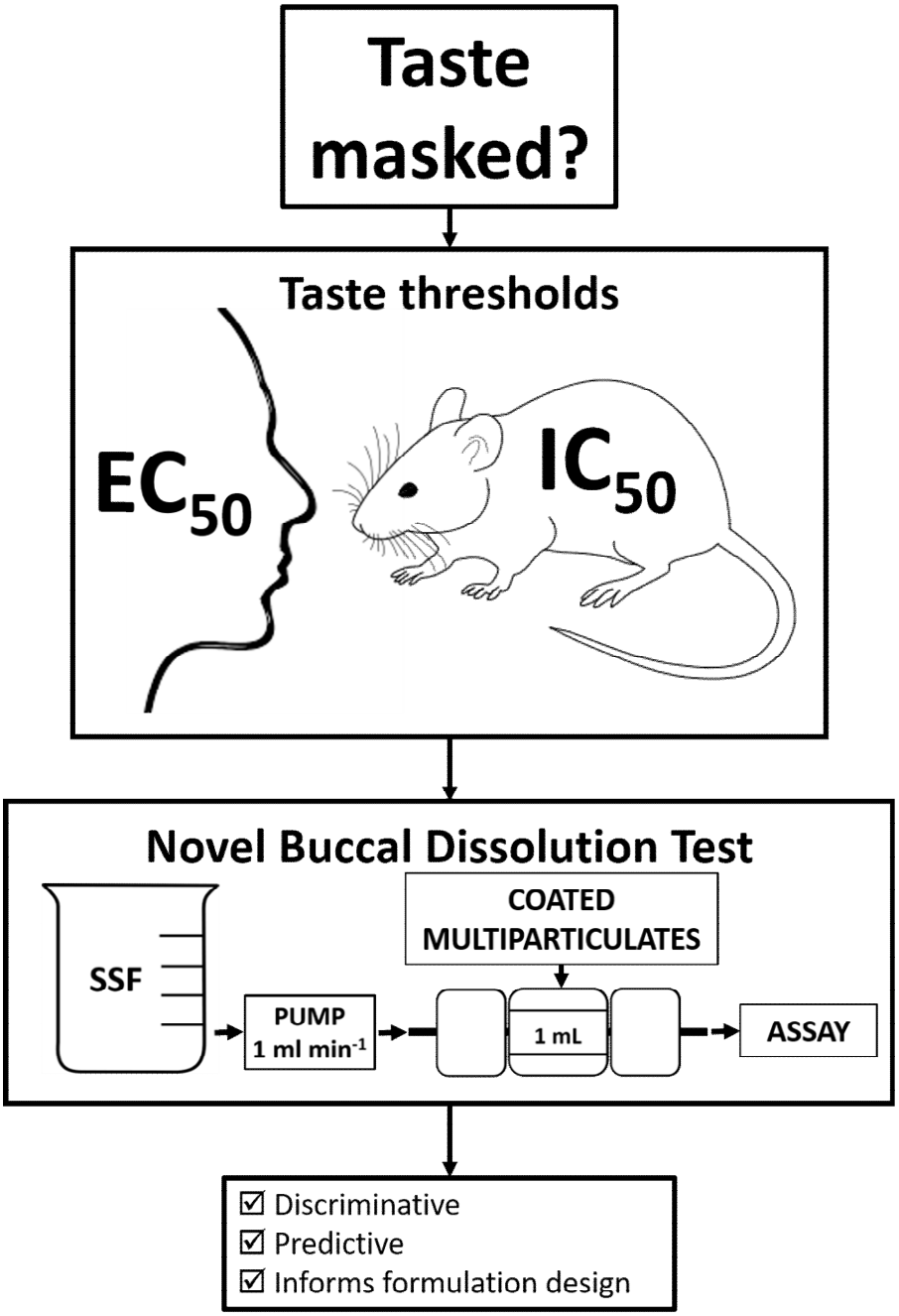
Title:
In vitro dissolution model can predict the *in vivo* taste masking performance of coated multiparticulates

Key words: taste, taste masking, human taste panels, brief-access taste aversion model, dissolution

Funding: This work was supported by the Engineering and Physical Sciences Research Council (EPSRC) Centre for Doctoral Training (CDT) in Advanced Therapeutics and Nanomedicines [grant number EP/L01646X/1] and Colorcon Ltd.

Visual Abstract



ABSTRACT

The majority of active pharmaceutical ingredients (APIs) are bitter. Therefore, compliance can be a problem where adequate taste masking has not been achieved; this is most problematic in paediatrics. Taste masking is thus a key stage during pharmaceutical development with an array of strategies available to the formulation scientist.

Solid oral dosage forms can be taste-masked quite simply by polymer coating, which prevents drug release in the mouth, without unwantedly impairing drug release further down the

gastrointestinal tract. At the early stages of pharmaceutical development, an *in vitro* method for assessment of taste masking is necessary given the lack of toxicological data preventing the use of human taste panels. Currently there is no such tool allowing prediction of taste masking efficiency.

In this study, drug dissolution in the context of aversive taste thresholds was proposed as a means to bridge this knowledge gap. Thus, a biorelevant buccal dissolution test was developed in which previously determined taste thresholds *in vivo* were used to evaluate taste masking efficiency: if drug release exceeded said thresholds, the formulation was deemed to be poorly taste-masked, and vice versa. This novel dissolution test was compared to the USP I (basket) dissolution test, and the biopharmaceutical implications of taste masking were also assessed by performing USP I (basket) dissolution testing in simulated gastric fluid (SGF).

Chlorphenamine maleate (CPM), a model bitter BCS class 1 API, was layered onto sugar spheres and taste-masked using polymer coatings. An array of coating technologies were employed and assessed single blinded: two pH independent water insoluble coatings (Surelease®:Opadry® at 8, 12 and 16% weight gain and Opadry EC ® at 4, 6 and 8% weight gain) and a pH dependent water insoluble reverse enteric coating (developmental fully formulated system based on Kollicoat® Smartseal 100P® at 10% weight gain).

Both the biorelevant buccal and the USP I dissolution tests were capable of discriminating between both type and level of coating used. However, only the buccal dissolution test was able to provide absolute quantification of the level of taste masking achieved in the context of previously determined taste thresholds, while the USP I test merely provided a relative comparison between the different technologies assessed. When the release data from the buccal test were assessed in parallel to that in SGF, it was possible to predict *in vitro* optimised taste masking without compromising bioavailability. The fully formulated system based on Smartseal 100P® was identified as the most effective coating and Surelease:Opadry® the least effective.

The developed methodology provides true insight for the formulator, enabling more informed patient-centric formulation decisions, better taste masking and ultimately more effective medicines.

Introduction

Medicines taste bad. Indeed, this is particularly pertinent to children who are often provided with poor-tasting liquid dosage forms as an alternative to more easily taste masked solid dosage forms. Given that children may lack the understanding of their adult counterparts that medicines are of benefit and thus worth the momentary displeasure upon administration, compliance may be affected. Indeed, in a recent survey of 153 children [1], the principle reason for medicine rejection was confirmed to be bad taste.

The importance of the taste of medicines in this population has been acknowledged by pharmaceutical regulatory authorities. The European Medicines Agency (EMA) and the Food and Drug Administration (FDA) now require the inclusion of Paediatric Investigation Plans (PIPs) and Paediatric Study Plans (PSPs), respectively, as part of a new drug application (NDA), unless there are grounds upon which such studies can be waivered, e.g. a lack of paediatric indication [2]. Therefore, there is no longer just a need to produce better tasting medicines for children; now, there is also a requirement.

There are several methods that can be employed to mitigate poor tasting medicines, and all fall under the umbrellas of either masking the taste of the bitter active pharmaceutical ingredient (API) or reducing the contact of the API with the taste receptors. Indeed, one could employ bitter blockers, taste modifiers, sweeteners, flavours, solubility-modification of the API, ion-exchange resins, cyclodextrins or different physical barriers such as polymer film coats or lipidic barrier systems [3]. However, there is a combination of technical, safety and regulatory challenges for the use of excipients in paediatric preparations. Therefore any additional excipients can yield issues given the associated regulatory constraints, particularly when considering use in younger children [3] while the use of more complicated techniques introduces challenges in manufacture and product development, which may affect the commercial viability of a product [4].

Multiparticulates as a dosage form is a platform technology providing means to overcome the inability of children to swallow monolithic dosage forms, the innate foul taste of many APIs and the aforementioned complications of producing a commercially viable taste masked formulation [5]. These are drug delivery systems (DDSs) comprised of multiple solid units, such as pellets or minitablets [6]. Such systems can easily be coated for taste masking. A variety of coating systems are available, which differ in terms of their composition and their water-solubility, either

dependent or independent of pH of the media. These coating materials can include lipids, sugars and polymers, which include water insoluble, water soluble and blends of water insoluble and soluble polymers with or without organic and inorganic pore formers. Water insoluble polymers include both pH dependent and pH independent water insoluble polymers. Further, the pH dependent water insoluble polymers can be further classified based on their release profile within the stomach, and include reverse enteric, enteric and their combinations [7].

However, taste masking using coating technologies yields one challenging question: how can we be sure that adequate taste masking has been achieved, particularly during early drug development when insufficient toxicological data prevents the use of human taste panels? Dissolution testing may provide some of the answers given that it stands to reason that only that which is dissolved is capable of interacting with the taste receptors within taste buds of the tongue and thus elicit a taste. However, there is currently no such dissolution test that replicates closely enough the human oral cavity and enables the prediction of *in vivo* taste masking efficacy. Such a test would have to enable drug release to be assessed in the context of taste and therefore linked to previously determined taste thresholds with conditions replicative of the human oral cavity, namely volume (1-2 mL), temperature (35-36 °C), pH (5.7-7.5) and osmolarity (50-100 mOsmole/Kg) of saliva [8,9]. Furthermore, such a test would have to be able to discriminate between different coating technologies, predictive of taste and inform formulation design.

There is currently no standardised pharmacopoeial dissolution test for taste-masked dosage forms, and as such there is great variation among the methods employed by researchers working in this area [8]. The methods identified differ in terms of the media employed, with phosphate buffer at pH 6.8 being frequently observed [10–14], while some researchers have simply opted for water [15–18]. The pH of the media has also been debated with researchers employing phosphate buffers at varied pH values, from 5.6-8.0 [19–22].In all instances, the volume of media used was 900 mL, in line with conventional dissolution testing and is thus physiologically irrelevant, particularly given that no increase in dose was observed to account for this volume discrepancy. Better attempts have, however, been observed with Guhmann *et al.,* who used simulated salivary fluid (SSF) at pH 7.4 as the dissolution medium and a volume of 50 mL, which is improved compared to the aforementioned but still lacks relevance to the human oral cavity [23]. Thus, it is clear that there is no concordance among researchers assessing taste-masked dosage forms, but it stands to reason that to assess taste-masking, the scientist must replicate the conditions of the human oral cavity as closely as possible [8].

The present study aims to evaluate a novel flow-through dissolution column replicative of the conditions experienced by a dosage form in the human mouth. Taste masking efficacy of various coating technologies was assessed by linking drug release data within this novel flow-through dissolution column replicative of the conditions experienced by a dosage form in the human mouth to the aversiveness taste thresholds of the model bitter API acquired from a human taste panel and the rat brief–access taste aversion (BATA) model [24]. This was compared to traditional pharmacopoeial dissolution methods.

Materials and Methods

Materials

The chlorphenamine maleate (CPM) used in the BATA was purchased from Sigma Aldrich (St Louis, Missouri, USA), while that for the human taste panel was purchased from Fagron (Rotterdam, The Netherlands). The CPM loaded multiparticulates were prepared as described below in methods. Potassium dihydrogen phosphate analytical reagent grade, acetonitrile HPLC gradient grade, methanol HPLC grade, orthophosphoric acid HPLC electrochemical grade and sodium hydroxide pellets from Fisher Chemical (Leicestershire, England); sodium chloride from Fagron (Newcastle-upon-Tyne, England); calcium chloride from Sigma-Aldrich (St. Louis, USA); dipotassium hydrogen phosphate trihydrate reagent grade from Alfa Aesar (Massachusetts, USA); triethylamine from Alfa Aesar (Heysham, England).

Methods

1] Taste Thresholds

a) Rat Brief-Access Taste Aversion model (BATA)

BATA procedure

During the BATA procedure, ten rats were deprived of water for 22 hours prior to commencement of the experiment. A lickometer (Davis MS-160, DiLog instruments, Tallahassee, Florida, USA) was used to record the number of licks taken by each rat for each presented sample. Each rat underwent a single training day, in which all presented samples contained water, and two test days, during which the chlorphenamine maleate samples were presented at concentrations ranging from 0.005 to 18 mg/mL in triplicate and at random. During the testing days, the samples were presented to the rat for 8 seconds (S) after the initial lick, followed by a 2 s water rinse presentation. Between each presentation, a 5 s inter-presentation interval was observed [24]. All the procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668).

Data analysis

Data were visualised as notched box-plots consisting a central line indicative of the median, the box indicative of the interquartile range and the whiskers being 1.5 times the 25th and 75th percentile, respectively. The notches are indicative of the 95% confidence interval of the median,

such that if the notches of respective boxes do not overlap, there is strong evidence that their medians differ significantly – see figure 1.

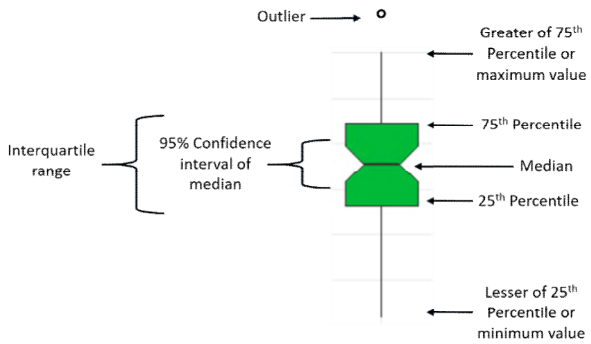


Figure 1 The elements of a notched box-plot

The distribution of the data was assessed using the Shapiro-Wilk test [25]: if non-normal, statistical significance between concentration ratings was determined using Kruskal-Wallis rank sum test followed by post-hoc analysis using Xin Gao et al's non-parametric multiple test procedure [26]. If the distribution of data was normal, the one way analysis of variance (ANOVA) was performed with Tukey's honest significant difference (HSD) as post-hoc analysis. All data visualisation, analysis and statistics were performed using R software (open source). The data were also pooled and used to calculate the IC_{50} using non-linear mixed effects (NONMEM) tool (version 7.3, ICON Development Solutions, Dublin, Ireland) [27].

b) Human Taste Panel

Participants

Twenty-four healthy volunteers between the ages of 18 and 47 years old (median 22 years old; 12 males and 12 females) were enrolled in a randomised single-blind study. The protocol was approved by the UCL Research Ethics Committee (REC) (ID: 4612/017).

Taste panel procedure

The 'swirl and spit' methodology as described in [24] was employed, whereby the participants were presented with 10mL of the following CPM concentrations: 0.05, 0.15, 0.5, 1.5 and 2.4 mg/mL (selected based on aversiveness findings from rat BATA study and toxicity considerations), which they were then instructed to swirl around their mouth for 10 seconds, before spitting. The solutions – each labelled with a random 3-digit code – were presented at random and in triplicate, with a 10-minute washout period between each presentation to allow for taste neutralisation.

During this inter-presentation interval, participants were also able to consume a plain, non-salty cracker in order to neutralise their palate (Figure 2).

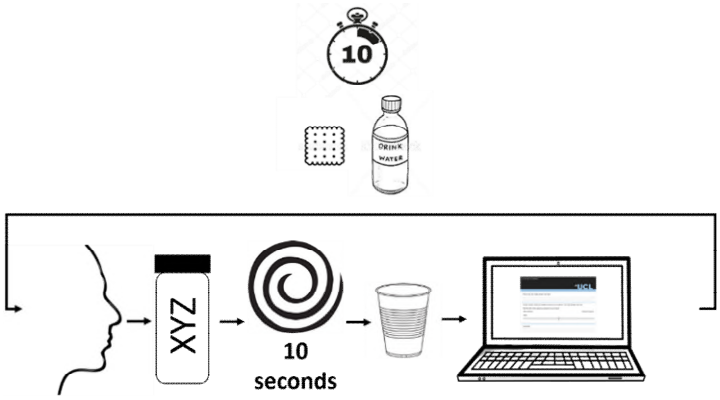


Figure 2 Flow diagram representing the 'swirl and spit' methodology steps in a human taste panel

Participant assessment of each sample was achieved using the online survey software Qualtrics (Provo, Utah, USA; version: November 2017), which calls on the participant to rate a given sample's intensity on a 100 mm visual analogue scale (VAS) from 'not aversive' to 'extremely aversive'.

Data analysis

The human data were treated and analysed in an identical way to the rat data, with the exception of the taste threshold, which was calculated in an identical way using NONMEM, but is referred to as the EC_{50}.

2] Taste masking of CPM

Chlorphenamine maleate (CPM), a BCS class 1 API, was used as the model bitter drug [3,28,29]. Sugar sphere pellets (Suglets®; 850-1000µm) were drug layered at 0.03g/1g and used in this study. Two coating system approaches were used to coat the drug layered pellets: two pH independent water insoluble coatings (Surelease:Opadry® and Opadry EC ®[30,31]) and a pH dependent water insoluble reverse enteric coating, which prevents release at neutral pH of the oral cavity, but allows release at low gastric pH [32] (developmental fully formulated system based on Kollicoat Smartseal 100P® [33,34]).

Drug layering and barrier membrane taste mask coating of the sugar spheres were performed at Colorcon. The Surelease:Opadry®, an aqueous ethylcellulose-based coating system, was applied using the Glatt GPCG 1 Fluid Bed coating machine with: inlet temperature of 60-69°C, product temperature of 45-47°C, spray rate of 6.5g/min and 95-103 m^3/hr airflow. The Opadry EC ®, an ethylcellulose-based coat, was applied using the Vector VFC Lab 1 with an industrial methylated spirit (IMS):water (90:10) solvent, an inlet temperature of 40°C, a product temperature of 32-35°C, a spray rate of 4.7 g/min and an airflow of 70 m^3/hr. The coating with Kollicoat Smartseal 100P® – an aqueous dispersion of a co-polymer comprising methyl methacrylate (MMA) and diethylaminoethyl methacrylate (DEAEMA) was applied using the Vector VFC Lab 2 with an isopropyl alcohol (IPA):water (85:15) solvent, an inlet temperature of 38-44°C, a product temperature of 32-33°C, a spray rate of 3.3 g/min and an airflow of 75 m^3/hr. Samples were taken at intervals according to the desired theoretical % weight gain for each coating system as shown in Table 1.

These film coats were applied at various thicknesses as expressed in % theoretical weight gains (Table 1.) The research team received them labelled randomly A to G to perform the dissolution experiments blindly.

Table 1 Coatings types and coating levels investigated

Coating	% Weight gain
Opadry EC®	4
	6
	8
Developmental Smartseal® coating	10
Surelease:Opadry® (70:30)	8
	12
	16

3] Drug release assessment

a) USP I (Basket) Dissolution

The USP I (basket) apparatus was used to assess blindly the drug release from the CPM layered sugar spheres using a *conventional* dissolution test. The Caleva ST7 dissolution bath was used, with basket rotation set to 50 rpm and temperature to 37°C. Each dissolution vessel (n=6) contained 900 mL of phosphate buffer (adjusted to pH 6.5) as dissolution media, and each basket was loaded with 600 mg of CPM sugar spheres for assessment. 2 ml of media was sampled with volume replacement and assayed at 0, 2, 4, 6, 8, 10, 20, 30, 45 and 60 minutes.

Sample assay

All samples were assayed using ultraviolet (UV) spectrophotometry at 261 nm. Prior to assay, each sample was filtered using a 0.45 µm membrane filter. A calibration curve with an R^2 of 0.9999 was used to determine the CPM concentration within each sample.

Data analysis

For taste masking consideration, the concentration of drug released within the simulated oral cavity is of greatest concern and most relevant in terms of taste. As saliva is constantly produced in the mouth and swallowed, therefore the dissolution data were generated as non-cumulative concentrations over time. This was to simulate the concentrations likely to be observed in the oral cavity over time, thus providing the best means of potentially predicting the taste. The efficacy of taste masking of all coated beads formulations was tested using this method. The mean concentration of drug at each time point was calculated (n=6). The standard deviation and standard error of the mean were also calculated.

b) Novel Dissolution Apparatus

A novel dissolution method was developed in which simulated salivary fluid (SSF) (table 2) was used as the dissolution medium and pumped through a bespoke dissolution column using a peristaltic pump at a rate of 1 mL min^{-1} (Figure 3). The column was manufactured in house using acrylic tubing. The column was loaded by removing one end of the bespoke column (shown in figure 3 as squares either side of the central column lumen) and hand-loading CPM-loaded multiparticulates (600 mg) (drug loading: ~30 mg/g) into the lumen, which has a calculated internal volume of 1 mL, before re-sealing the column and attaching it to the peristaltic pump. The multiparticulates are retained in the column by wire mesh discs placed either side of the lumen. Samples (n=6 per coating) were taken at 60, 80, 100, 120, 180, 240 and 300 s and assayed by high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector.

Table 2 Composition of SSF (in full) as per Guhmann et al. [23]

Compound	Concentration
Potassium dihydrogen phosphate	12 mM
Sodium chloride	40 mM
Calcium chloride	1.5 mM
Sodium hydroxide	To pH 7.4
Deionised water	To 1 L

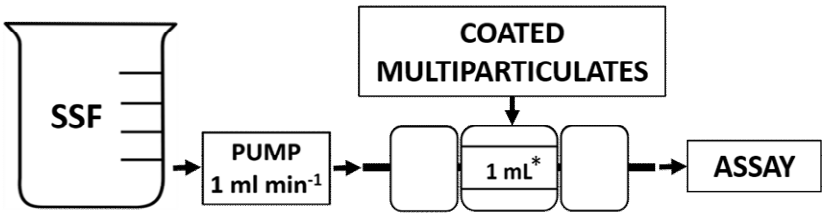


Figure 3 Flow diagram representing the biorelevant buccal dissolution test (*calculated internal volume of the column)

Sample assay

Samples (n=6 per coating) were taken at the aforementioned time points and diluted 15-fold with 20 % v/v methanol before being analysed using HPLC-UV, equipped with an Agilent Technologies 1200 series degasser, quaternary pump, auto-sampler, thermostatted column compartment set at 40°C and a variable UV wavelength detector set to a wavelength 265 nm. Chromatography was performed using a Synergi 4u Polar-RP 80A column (4µm, 150×4.60 mm). Two mobile phases were used:

- Mobile phase A was potassium phosphate buffer – containing potassium dihydrogen phosphate (2.6 ± 0.2 g/L), dipotassium hydrogen phosphate trihydrate (1.4 ± 0.2 g/L), acetonitrile (50 ml/L), triethylamine (1.5 ml/L) and adjusted to pH 6.5 with orthophosphoric acid
- Mobile phase B was acetonitrile. An isocratic method was employed in which mobile phases A and B were set to 35% and 65%, respectively at a flow rate of 1.0 mL min^{-1} and a needle wash containing 100% methanol.

The volume of each sample injected was 10 µL. The retention time was 5.5 mins.

Data analysis

Average CPM concentration (n=6) were presented in the same way as that for the USP I (basket) dissolution test for reasons outlined in section 3a: data analysis.

c) Drug release post taste masking - biopharmaceutical consideration

In order to ascertain the biopharmaceutical implications of taste masking, the drug release from 600 mg CPM coated sugar spheres (n=6 per coating) was assessed in simulated gastric fluid (no

pepsin) (SGF) following soaking in 10 mL SSF for 1 minute. The Caleva ST7 dissolution bath was used, with basket rotation set to 50 rpm and temperature to 37°C. Following soaking, the entire contents were added to 890 mL of SGF. 2 ml of media was sampled – with volume replacement – and assayed at 0, 2, 4, 6, 8, 10, 20, 30, 45 and 60 minutes.

Sample assay

All samples (n=6 per coating) were assayed using ultraviolet (UV) spectrophotometry at 261 nm. Prior to assay, each sample was filtered using a 0.45 µm membrane filter. A calibration curve with an R^2 of 0.9999 was used to determine CPM concentration within each sample.

Data analysis

Average cumulated CPM concentration (n=6) were presented as cumulative concentration against time and plotted alongside the USP I dissolution data as a means of assessing change, if any, in release behaviour as the formulation enters the simulated stomach.

Results

Taste Thresholds

Rat BATA

Rat BATA analysis of increasing concentrations of CPM in water was successfully carried out with the results shown in figure 4. Gao's post-hoc analysis revealed that concentrations ranging from 0.005-0.15 mg/mL did not differ significantly from each other or from water ($p>0.05$), while concentrations exceeding 0.5 mg/mL did differ significantly from water. Concentrations 0.5 and 1.5 mg/mL differed significantly from all other concentrations assessed ($p<0.05$), while concentrations 3-18 mg/mL differed significantly from all other concentrations assessed ($p<0.05$), but did not differ significantly from each other ($p>0.05$).

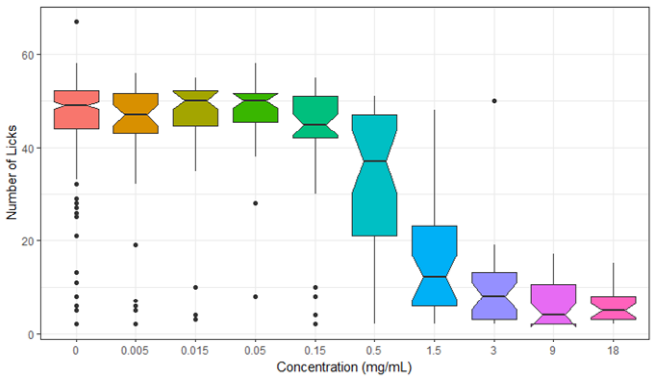


Figure 4 Rat response (number of licks) to increasing concentrations of CPM in water.

Figure 5 demonstrates the average response of the rats to increasing concentrations of CPM. Nonmem was also used to ascertain the IC_{50} – the concentration eliciting half the maximum (water) lick response of the rats[27]. This was found to be 0.788 mg/mL, and formed the rat taste threshold that was later utilised in the taste assessment of the CPM sugar spheres by dissolution.

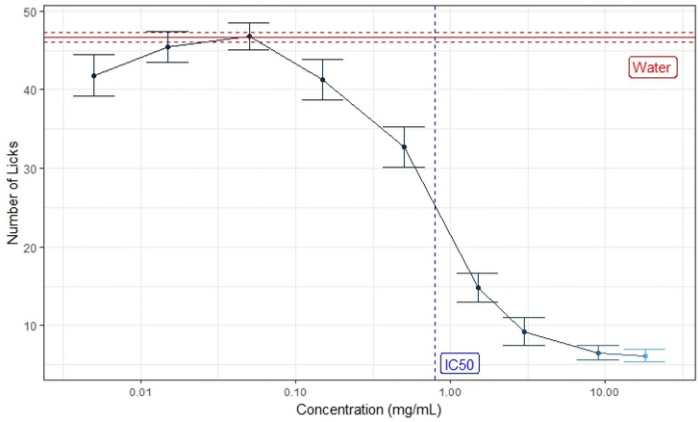


Figure 5 Mean number of licks [+/- standard error of the mean (SEM)] as a function of increasing CPM concentration (mg/ml). *The water control is shown as a solid red line (mean number of licks), with the SEM as dashed red lines. The IC_{50} is shown as a vertical blue dashed line.*

Human Taste Panel

The human taste panel assessing increasing concentrations of CPM was successfully carried out, with results shown in figure 6. Significant differences were observed between all concentrations, with the exception of the uppermost concentrations (1.5 and 2.4 mg/mL), and this was confirmed with Gao's post-hoc analysis ($p<0.05$).

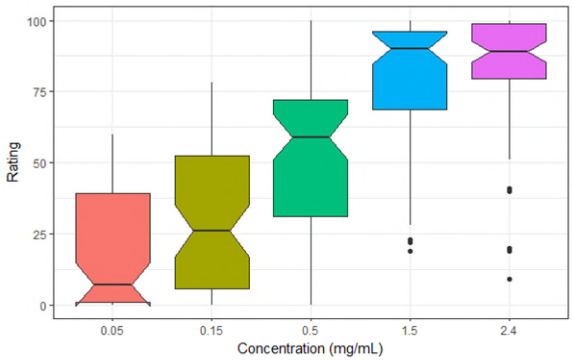


Figure 6 Participant aversiveness response to increasing concentrations of CPM in water.

Nonmem was used to calculate the EC_{50} – see methods section – which was found to be 0.506 mg/mL. This formed the human taste threshold that was later used to assess the taste of the CPM sugar spheres by dissolution.

Taste masking assessment of CPM

USP I (Basket) Dissolution

Dissolution testing using a conventional USP I system was blindly conducted on all coated CPM layered sugar spheres in order to set the benchmark for future comparison. Figure 7 summarises the findings and includes the taste thresholds, IC_{50} and EC_{50}, indicated as grey and black dashed lines, respectively. As shown in figure 7, when drug release is considered in the context of the human and rat aversiveness thresholds, taste masking efficacy as a function of coating cannot be determined.

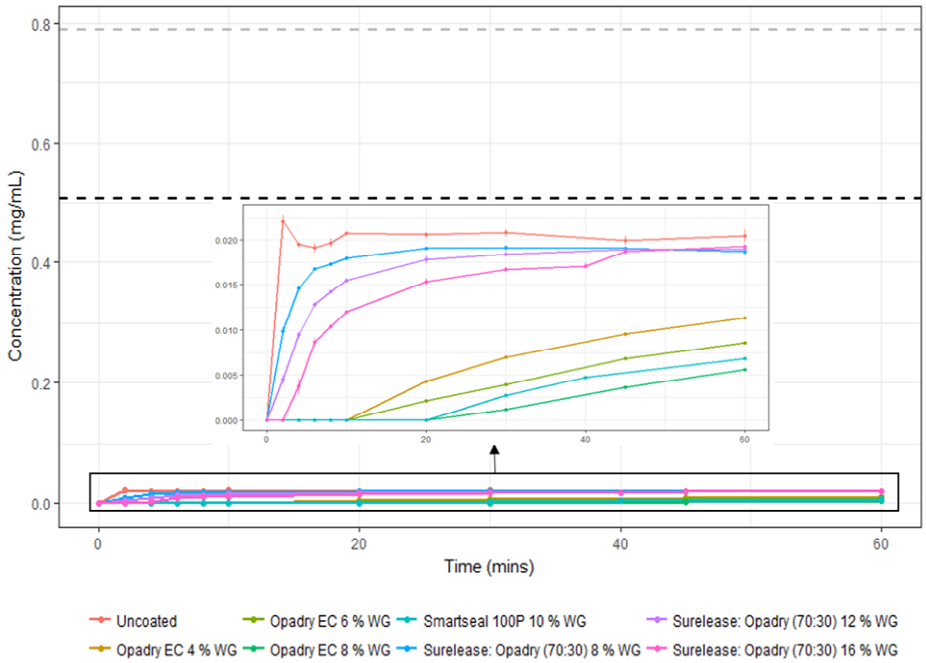


Figure 7 Drug release [mean +/-SEM] from CPM loaded sugar spheres with varying coatings in PBS using USP I dissolution apparatus. Taste thresholds are shown as grey and black dashed lines; the IC_{50} and EC_{50} respectively. The inlay magnifies the plot for relative comparison.

However, when the taste thresholds are disregarded as shown in the inlay in figure 7, distinction between both the type and extent (% WG) of coating is possible. Throughout the entire 60 min duration of the experiment, the best performing coating was Opadry EC ® at a level of 8% WG, minimising drug release to such an extent that a final concentration of approximately 0.005 mg/mL was observed and negligible release was observed up to 20 minutes (Figure 7 inlay). Pellets coated with developmental formula using Smartseal 100P® also showed negligible release up to 20 minutes, but released drug at a greater rate than Opadry EC ® 8% WG, but was nevertheless the second best performing coat under scrutiny. As the Opadry EC ® coating WG (%) was reduced, the

amount of drug released, increased. However, the lowest % WG Opadry EC ® coating was still sufficient to minimise drug release to a level significantly lower than the highest % WG Surelease:Opadry® (70:30). Nonetheless, an inverse relationship between Surelease:Opadry® (70:30) coating level and drug release was observed up to 40 minutes, with Surelease:Opadry® (70:30) 8% WG allowing the greatest amount of drug release Surelease:Opadry® (70:30) 16% demonstrated a lag time of approximately 2 minutes, followed by drug release. After 40 minutes, no significant difference in drug release was observed for all coating levels of Surelease:Opadry® (70:30).

Novel Dissolution Apparatus

A bespoke flow-through oral dissolution apparatus was used to evaluate the release of CPM from sugar spheres coated with different coating technologies and to different extents. Figure 8 summarises the findings from each coating including the uncoated sugar spheres, with the taste thresholds – EC_{50} and IC_{50} indicated as black and grey dashed lines, respectively, thus enabling drug release to be evaluated in the context of taste. It shows that the dissolution test was capable of distinguishing between both different coating technologies and coating levels. The greatest CPM release was observed from the uncoated sugar spheres, with concentrations exceeding 10 mg/ml seen within the first 75 seconds which, in the context of the EC_{50} and IC_{50}, indicate a very aversive taste. The sugar spheres coated with Surelease:Opadry® (70:30) also demonstrated CPM release exceeding both the EC_{50} and IC_{50}, thus indicating insufficient taste masking. An inverse relationship between coating level and CPM release was observed for this coating technology, allowing an approach to achieve acceptable taste masking by either higher coating weight gain or reduce the amount of pore-former (to reduce permeability of the film). Sugar spheres coated with Opadry EC ® and Smartseal 100P® did not allow release of CPM sufficient to exceed the EC_{50} or IC_{50}, thus indicating that adequate taste masking has been achieved.

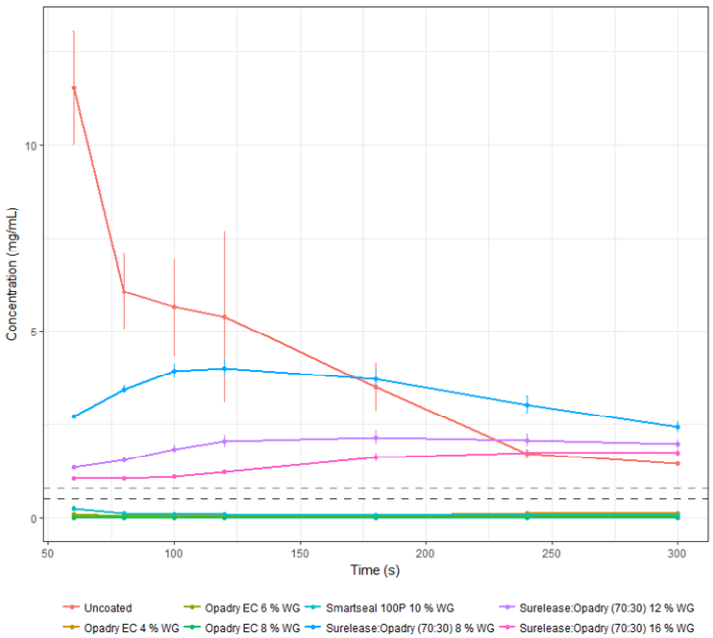


Figure 8 CPM release (mean +/- SEM) as a function of both type and level of coating technology. *The taste thresholds are represented as grey and black dashed lines; the IC_{50} and EC_{50} respectively.*

Opadry EC ® coated CPM sugar spheres

As indicated previously, the sugar spheres coated with Opadry EC ® did not allow CPM release sufficient to exceed the EC_{50} or IC_{50}, thus indicating adequate taste masking. The greatest CPM release was observed from the lowest coating level: 4 % WG at 0.13 mg/ml, while the highest coating level – 8 % WG – did not exceed 0.015 mg/ml, thus indicating exceptional taste masking (Figure 9).

Thus, the dissolution test enabled distinction between increasing levels of coat (% WG), with an inverse relationship between % WG and CPM release observed.

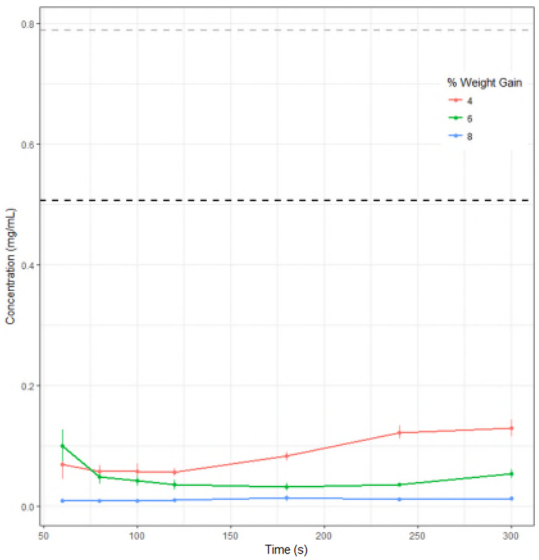


Figure 9 CPM release (mean +/- SEM) as a function of Opadry EC ® coating level. *The taste thresholds are represented as grey and black dashed lines; the IC_{50} and EC_{50} respectively*.

Surelease:Opadry® (70:30) coated CPM sugar spheres

Surelease:Opadry® (70:30) was observed to function inadequately as a taste masking coat with all coating levels allowing CPM release sufficient to exceed both the EC_{50} and IC_{50} (Figure 10). Indeed, a burst release of CPM was observed with the 8 % WG coating level, peaking at a mean of 4 mg/mL at 120 s. However, CPM release did reduce as a function of coating level, with the lowest CPM release observed with the highest coating level – 16% WG. Indeed, at a coating level of 16 % WG, the CPM release did not exceed 1.75 mg/mL over the course of the experiment. For this coating system to produce acceptable taste masking for CPM loaded pellets, a higher coating weight gain or a different Surelease:Opadry® ratio would be required. Thus, this provides a further demonstration of the ability of the dissolution test to distinguish between different coating levels.

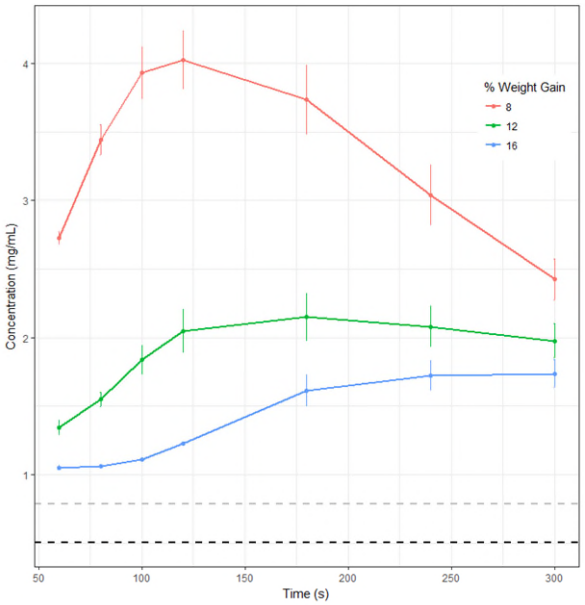


Figure 10 CPM release (mean +/- SEM) as a function of Surelease:Opadry® coating level. *The taste thresholds are represented as grey and black dashed lines; the IC_{50} and EC_{50} respectively.*

Developmental formula based on Smartseal 100 P coated CPM sugar spheres

Throughout the 300 s timeframe of the dissolution test, the Smartseal 100P® coat inhibited release of CPM to such an extent that the concentrations observed stayed below both the EC_{50} and IC_{50} for the duration (Figure 11). A moderate burst release was observed during the initial seconds of the experiment with 0.25 mg/mL mean release observed at 60 s; this may be a function of drug contamination on the surface or inadequate coating thus exposing the drug coating.

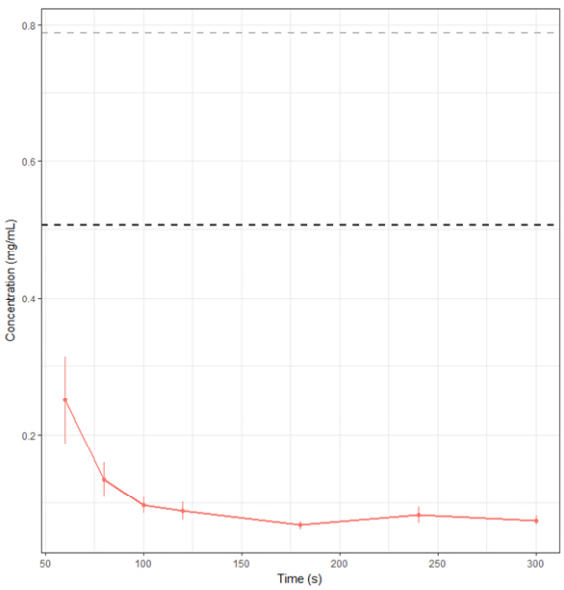


Figure 11 CPM release from a developmental formula based on Smartseal 100P® coated sugar spheres, showing the taste thresholds as grey and black dashed lines; the IC_{50} and EC_{50} respectively. The error bars are indicative of the mean +/-SEM.

Drug release post taste masking - biopharmaceutical consideration

In order to ascertain the biopharmaceutical implications of taste masking, the release of CPM from sugar spheres was assessed in SGF following soaking in SSF for 1 min (Figure 12).

Uncoated sugar spheres demonstrated significantly different drug release in PBS and SGF with greater release observed in PBS, however the general pattern of release observed was the same. While, the Surelease:Opadry® (70:30) coating showed some significant differences in release at certain time points, the general patterns of release observed were also the same. CPM release was only slightly hindered by the Surelease: Opadry (70:30) coat.

Opadry EC ®, a pH independent water insoluble barrier membrane, showed no significant difference in drug release over time as a function of dissolution medium. Importantly, however, the final concentrations observed after 60 minutes of dissolution of the Opadry EC ® sugar spheres were small relative to that observed for the uncoated sugar spheres, demonstrating a negative biopharmaceutical impact of taste masking by this coat proportional to increasing coating level (% WG).

The developmental formula based on Smartseal 100P®, a pH dependent water insoluble reverse enteric coating, was the only coating that showed a marked difference in the pattern of CPM release overtime as a function of dissolution medium. Indeed, negligible release was observed in PBS up to 20 mins, while after 6 mins in SGF, the plateau was reached (0.0154 mg/mL). Thus, the biopharmaceutical impact of this particular coat was minimal given that one can deduce that once in the stomach, the reduction in pH will yield release comparable with uncoated sugar spheres.

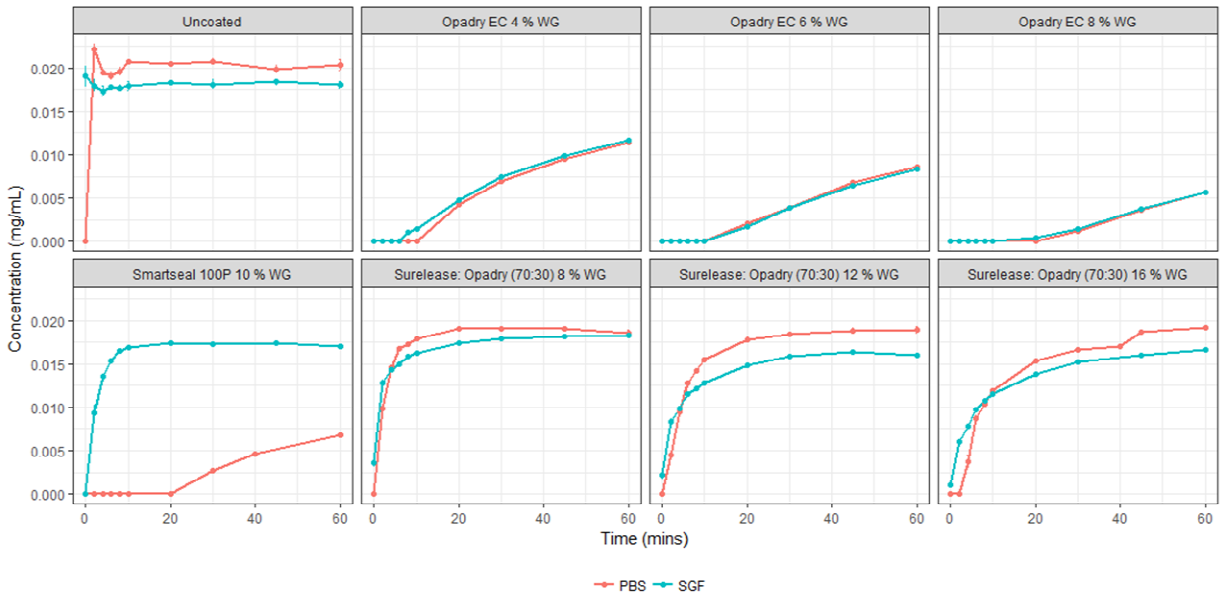


Figure 12 Drug release [mean +/- SEM] of CPM from sugar spheres in SGF following pre-soaking in SSF (blue) and in PBS (red).

Discussion

The lack of dissolution methodology for taste masked oral dosage forms was highlighted by Gittings *et al* [8]. The present study is the first study of its kind to assess drug release in a system biorelevant to the human oral cavity and draw real conclusions as to the taste using previously determined aversive taste thresholds. Several attempts have, however, been made in the literature, but fail due to a lack of biorelevance and/or lack of correlation to real taste data. For example, in a study assessing the taste of a novel midazolam formulation for children, the authors utilised dissolution as one of their means to assess taste [35]. While simulated saliva was used in this study, release was assessed in a fixed 300 mL of media, which far exceeds the volume of saliva normally observed in the human oral cavity at any given time, and does not account for saliva production and swallowing. Furthermore, sampling occurred at 0 and 5 minutes initially and up to 60 mins, thus the initial window of release that is so crucial to taste, as feasibly a patient will not have a dosage form in their mouth beyond this point, was missed. Finally, although taste assessment using the BATA model was performed in this study, the dissolution data were not assessed in the context of taste thresholds, thus conclusions as to the absolute taste were not possible [35]. In another example, film coating of granulated core particles was investigated as a means of taste masking [36]. In this study, the efficacy of taste masking of ibuprofen was assessed using a 'rapid dissolution test', in which the coated granules were added to 20 mL of Japanese Pharmacopoeia XV (JPXV) dissolution media 2 at pH 6.8 with mixing for 7-10 s before withdrawing 7 mL, filtering and administering to human volunteers (n = 3) previously calibrated with increasing concentrations of the ibuprofen in JPXV dissolution media 2. If one can ignore the inadequate sample size, poor taste assessment methodology and excessive volume of dissolution media, this study lacks the elegance demonstrated by our buccal dissolution test given that it requires repeated exposure of human participants to experimental formulations in order to gain an insight into taste masking. Indeed, use of this methodology during early drug development would be impossible given the lack of toxicological data at this stage [36].

Presently, the USP I (basket) dissolution test proved an ability to discriminate both between different types and extents of coating in terms of extent to which drug release is prevented.

However, if considered in the context of taste masking, one can draw no conclusion from the results, in a similar way to the aforementioned studies [35,36]. Indeed, it identified Opadry EC® at a level of 8 % WG as the most effective coat in terms of inhibiting drug release regardless of the dissolution media. It is a fully formulated solvent based coating system with ethylcellulose as the

barrier membrane film former and HPMC as a soluble pore-former. One cannot conclude whether or not such a release-limiting coat is necessary for taste masking, particularly when considered in terms of the biopharmaceutical impact of taste masking by the Opadry EC ® coat as discussed previously; this polymer system has primarily been developed for extended release applications. Testing using the USP (I) basket apparatus demonstrated that Opadry EC ® at a level of 4 % WG yielded negligible release up to 20 mins, thus perhaps this level of coating is sufficient to achieve taste masking, with reduced biopharmaceutical implications, but there is no absolute quantification. Conversely, when formulated with Surelease – a fully formulated aqueous dispersion consisting of ethylcellulose, ammonium hydroxide, medium chain triglyceride, oleic acid and water – to yield the Surelease:Opadry coat, which has previously been used for taste masking in marketed paediatric medicines [37], drug release is less inhibited regardless of dissolution media. Thus, a relative comparison as achieved by the USP I dissolution test would lead one to define Surelease:Opadry as the least effective coat at inhibiting drug release of those assessed, but perhaps still sufficient enough for taste masking. However, no absolute quantification was provided by the USP I test, thus no conclusion can be drawn.

The fully formulated developmental Smartseal system performed well in the USP I dissolution tests with inhibited release up to 20 mins in PBS and full immediate release in SGF given its pH dependent nature. It is based on a novel spray dried copolymer of methyl methacrylate and diethyl aminoethyl methacrylate (Kollicoat Smartseal). However, one still cannot conclude that inhibited release up to 20 mins in 900 mL PBS correlates sufficiently to what one might observe in 1 mL of saliva within the human mouth.

Therefore, the formulator is provided with very limited information from the USP I dissolution test for consideration of the coating technology and level necessary. Put simply, this test, while discriminative, is not predictive of taste masking and cannot provide the necessary information to inform the formulation scientist on choice and level of taste masking technology.

The novel buccal dissolution test, on the other hand, serves as a predictive as well as a discriminative dissolution test in the context of taste masking. Unique to any other previous attempts to assess taste masking from *in vitro* dissolution data, it linked drug release data from multiparticulates coated using a range of technologies and coating levels to taste by considering release in the context of human and rat taste thresholds: EC_{50} and IC_{50}, respectively. It predicted that the Surelease:Opadry® (70:30) coating would allow release of CPM to a point deemed aversive by the patient, given that after 60 s in the simulated oral cavity, non-cumulative

concentrations exceeded both the EC_{50} and IC_{50}. While, it predicted that Opadry EC ®, even at the lowest coating level (4 % WG) prevented release sufficient to exceed the taste thresholds. If these data are considered alongside release data in SGF, it is possible to maximise taste masking without inhibiting drug release to such an extent that bioavailability is hindered. Indeed, Smartseal 100P® demonstrated excellent taste masking comparable to that of Opadry EC ® but, being a pH dependent water insoluble reverse-enteric coating, release was not hindered in SGF. The absolute quantification of taste masking in vitro, as demonstrated here, has not been achieved in any other study.

Using this novel dissolution method, the formulator can optimise the coating type and level for taste masking for specific drug formulations. Indeed, it can be used to minimise the use of taste-masking excipient, which is of significant benefit given the conservative approach in case of limited safety data relevant to the use of an excipient, particularly in infants and the regulatory framework requesting thorough justifications [3]. The conventional USP dissolution method or other proposed tests found in the literature are unable to predict taste masking adequately, instead they may only allow relative comparisons to be made amongst different formulations. However, adjustment of the USP I dissolution test may be possible to achieve a more biorelevant system, e.g. the use of mini vessels and a smaller amount of adequate medium. Indeed, this may form part of the future work in which such an altered USP I methodology would be compared to the novel buccal methodology discussed here.

Additionally, the assessment of taste *in vitro* could feasibly be performed without the need for a human threshold value (EC_{50}), thus using the IC_{50} alone as the taste threshold [24]., The novel dissolution method offers great potential as an early-stage *in vitro* taste assessment methodology with minimal animal experimentation (1 API dose-aversiveness response curve).

Attention must be drawn to a key limitation that currently exists for the novel buccal dissolution test; that of size limitation. Given the small internal volume – 1 mL chosen to enhance the biorelevance given that approximately 1 mL of saliva is present at any given time in the human oral cavity – the size of dosage form that can be assessed is limited. Thus, while it is sufficient to assess multiparticulates, assessment of other oral dosage forms such as tablets or capsules is currently not possible. Therefore, future work will also involve the modification of the apparatus to accommodate larger oral dosage forms, while maintaining an internal volume relevant to that of the human oral cavity and saliva content.

In order to better understand the benefits and challenges of this novel dissolution test, it must be further tested using other APIs of varying solubility, a wider range of coating technologies and a wider range of dosage forms, e.g. orally-dispersing tablets and ion-exchange resins. The benefits of this novel test are however clear and point to a place where taste masking efficacy can be more accurately determined *in vitro*, and where the formulator can make better formulation decisions, balancing both compliance and bioavailability.

Conclusion

An *in vitro* methodology for taste assessment is required allowing informed formulation design in the context of taste masking. As yet, this goal has not been achieved in the literature. This study sought to achieve this goal by developing a dissolution methodology replicative of conditions encountered within the human oral cavity and assessing drug release in the context of taste by using previously determined taste thresholds taken from human and rat studies. In order to test the feasibility of this model to assess taste-masked pharmaceutical formulations, multiparticulates taste-masked using various polymer technologies and coating thicknesses were assessed for their '*in vitro* taste' masking properties. In contrast to conventional USP dissolution methodologies which provided no absolute assessment of taste, only relative distinction between technologies/coating thicknesses, the novel buccal dissolution test developed here enabled both discrimination and prediction in a quantitative manner. Thus, the developed methodology provides true insight for the formulator, enabling more informed patient-centric formulation decisions, better taste masking and ultimately more effective medicines.

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