Enhancing the understanding of palatability assessment used in the development of paediatric medicines

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Declaration

I, Alexander Joseph Keeley, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Children are averse to unpalatable medicines. A medicine will only elicit its desired effect if it is taken by the patient, therefore unpalatable medicines threaten the effective treatment of paediatric indications. Regulators thus now require all new medicines to have associated plans for paediatric formulation development; key to which is palatability testing. Therefore, there is a real need to enhance our understanding of the nascent area of pharmaceutical palatability testing.

Much of this research has focused on the rat brief access taste aversion (BATA) model, which uses water-deprived rats to evaluate aversiveness of a given sample by counting the number of rat licks relative to water and has the distinct advantage of being used pre-clinically due to the absence of human participants. The overall aims of this research were to: explore the methodological limitations of promising palatability assessment methodologies; expand the formulation repertoire and push the limits of the BATA model; and leverage the data from the BATA model to minimise animal use.

Our understanding of pharmaceutical palatability testing has been enhanced. Key questions such as the number of participants necessary for a human pharmaceutical taste panel are now known. The limits of the BATA model have been explored, and we now know that it can provide information on mouthfeel as well as taste, enabling assessment of more complex liquid oral dosage forms such as suspensions. Furthermore, by leveraging the data from the BATA model, a methodology for assessing solid oral dosage forms and an in silico model for prediction of palatability were developed.

This work has both answered and yielded questions and more work is need to improve pharmaceutical palatability assessment and thus children's medicines. However, it is clear we are on a path towards more palatable children's medicines and thus more effective treatment of paediatric diseases.

Impact statement

A medicine must be acceptable to the patient for it to function as such. Indeed, without adequate acceptability, compliance issues may arise, most notably in children. Thus, a lack of acceptable dosage forms is problematic to caregivers, healthcare professionals and ultimately patients, who may not be receiving a dose sufficient to the therapeutic requirements.

The importance of acceptability has been identified by pharmaceutical regulators around the world, such as the FDA, who require Paediatric Study Plans (PSPs), as part of all New Drug Applications (NDAs) unless a waiver has been granted. A key aspect of PSPs is palatability testing.

Previous research has demonstrated the feasibility of the rat brief-access taste aversion (BATA) as a tool for taste assessment with excellent human correlation and, being an animal model, allows taste assessment early on in the drug development process, when insufficient toxicological data proscribe taste assessment by human subjects.

The research described in this thesis serves to critique the BATA model. Indeed, for a pharmaceutical taste assessment methodology to be utilisable by both the pharmaceutical industry and the regulatory agencies alike, it must be correlated to human taste panels of sufficient sample size and not be limited by dosage form. Prior to this work, the BATA model was only capable of assessing active pharmaceutical ingredients (APIs) dissolved in water, however this research attempts to expand the formulation repertoire of the BATA model by assessing more complex liquid dosage forms and solid dosage forms.

The knowledge presented in the proceeding pages is of value to those working on developing more acceptable medicines in both academia and the pharmaceutical industry, and those assessing PSPs and PIPs in the regulatory agencies. It provides evidence for optimisation of taste assessment methodologies in humans and rats. Further, it presents a tool that can be used by the formulation scientist early on in the drug development stages to assess acceptability of a range of dosage forms. Ultimately, it is hoped that the outcome of this research will foster more informed patient-centric formulation decisions, better taste masking and ultimately more effective medicines.

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1 Introduction

This chapter serves to introduce the importance of palatability in medicine, particularly in children and thus the need for palatability testing. Current regulatory requirements pertinent to pharmaceutical companies in the introduction of new medicines to the market are discussed, with particular focus on those relating to children. Following this, the different methods – both *in vitro* and *in vivo* – by which palatability is tested are discussed, before finally identifying current knowledge gaps and thus research needs.

1.1 Paediatric medicines

It has long been known that children, as patients, differ to adults. Indeed, children differ to adults in their ability to swallow monolithic dosage forms such as tablets, their aversion to bitter taste and frequently the dosage required for a therapeutic effect [1]. Nonetheless, children continue to be failed as patients, receiving medicines that have not been evaluated as suitable for this patient population, instead receiving medicines 'off label' or without license or marketing authorisation [2]. Historically, paediatric doses were calculated by extrapolation from adults without adequate testing in children, thus posing real risk to children resulting from overdosing leading to excessive unwanted side effects with the possibility of toxicity or under-dosing leading to a lack of therapeutic effect [2].

With neither a legal obligation nor a financial incentive to produce more patient-centric paediatric medicines, the pharmaceutical industry have neglected this group, with the obvious exceptions of vaccines and paediatric-only indications [3]. Naturally, pharmaceutical companies have fabricated their pipelines based on market forces, thus driving the development of medicines that target the most profitable patient population: adults. The result of this is that, for example in the USA, most drugs (75 %) do not have approved paediatric formulations [4].

One of the key barriers to the development of paediatric medicines is that of taste. Active Pharmaceutical Ingredients (API) – the component of a formulation that elicits a pharmacological effect – are frequently aversive in taste with bitterness being the primary cause of aversion [5]. The majority of APIs are exogenous and in sufficient doses can be toxic. It is thought that evolution has facilitated the development of bitter taste to

protect against poisoning by such compounds [6]. Of course, medicines consist of said APIs at doses sufficient to treat a given disease but low enough to not induce harm to the patient. Such a concept can be easily understood by adult patients, but children, particularly very young children, may be less able to grasp this concept and thus reject the medicine. Indeed, in a survey, 90 % of paediatricians identified taste and palatability as the greatest barrier to treatment completion among their patients [7]. Furthermore, a more recent study which asked children directly their views on taking medicines identified taste as the most commonly reported reason for problems in taking medicines with 416/653 respondents between 10 and 18 years old stating 'don't like the taste' when asked 'why do you find some of the medicines difficult to take?'[8].

The problem of bitter tasting APIs is compounded by children's age-dependent inability to swallow tablets or capsules, thus necessitating the administration of alternative liquid dosage forms. Oral solid dosage forms, such as tablets or capsules offer the formulation scientist a distinct advantage over oral liquid dosage forms in that they may be taste-masked with ease by simply coating with an appropriate polymer film coat or lipidic barrier system [9]. It is generally regarded that children require a liquid dosage form prior to the age of between 6 and 8 years old, but variability in older children in their ability to swallow monolithic oral dosage forms has been noted [10–12]. A further contraindication of oral solid dosage forms in children is their poor flexibility of dose, resulting in tablet/capsule splitting in order to obtain the required strength, which inevitably puts patients at risk from under- or overdosing [13,14]. Thus, liquid dosage forms are administered, but these are far more difficult to taste-mask, requiring the addition of multiple excipients to mitigate aversion. However, the toolkit of excipients available to the formulation scientist is limited for children's medicines. Indeed, some are contraindicated given their link to the retardation of organ development, such as ethanol, propylene glycol, benzyl alcohol and ethanol [15]. While others are contraindicated due to their link to the increased incidence of dental caries, for example sucrose and acids achieving a pH < 5.5 [16–19]. Therefore, medicines may be poorly taste-masked and, as highlighted above, this may cause problems with patient compliance.

1.2 Case studies: the Importance of taste

To further highlight the importance of bad taste and its effect on patients, two key paediatric indications where taste plays a significant role in poor patient adherence and thus treatment failure will be discussed as case studies. The selected indications cover both acute and chronic conditions – bacterial infection and HIV – to demonstrate that the problem of bad taste spans paediatric medicine, affecting both acute and chronic paediatric indications.

1.2.1 Chronic: Paediatric antiretroviral therapy (ART)

More than 3.2 million children worldwide are infected with human immunodeficiency viruses (HIV), the majority of whom live in sub-Saharan Africa, and with only 24 % of these children taking antiretroviral therapy (ART), HIV/AIDS stands as the biggest killer of adolescents in this region [20,21]. The World Health Organisation (WHO) recommends that, regardless of age, all HIV-infected and HIV-exposed individuals must receive ART for treatment and prophylaxis, respectively [22]. Thus, the need for paediatric formulations to treat HIV is greater than ever.

Children with HIV can live long, healthy lives but only if the viral loads are controlled, which can only be achieved with adherent ART. Therefore, it is of critical importance that the causes of non-adherence are elucidated and addressed. Indeed, these include the cost and access to medicines, stigma surrounding the condition and diagnosis [23,24]. However, the most common issue identified in studies of paediatric ART adherence is the lack of paediatric formulations; those ART currently available are frequently unpalatable to children regardless of age [25].

The bitterness of ART has a direct impact on caregivers who have to undergo a daily routine of negotiation in order for the child to take the medicine, often with little success, thus creating major issues with ART compliance and thus control of viral load, symptoms and viral resistance [26–30]. Therefore, it is of no surprise that in a study assessing caregivers' opinions on the most important innovations in ART, which ranged from less complicated dosing regimens to the reduced need for refrigeration to a daily visit by a nurse, the majority (81 %) of respondents stated that improving the taste of medicines is the single

most important strategy to enhance ART compliance [31].The aforementioned displays the importance of taste in paediatric medicine and the life-threatening consequences that inappropriate, non-patient-centric paediatric medicines can have. For the 3.2 million children infected with HIV, and the many more suffering with other medical conditions, it is critical that the poor taste of paediatric medicines is adequately addressed during drug development to achieve the greatest patient adherence and thus the best treatment outcomes and minimise suffering.

1.2.2 Acute: Paediatric antibiotic treatment

Adequate management of a paediatric bacterial infection, like any other infection, is reliant on adherent antibiotic treatment [23,32,33]. The selection of an antibiotic for a given infection is governed by the suspected bacterial cause, location and severity, age, known allergies, side effects and toxicology [34]. The omission of palatability must be noted here despite taste, texture and aftertaste being cited as important considerations for children taking medicines throughout the literature [32,33]. Indeed, a study in Japan assessing antibiotic adherence among 192 families in Japan revealed approximately 25 % non-adherence to the full course, with the second-most common reason for non-adherence being the child's refusal to take the drug [35]. Another study in Saudi Arabia assessing adherence to short-term antibiotic therapy among 414 patients, the majority of whom were children, found bitter taste to be one of the key drivers to non-adherence [36]. Thus, it stands to reason that better-tasting antibiotics will positively correlate to better adherence. However, despite a wealth of evidence that palatability is a key driver of compliance, poor-tasting antibiotics are still prescribed, with some being first-line treatment. For example, in the UK, the National Institute for Health and Care Excellence (NICE) cite flucloxacillin as unpalatable to children, yet it is recommended as first-line treatment for *Staphylococcus aureus* and streptococcal skin infections, among others [34].

Clearly, taste is a critical attribute to medicines, particularly those intended for children, be they for acute or chronic conditions. The evidence is clear: if a medicine tastes bad, the child will not take it leading to treatment failure and thus patient suffering. Therefore, taste must be regarded as a key attribute to the efficacy of a medicine and properly addressed during the drug development process. The ways in which the regulators are

addressing this key failure in drug development will now be discussed before identifying emerging paediatric dosage form technologies.

1.3 The regulatory requirements

In order to overcome the barriers to paediatric drug development – economic as mentioned and ethical as until 1980s it was thought that children should be protected from such research – the Paediatric Regulation (EC) 1901/2006 was adopted, which was largely inspired by developments in the USA addressing paediatric drug development [37]. This legislation provided a legal obligation for pharmaceutical companies to develop paediatric medicines and established incentives for doing so [38]. The objectives of the regulation were threefold:

1. Encourage and enable high-quality research into the development of medicines for children.
2. Ensure, over time, that all medicines used by children are authorised for such use with age-appropriate forms and formulations.
3. Increase the availability of high quality information about medicines used by children [39].

The regulation achieves the aforementioned objectives by legislating that pharmaceutical companies assess each new medicine for its potential paediatric indication thus gradually polluting the market with child-appropriate medicines [39]. Indeed, a pharmaceutical company must submit a paediatric investigation plan (PIP) to the European Medicines Agency (EMA) Paediatric Committee (PDCO) [38]. The PIP consists a collection of studies proposed by the pharmaceutical company that will serve to demonstrate safety and efficacy in children. It includes both clinical and non-clinical studies and patient-centric paediatric formulations. The incentive for fulfilling such obligations is a six-month extension of the supplementary extension medicines indicated for non-orphan disease and a further two years of market exclusivity for orphan drugs. Furthermore, for those compounds that are off-patent, it is possible to apply for paediatric-use marketing authorisation or PUMA [37]. Of course, not all conditions for which medicines are developed affect children, and further, some drugs may be ineffective or unsafe in children. In such instances, a pharmaceutical company may apply for a waiver thus removing the need to

comply with the paediatric regulation. Such waivers are granted by medical condition rather than broadly for all indications of a drug [38].

1.3.1 Impact of the paediatric regulation

There are now more medicines indicated for children available in the market, with 267 such medicines including new marketing authorisations and indications authorised in the EU between January 2007 and December 2016 [38]. Furthermore, the number of PIPs submitted exceeded 1000 in 2017, thus the number of paediatric medicines available is certainly set to increase [39]. To provide evidence to support that this increase in paediatric medicines is due to the introduction of the Paediatric Regulation, Tomasi *et al.* compared two 3-year periods (January 2004 – December 2006 and January 2014 - December 2016), finding that there was a 147 % increase in the number of new medicines with a paediatric indication both new and re-purposed: from 30 to 74. These figures are especially impressive given the time it takes to bring a drug to market: approximately 10 years.

1.3.2 Patient acceptability

A key component of the Paediatric Regulation is patient acceptability, defined by the EMA as the overall ability and willingness of the patient to use and its care giver to administer the medicine as intended [40]. It encompasses the following:

- Palatability, swallowability
- Appearance
- Complexity of the modification to be conducted by the child or its caregivers prior to administration
- Dose
- Dosing frequency and duration of treatment
- Administration device
- Primary and secondary container closure system
- Mode of administration and any related pain or discomfort [40].

Palatability, listed first above, is regarded as the most important aspect of acceptability for paediatric medicines, and is defined as the overall appreciation of a medicinal product in relation to its smell, taste, aftertaste and texture [40,41]. The EMA has outlined the

presence of a variety of methods for assessing patient acceptability in the literature, with sometimes conflicting outcomes for the same product [40]. There is currently no specific guidance on which methodology to use to demonstrate acceptability of medicines. Nonetheless, the Paediatric Regulation has made an impact on the provision of more acceptable paediatric medicines, as discussed above. The formulation approaches to paediatric oral drug delivery will now be discussed, outlining the advantages and disadvantages of each technology, before discussing how acceptability can be tested, both *in vitro* and *in vivo*.

1.4 Current formulation platforms for paediatric oral drug delivery

Children have distinct needs as patients, making their requirements for medicines more complex relative to their adult counterparts. For example, the effect of the drug on the body, and the body's effect on the drug – pharmacodynamics and pharmacokinetics, respectively – differ from adults but also among different paediatric age groups. Thus, dosing flexibility is a key attribute to a paediatric formulation [42]. Excipients provide a further consideration given that additional safety concerns may be present for some excipients even where their use in adults is well documented [43]. As mentioned, a paediatric medicine must also be palatable, suitable for those who are unable to swallow and compatible with the caregiver who may have to administer the medicine [44]. However, all of the aforementioned must be achieved while also being economically viable to manufacture [45]. All aspects pertaining to the development of oral formulations for paediatrics are detailed in Table 1-1.

Table 1-1 Requirement for age-appropriate oral drug delivery platforms; adapted from [45]

Benefit/risk	Criterion for drug product	Requirements
Efficacy/acceptability	Dosage	Dose flexibility
		Acceptability of size/volume
	Preparation/administration	Easy and convenient handling
		Easily administered
	Compliance	Minimal impact on lifestyle
		Acceptable appearance and taste
		Minimal administration frequency
Patient safety	Bioavailability	Sufficient
	Excipients	Minimal
		Safe level
	Stability	Stable during shelf-life
		Stable in-use
	Medication error	Minimal risk of dosing error
Patient access	Manufacturability	Robust manufacture process
		Commercially viable
	Affordability	Acceptable cost
		Easily transported and stored
		Low environmental impact

1.4.1 Liquid dosage forms

Less focus has been observed on the development of liquid dosage forms for children given their inferiority to solid dosage forms in terms of the stability, ease of transport and manipulation of drug release. However, their innate advantages of ease of swallowing and dosing flexibility mean liquid dosage forms will be omnipresent in paediatric pharmaceuticals [45].

Developments have been made to address the limitations of liquid dosage forms as highlighted above. Indeed, controlled-release liquid preparations are being gradually introduced to the market with both azithromycin and methylphenidate hydrochloride extended release preparations now available [46,47]. However, the difficulty in taste-masking liquid oral dosage forms remains a challenge, necessitating adequate testing to ensure palatability and thus adherence [45].

1.4.2 Solid dosage forms

Solid dosage forms have, and may always be, the formulation of choice for the pharmaceutical industry given the wealth of research in delivering drugs in this fashion, ease of manufacture, inherent stability, ease of transport and low cost [45]. However, solid oral dosage forms pose a significant problem to children who struggle to swallow tablets or capsules, and require dose flexibility, which is naturally difficult with a monolithic dosage form [45]. To mitigate the highlighted issues, smaller solid oral dosage forms have been developed, which are inherently easier to swallow and consist a fraction of the dose allowing flexible dosing by administering multiple units in order to achieve the desired dose. Indeed, studies have demonstrated that 6 month old children are capable of swallowing single minitablets, with some children perceiving said minitablets to be more acceptable than syrups [48,49]. Minitablets will be discussed further below. In addition, devices to mitigate swallowing issues have been developed such as 'pill swallowing cups', while education and training of patients to enable them to swallow solid oral dosage forms has also provided some benefit [50,51].

1.4.3 Emerging paediatric dosage form technologies

1.4.3.1 Multiparticulate drug delivery systems

Multiparticulates include minitablets, as highlighted above, but also extend to other discrete units such as powders, granules and pellets [45]. These confer the same advantages as minitablets over more conventional monolithic oral solid dosage forms through their relative ease of swallowing and dosing flexibility. Importantly, multiparticulates have a distinct advantage in their ease of taste-masking as this can be achieved by the application of a film coat to limit drug release in the mouth without hindering release further along the gastrointestinal tract [45].

While, multiparticulates confer a distinct advantage in their ease of taste-masking, they may present an additional acceptability issue: mouthfeel. The administration of multiple small units into the human oral cavity will feasibly induce a gritty sensation, however the consequences to patient acceptability are as yet unknown [52,53]. This highlights the importance of testing palatability of new dosage form platform technologies; currently there is no such validated tool for assessing mouthfeel without a human taste panel.

1.4.3.2 Orodispersible formulations

1.4.3.2.1 Orodispersible tablets (ODTs)

Orodispersible tablets offer the significant advantage over monolithic dosage forms given that they do not require swallowing, but instead rapidly disintegrate in the oral cavity to be absorbed in the gastrointestinal tract following swallowing, or buccally if the ODT is retained in the mouth [54]. A key limitation of this technology is taste; as the dosage form is dispersed in the mouth, this technology is subject to acceptability problems if the API is bitter or aversive thus requiring advanced technologies to mitigate any possible taste. Sweeteners and/or flavours may be added, but their efficacy may be questionable and their use may be limited in paediatric indications [9,45]. Thus, acceptability testing is also critical if this technology is chosen to ensure adherence is not affected.

1.4.3.2.2 Orodispersible films (ODFs)

ODFs share similarities to ODTs, but rather than the API being incorporated in a tablet, it is instead incorporated in a polymer matrix, which rapidly dissolves when placed on the tongue, thus mitigating swallowing issues that may be present in paediatric patients. Thus, ODFs also share the primary drawback of ODTs: taste. Taste-masking of bitter APIs is highly problematic in a formulation designed to disperse on the tongue, making issues with poor acceptability problematic [45]. Thus, it must be reiterated that adequate taste assessment methodologies are utilised early on in the drug development process to ensure acceptability issues do not lead to product failure post-marketing.

1.4.3.3 Chewable formulations

Chewable formulations are those that require mechanical processing in the mouth by chewing to release the API and thus enable absorption and a pharmacological effect [45]. Such formulations are advantageous in that they may not require swallowing, may be administered without water and may be preferred by patients over conventional tablets given their aesthetic properties [45]. However, the omnipresent issue of taste remains: API is released directly into the oral cavity, and may therefore elicit a taste, which may prove problematic if the API is bitter, which it most likely will be [45,55]. Palatability testing is therefore essential during drug development to enable proactive formulation development to mitigate adherence issues.

Having now introduced the problem of palatability in paediatric medicine and the technologies that are being developed to address acceptability in children, the physiology of taste will be discussed before identifying how taste can be tested.

1.5 A brief insight into taste physiology

The sense of taste or gustation results from the interaction of a chemical species with the taste bud cells (TBCs), principally found on the tongue [56]. It is a critical sense, acting as the final point of reference prior to ingestion of an exogenous substance [57].

Mammals are capable of distinguishing between five principal taste sensations – sweet, sour, salty, umami and bitter – however a growing body of research suggests the existence of a sixth taste modality – fat [58,59].

1.5.1 The anatomy of taste

The taste buds – shown in Figure 1-1 – have a structure that is frequently compared to a garlic bulb and are principally found in areas of the tongue known as papillae, specifically the fungiform (central and anterior tongue), foliate (lateral tongue) and circumvallate papillae (posterior tongue) [56]. However, taste buds are also located to a lesser extent in other areas of the mouth, for example the palate, nasopharynx, epiglottis, larynx and nasoincisor duct of rats [60]. Furthermore, hormone synthesising TBCs – producing hormones such as grehlin and GLP-1 (glucagon-like peptide-1) – are located in the brain and the gut [61,62].

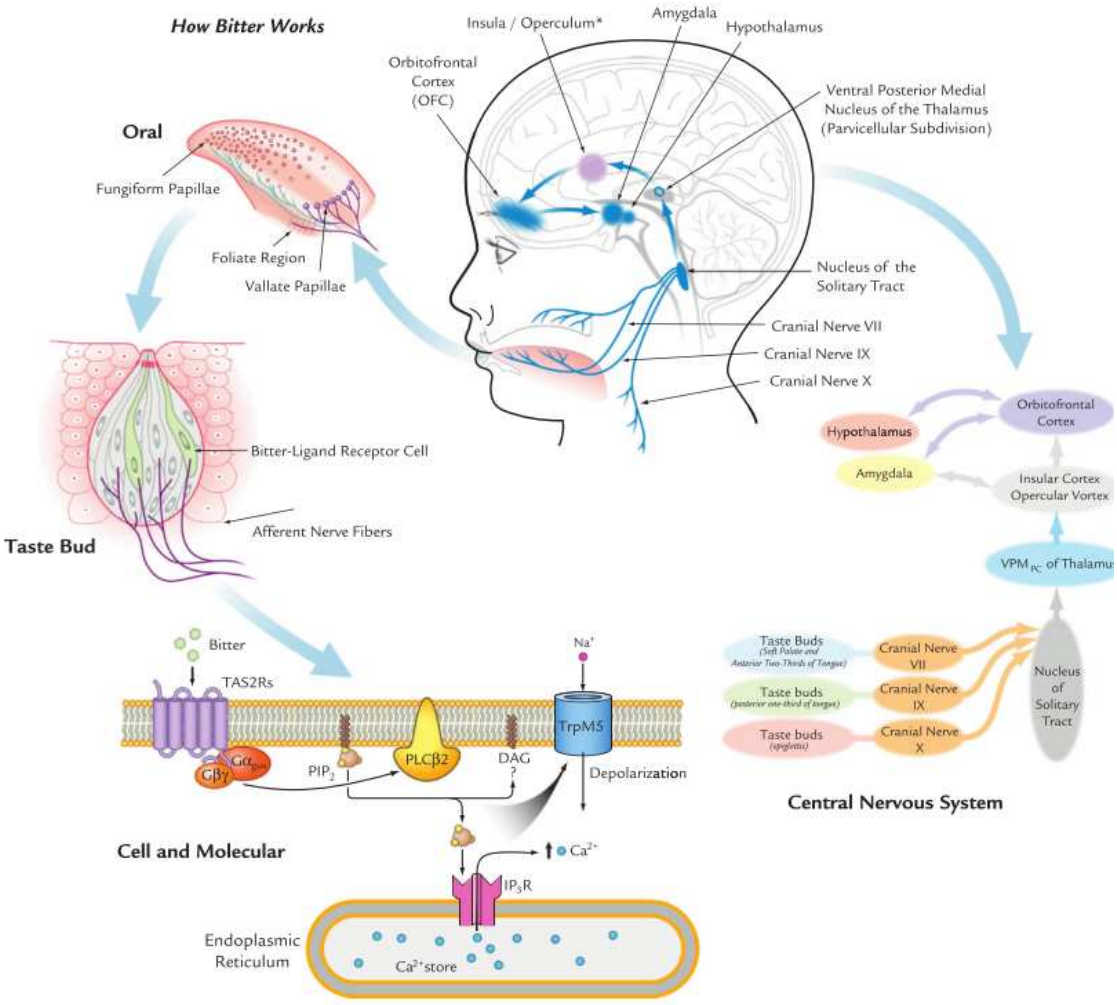


Figure 1-1 The physiology of bitter taste (adapted from [5])

If we imagine the taste bud as a garlic bulb, each TBC is a clove within the garlic bulb, with between 50-100 TBCs making up a single taste bud. TBCs are of five types (I-V). All taste buds consist all five subtypes of TBC [56].

1.5.1.1 Type I (Glia-like cells)

Type I TBCs are the most abundant TBC within taste buds and are believe to act principally as supporting cells, enveloping type II and type III cells in a Schwann-cell like manner [63]. These cells may serve to secrete and phagocytose, probably acting to produce the amorphous material found within the pit of the taste bud [64,65]. As alluded to in the above title, they also function in a similar way to the glial cells of the central nervous system (CNS) by clearing neurotransmitters and redistributing ions [63]. Taste I cells are however

thought to facilitate the perception of low salt tastants through the expressed amiloride-sensitive sodium channel subunit α (otherwise referred to as the epithelial sodium channel subunit α or α-ENaC) (fig. 3), although the exact mechanism of downstream signalling to achieve this function is as yet unknown [66,67].

1.5.1.2 Type II (Receptor cells)

Type II cells, or receptor cells, function to express receptors capable of binding sweet, umami and bitter tastants. Neither sour nor salty tastes are transduced by these cells [68,69]. A family of three G-protein coupled receptors (GPCRs): the taste receptor type 1 (TAS1Rs) – taste receptor type 1 member 1 (TAS1R1), taste receptor type 1 member 2 (TAS1R2) and taste receptor type 1 member 3 (TAS1R3) – enable the recognition of sweet and umami modalities by heterodimerising in various combinations depending on the modality transduced [56]. For example, umami tastants activate the heterodimeric receptor formed by TAS1R1 and TAS1R3, while a sweet tastant activates heterodimeric receptors formed by TAS1R2 and TAS1R3 – see Figure 1-2 [70].

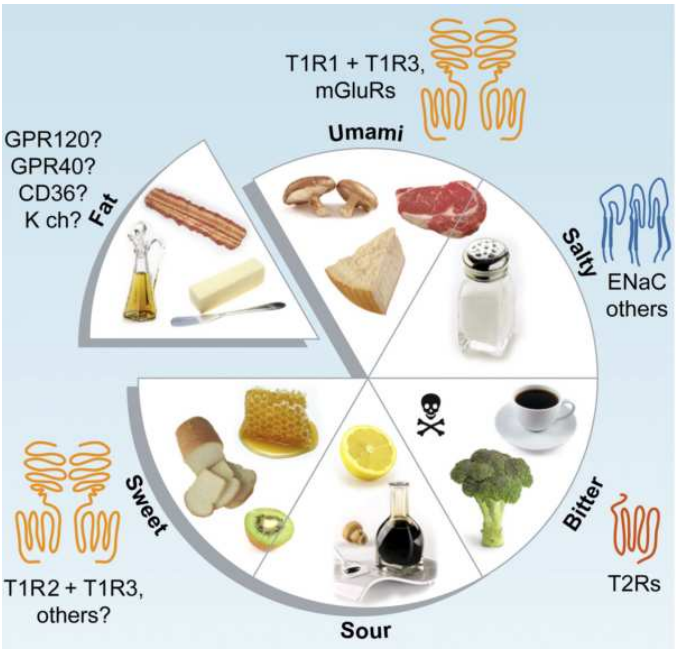


Figure 1-2 Taste modalities and the cognate receptors that aid in their detection. Adapted from [70]

Taste receptor type 2 (TAS2R) – also a family of GPCRs – are responsible for the transduction of bitter tastants (fig. 3) [70]. Approximately 30 members comprise this family

of taste receptors, meaning the diversity of chemical structures that elicit bitterness approaches infinity, making prediction of such very complicated [71–73].

A type II TBC will either express TAS1Rs or TAS2Rs, such that it will respond solely to sweet and umami or bitter tastants.

1.5.1.3 Type III cells (presynaptic cells)

A mere 5-7% of the cells that consist a taste bud are type III cells, but these are the only cells with synaptic connections to intragemmal nerve fibres [63]. These cells have processes extending basally and apically [63]. Indeed, it appears that their apical portion consists channels that enable transduction of the sour taste modality [74].

1.5.1.4 Type IV cells (basal cells)

Basal cells are undifferentiated cells located in the basal area of the taste bud. These are not, however, stem cells but rather postmitotic, immediate precursors of the aforementioned type I-III TBCs, as indicated by the presence of sonic hedgehog protein (SHH) in most basal cells, which regulates the differentiation of TBCs [75].

1.5.1.5 Type V cells (marginal cells)

Type V cells, perigemmal cells or marginal cells are thought to be taste bud stem cells [63].

1.5.2 Taste transduction

As highlighted above, type III cells are the only taste cells with conventional neuronal synapses, and as such one would expect that in order to achieve taste transduction, there would have to be some form of communication between the receptor (type II) cells and the pre-synaptic (type III) cells. However, remarkably genetic ablation of type III cells from mice does not lead to a disruption of taste signalling for sweet, bitter and umami modalities [76]. Indeed, type II cells achieve taste signalling by the direct release of ATP, which activates purinergic receptors on nerve fibres thus eliciting a response [77,78]. Furthermore, these cells also release hormones, e.g. glucagon-like peptide 1 (GLP-1) and neuropeptide Y (NPY), capable of communicating with both neighbouring taste cells and afferent nerve fibres within the taste bud, thus enabling taste modulation [61,79,80].

Transmittance to higher brain regions is achieved by cranial nerves VII, IX and X, going via the nucleus of the solitary tract and into the thalamus, as indicated in Figure 1-1.

Given that sweet, umami and bitterness taste receptors are GPCRs, the downstream signalling pathways overlap. As summarised in Figure 1-1, when a sweet, umami or bitter tastant binds to its cognate receptor, PLCβ2 (1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-2) is activated and second messengers, e.g. IP_3 (inositol triphosphate), are produced, leading to intracellular Ca^{2+} release and subsequent gating of TRPM5 (transient receptor potential cation channel subfamily M member 5), thus the cell depolarizes and non-vesicular release of ATP through voltage-gated CALMH1 (calcium homeostasis modulator 1) channels occurs [81–85].

Having now addressed the importance of taste and its physiology, the regulatory requirements and the plethora of formulation technologies being developed to enable better, more patient-centric medicines, the testing of palatability will now be discussed.

1.5.3 The need for palatability testing

Clearly, significant efforts in both academia and the pharmaceutical industry have resulted in a plethora of age-appropriate formulation technologies. However, as highlighted in section 1.4.3, neither of the presented technologies are a panacea. Indeed, it seems that where one issue is solved, another is created. Indeed, by solving the issue of swallowability by developing ODFs, ODTs and chewable formulations, the issue of taste becomes the limiting issue on patient adherence. And by solving taste and swallowability using coated multiparticulates, the issue of unpleasant mouthfeel becomes the main driver of poor compliance. Thus, it is critical that formulation development for paediatric indications works in tandem with psychophysical assessment of developed dosage forms, which includes assessment of both taste and mouthfeel to gauge an holistic representation of the acceptability. Furthermore, the earlier such testing can occur during the drug development process, the more proactive the formulation scientist can be in addressing potential acceptability issues, enabling more efficient drug development and better, more patient-centric medicines. Methods by which palatability assessment may be achieved will now be discussed.

1.6 Palatability testing

1.6.1 Human tools for taste assessment

The multitude of psychophysical methods that can be utilised to assess taste will now be discussed, with a focus on those that may be suitable in children, as a demonstration of the complexity of the field.

1.6.1.1 Taste Reactivity

Taste reactivity, in which the response to a given oral stimulus is assessed by analysing orofacial responses from slow-motion video footage of participants is perhaps one of the more intuitive methods of taste assessment [5]. This method relies on the work of Oster and Rosenstein [86] who, in 1988, developed the anatomically-based Facial Action Coding System of Ekman and Friesen [87]. Here, facial expressions are dissected into constituent action units (AUs), with the number of affective reactions revealing the valence and intensity of reaction [5]. Such a methodology is ideal for children given that minimal participant understanding is necessary, however, requires researchers to be trained in the Facial Action Coding System, which can be both time-consuming and costly [88].

1.6.1.2 Forced-choice tracking procedures/thresholds

Forced-choice tracking procedures/thresholds exist that enable the researcher to establish the sensitivity of the participant to a given tastants(s) [5]. Where the researcher wishes to determine the potency of a given tastant, a forced-choice paired-comparison procedure with water may be used in which the lowest concentration at which the participant is capable of distinguishing the sample from water thus providing the detection threshold and hence the potency [89]. However, where preference is being measured, this method may be adapted by asking the participant which sample they prefer in a forced-choice paired-comparison method, in which samples of varying concentrations are presented to the participant and their most preferred concentration is established [90]. Such a methodology may be useful in determining, say, the necessary concentration of a sweetener to be used in a paediatric formulation.

1.6.1.3 Scales

Scales may also be used in which the participant evaluates a given sample using a given type of scale. There are a multitude of such scales, but are usually presented as a line fragmented by either verbal descriptors or pictures representing how the participant feels [5]. The most common scales used by pharmaceutical companies are the facial hedonic scales and 10 cm visual analogue scales (VAS) [34].

However, despite the existence of multiple methods for taste evaluation, there is currently no guidance on which methodology should be used to evaluate the taste of pharmaceuticals, how many participants are necessary or even clearly defined acceptance criteria [41]. Indeed, the lack of available guidance on acceptability testing can lead to challenges during formulation development as demonstrated by Walsh, J [41] in a reflection paper outlining the challenges encountered with a PIP for an off-patent drug; in which Walsh outlined the lack of methodological guidance despite the EMA's stipulation that acceptability must be demonstrated, necessitating the use of multiple methods to demonstrate acceptability in children of multiple ages: facial expressions were recorded in children below 5 years, while those aged 6 years and above were given a short and simple questionnaire [41].

1.6.2 Non-human tools for taste assessment

Human taste panels are the gold standard of taste assessment, however their use is restricted to those compounds who have sufficient toxicity data to allow administration to humans [91,92]. During early drug development, such data do not exist, thus preventing taste assessment by humans due to safety concerns. Therefore, there has been great interest in the development of non-human tools for the taste assessment of pharmaceuticals [93].

1.6.2.1 Electronic taste sensors

It has been proposed that taste can be approximated *in vitro* by assessing a multitude of physicochemical properties of a given tastant solution, e.g. voltammetry, potentiometry, impedance, optical techniques and mass change [92]. However, in the pharmaceutical industry, the most frequently in encountered systems are the Insent® (AtsugiChi, Japan)

and α-Astree® e-sensors (Alpha M.O.S., Toulouse, France), which utilise lipid membrane sensors and polymeric sensors based on chemical modified field effect technology (ChemFET), respectively. The aforementioned differ in their cross-selectively, with the Insent having only partial cross-selectivity among sensors assigned to specific taste modalities while the α-Astree consists seven sensors that differ in composition but are cross-selective [94]. The α-Astree also differs from the Insent in that it measures only the initial taste, while the Insent also provides information on the aftertaste [93]. The reliability of these sensors is questionable. Both the α-Astree and Insent e-sensors have shown acceptable reliability according to the International Conference for Harmonization guidelines Q2 (RSD < 4 % and r^2 > 0.98) over short time periods, but for experiments up to 6 months, and following storage of the sensors, both e-sensors did not meet this specification, showing that they are highly sensitive to minor changes in the analytical conditions, e.g. temperature and pH [94,95].

Both function well as assessors of relative taste, such as comparing taste-masked formulation to placebo, in which the Euclidean distance is measured on a principle component analysis (PCA) map, with a larger distance indicating a bigger difference in taste [95]. However, as assessors of absolute taste, their usefulness has yet to be demonstrated.

1.6.2.1.1 E-sensor to human correlation

When using e-sensors as predictors of human taste, bitter prediction models must be developed using a standard bitter compound previously assessed in humans, which correlate a human sensory output to a combination of e-sensor output thus providing a correlation curve as a predictor of human taste [96].

1.6.2.1.1.1 α-Astree

Results showing some predictability of human taste by the α-Astree e-sensor can be observed in the literature. For example, Wang *et al.* in the assessment of solutions of berberine hydrochloride demonstrated that no significant difference was observed between human taste scores and those predicted by the e-sensor with a human sample size of 129 [96]. However, it must be noted that the e-sensor was calibrated and model built with the same human participants and the same compound at the same concentrations,

thus anything other than a lack of statistical significance would be surprising. However, another study demonstrated that the bitterness of H1-antihistamines could be predicted with good precision using a model built with quinine hydrochloride scores [97]. Indeed, an r^2 of 0.9621 between predicted and actual human taste data was achieved.

1.6.2.1.1.2 Insent

The insent e-sensor has also shown promising results, but for the aftertaste output rather than initial taste [98,99]. For example, in one study the aftertaste of amlodipine orally disintegrating tablets (ODT) as assessed by a human taste panel was correlated to the aftertaste output of the Insent e-sensor with some success achieving an r^2 of 0.54 [98]. However, the participants were instructed to evaluate the bitterness of the ODT as a function of quinine hydrochloride bitterness and a sample size of merely six participants was used, thus questioning their methodology. In another study, six participants were also used to correlate human bitterness response to Insent aftertaste output, but in this case acidic non-steroidal anti-inflammatory drugs (NSAIDs) were evaluated [99]. By comparing to a quinine hydrochloride standard, the Insent e-sensor provided a good correlation using Pearson's test ($r = 0.83$).

1.6.2.1.2 Final remarks on e-sensors

Therefore, some promising results do exist for the use of the e-sensors for the taste assessment of APIs, however more studies are required in which larger sample sizes are used; six participants is surely insufficient to achieve statistically significant results, although the necessary sample size is not known. Furthermore, the use a quinine hydrochloride as a standard against which bitterness is evaluated is fraught with methodological considerations. The API under investigation must be used and evaluated alone using a VAS or hedonic scale as per conventional psychometric principles, and the data correlated to e-sensor output for said API. Moreover, quinine hydrochloride elicits a bitter taste, but in some of the aforementioned studies it was the palatability that was being assessed, thus using a bitter standard would therefore only capture a fifth of the taste as the participants cannot evaluate sourness, sweetness, saltiness or umami. Furthermore, aversiveness of an API may extend beyond the basic five tastes, with some APIs being described as metallic, astringent or providing a burning sensation [9]. Thus a

quinine hydrochloride does not provide a sufficiently wide pharmacophore range to enable correlation to aversiveness of an API to which the sensor is naïve. Further, e-sensors function by assessing the taste of the assessed compound with its own unique chemical nature, which may not be transferable to another compound with a different chemical nature. The use of quinine hydrochloride as a standard in e-sensor research, which is known to correlate well with human taste, shows a lack of confidence in the systems to evaluate bitterness of compounds to which they are naïve. Better, more methodologically robust studies are needed in order to justify the use of e-sensors in pharmaceutical psychophysics.

1.6.2.2 In vitro drug release and taste

Given that only that which dissolves can interact with the taste receptors within the taste buds of the tongue and thus elicit a taste, dissolution testing can feasibly provide some insight into the taste induced by a dosage form. 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 [100,101].

There is currently no standardised pharmacopoeial dissolution test for assessing the taste of dosage forms *in vitro*, and as such there is great variation among the methods employed by researchers working in this area [100]. The methods identified differ in terms of the media employed, with phosphate buffer at pH 6.8 being frequently observed [102–106], while some researchers have simply opted for water [107–110]. Such media are not relevant to saliva, and cannot feasibly be relevant to taste assessment. The pH of the media has also been debated with researchers employing phosphate buffers at varied pH values, from 5.6-8.0 [111–114].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 [115]. 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 [100].

1.6.2.3 Using animals to assess taste

Animal models of taste are of two types; those that provide information on the taste modality and flavour of the assessed sample (e.g. sweet, salty, sour, umami or bitter) known as taste-discrimination experiments and those that provide a more broad evaluation of the organoleptic properties of the sample including its smell, appearance aftertaste and mouthfeel as well as taste, which are referred to as taste-guided experiments [93]. In the literature, a variety of methodologies have been employed for pharmaceutical taste assessment and include conditioned taste aversion (CTA), operant taste discrimination, 96-well plate high-throughput taste assessment model, two-bottle preference tests and the brief-access taste aversion (BATA) model [116].

1.6.2.3.1 Conditioned taste aversion model

The CTA model enables the determination of taste modality or quality of an assessed pharmaceutical compound by first conditioning the animal to a reference stimuli, e.g. bitter compound by associating its administration to an unpleasant sensation, such as peritoneal injections of lithium chloride. Thus, if the animal is presented with a similar taste or taste intensity, the animal will demonstrate avoidance due to the close association with the reference [117]. However, such a procedure is very low throughput and may be considered unnecessarily cruel given the aversive conditioning required, particularly when other less harmful methodologies exist [118]. Further, this methodology only yields information on the taste modality, with no insight into the palatability.

1.6.2.3.2 Operant taste discrimination model

The operant taste discrimination model involves training an animal, usually a rat, to perform a certain task when administered a certain compound and another task if a second compound distinguishable from the first is administered [119]. Thus, when a compound to which the animal is naïve is administered, the task performed by the animal indicates the control compound to which the naïve compound is most similar, thus

yielding the taste modality of the naïve compound. This methodology suffers from its inability to only provide information on taste quality, its lengthy training schedule and low-throughput nature given that only pairwise comparisons can be made during each trial [118].

1.6.2.3.3 High-throughput taste assessment model

This model enables both taste quality and palatability to be assessed for a large number of compounds during a short trial. It first involves training a rat using a reference sample at a single concentration to perform a task, e.g. pressing a lever to yield a food pellet, in much the same way as the operant taste discrimination model. Following this training period, the rat can then be used for trials which involve sampling from a 96-well plate. Taste quality can be determined by whether or not the rat performs the trained task, and palatability is gleaned from the number of licks taken from each well by the rat [116]. However, the extensive training period of approximately 7 weeks must be noted. Furthermore, given that only a single concentration reference sample is used in training, there is a risk that the rat may provide information on the taste intensity rather than the modality [116].

1.6.2.3.4 Two-bottle preference test

This simple, elegant experiment involves providing animals with free access to two bottles, usually a taste solution and a water control for 48 hours, with a positional swap at 24 hours to mitigate any side-preference that can be pronounced in rodents. The relative volumes consumed from each bottle are compared, and preference ratio calculated [120]. Given that only a single concentration of tastant can be tested over a single 48-hour period, this experiment can be exceptionally long for a full concentration range of a single tastant, often extending to several weeks. It is also important to note that the volumes consumed are very much influenced by post-ingestive effects such as toxicity or satiety, which may skew conclusions of a purely chemosensory nature [93,121].

1.6.2.3.5 Brief-access taste-aversion (BATA) model

During the BATA procedure, rats are deprived of water for normally between 16 and 24 hours prior to commencement of the experiment in order to motivate the rats to drink.

A lickometer or gustometer is then used to record the number of licks taken by each rat from each presented sample. The samples (up to 16) are stationed on a rig which moves laterally to position each sample contained within a bottle with attached sipper tube to a shutter in a randomised sequence. The shutter opens for a specified amount of time, normally between 5 and 10 s, allowing the rat to lick the sipper tube. The number of licks are counted, with a high number of licks indicative of a neutral taste while lick suppression indicates an aversive sample [122]. Due to the large number of samples that can be presented to the rodent during a single BATA procedure, a full concentration range of a single tastant or multiple tastants for a single rat may be acquired in as little as 30 minutes [93]. Importantly, this methodology does not enable determination of taste quality, only palatability. Furthermore, due to the water-deprivation that this model necessitates, assessment of appetitive solutions can prove problematic [116]. However, due to the brief exposure time, the exposure encountered by the rodent is limited thus limiting harm and post-ingestive effects are mitigated [123]. The BATA model has been explored as a predictor of human taste response, with promising findings [124–126]. Indeed, we have recently published our findings in which nine marketed APIs of varying levels of bitterness (quinine hydrochloride dihydrate, 6-n-propylthiouracil, sildenafil citrate, diclofenac sodium, ranitidine hydrochloride, caffeine citrate, isoniazid, telbivudine and paracetamol) were investigated using the BATA model and a human taste panel. The concentrations eliciting half the maximal response were determined in rats and humans, IC_{50} and EC_{50} respectively with the rat IC_{50} being 89.6 % predictive of the human EC_{50} [127]. Indeed, such promising results make this the animal taste assessment methodology of choice for the pharmaceutical industry and, as such, the Sensory Pharmaceutics® research group at UCL School of Pharmacy conduct BATA studies on new molecular entities (NMEs) for the pharmaceutical industry; the data from which are used in new drug applications (NDAs) (unpublished proprietary work). However, it is essential that such research is conducted in as humanely.

1.7 Responsible animal research

The use of animals in research, although often essential, must be conducted in as humane a way as possible. Indeed, this sentiment is underpinned by the principles of the 3Rs: replacement, reduction and refinement; the foundations of which were developed more

than 50 years ago [128]. The principles of the 3Rs underpin legislation, both national and international, pertinent to animal research, which in the UK fall under the Animals (Scientific Procedures) Act 1986 (ASPA) [129]. In the UK, the National Centre for the Reduction, Refinement and Replacement of Animals in Research (NC3Rs) forms the national body for 3Rs.

1.7.1 Replacement

Those technologies that facilitate the avoidance of animals in research fall under the replacement arm of the 3Rs. Animal research is frequently time-consuming, expensive and, where they are used to model human physiology, may not be relevant and thus replacement with human volunteers, *in vitro* and *in silico* models is justified [128]. Replacement may be full or partial, in which animals are completely avoided or restricted to only those animals that are considered incapable of experiencing suffering (e.g. *Drosophila*, nematode worms and social amoebae), respectively [128].

1.7.2 Reduction

Reduction involves the continued use of animals in research, but the minimisation of the number used, whilst also continuing to achieve the scientific aims and statistical significance. This arm of the 3Rs may also involve maximising the information gained from each animal experiment, for example through the use of *in silico* models [128].

1.7.3 Refinement

Finally, refinement is where methods are used that serve to minimise pain, suffering, distress or lasting harm to the animal under experimentation. This principle applies to conduct during scientific procedures and during times of rest, thus extending to animal husbandry [128].

1.7.4 Applying the 3Rs to pharmaceutical animal taste research

Animal models for pharmaceutical taste have shown great promise, most notably the rat BATA model [93,122]. During early drug development, there is insufficient toxicological data to allow for assessment of taste by humans, thus animals or *in vitro* models must be used as an alternative. As discussed above, the currently available *in vitro* models of taste are

inadequate, particularly for new chemical entities (NCEs) and as such we believe that currently animal models cannot be replaced. However, the use of animals in the BATA model, for example, can be reduced by leveraging the data obtained. Indeed, much of this thesis will look at how the use of animals may be reduced by exploring *in vitro* and *in silico* models that can leverage animal data thus enabling the minimisation of animal use in pharmaceutical taste research. Of course, the refinement principle must be adhered to at all points during pharmaceutical animal taste research. Indeed, beyond the importance of animal welfare, animal taste experiments are behavioural in nature, thus the happier the animal subjects, the lower the variability in experimental results leading to greater reliability and repeatability of results [128].

1.8 Scope

This introduction has served to outline the need for age-appropriate paediatric dosage forms, both from a clinical and regulatory perspective. Platform technologies serving to circumvent issues surrounding paediatric administration were discussed, with a common issue identified: palatability, both in terms of taste and mouthfeel. Thus, there is a real need for taste testing of pharmaceuticals; indeed taste testing must occur in tandem with pharmaceutical development and as early on in the drug development process as possible to enable the delivery of the most effective, patient-centric and age-appropriate medicines. The following chapters will outline current knowledge gaps in the field of oral pharmaceutical taste assessment and strive to fill said gaps and push the boundaries of existing taste assessment methodologies.

1.9 Thesis aims and outline

1. To review current knowledge on the importance of palatability in paediatric medicine, how palatability may be assessed and identify where developments are required (chapter 1).

If a paediatric medicine is aversive, a child will not take it rendering it useless. The need for palatable children's medicines is clear and well documented in the literature. This goal will only be achieved with adequate palatability testing during drug development. While human taste panels form the gold standard of palatability testing, a lack of demonstrated

safety in humans during early drug development precludes their use. Other methodologies exist, each with varied success. The BATA model has proved to be the most promising taste assessment methodology without the use of humans, however further testing and development of the BATA model is required.

2. To explore the methodological limitations of palatability assessment methodologies (chapters 2 and 3).

While the BATA model has shown real promise as a predictor of human taste and therefore as a taste assessment methodology that may be used during early-stage drug development, there are still limitations that must be addressed both with the BATA model itself and the human taste panels to which the BATA model is correlated.

3. To expand the formulation repertoire and push the limits of the BATA model (chapters 4, 5, 6 and 7).

The BATA model, although a promising taste assessment methodology, has only been validated as a taste assessment tool for API alone dissolved in water, thus relying on the API being water soluble. The BATA model has not been explored as a methodology for assessing more complex systems incorporating more than merely an aversive API in water, certainly not fully formulated medicines or solid oral dosage forms.

4. To reduce the use of animals in pharmaceutical taste research by leveraging the data from the BATA model (chapters 5, 6 and 7).

The 3Rs must guide the use of animals in research at all times. Although the use of animals in the BATA model cannot be replaced, their use may be reduced by leveraging BATA data by exploring *in vitro* and *in silico* methodologies for taste assessment. Such methodologies may also serve to satisfy aim 3 by expanding the formulation repertoire.

Specific aims and objectives are outlined in each chapter.

2 Human Gustatory Tests

2.1 Introduction

As long as humans have been consumers, their opinion on the taste, smell or appearance of goods has been valued by producers and utilised to inform the manufacture of new products. Indeed, as trading between borders became commonplace, sampling of goods by buyers became more and more formal, resulting in the establishment of grading techniques for products such as tea, coffee and meat [130]. Such sensory testing now extends to almost any consumer product and, as mentioned in chapter 1, is a requirement for pharmaceutical companies as part of PIPs/PSPs when marketing a new drug.

Psychophysics is the field of study concerned with measuring sensory experience. Naturally, the measurement of an individual's experience is methodologically challenging, as by its very nature, it is the measurement of something entirely subjective [131]. The process of sensing, be it gustation or olfaction, is not a one-step process [132]. Indeed, when a participant tastes a stimulus, nerve signals are generated, integrated at the chorda tympani and sent to the brain, which processes the sensation into a perception [133134]. The participant's response might be one of simple objective identification, e.g. "this is bitter", and may or may not be accompanied by a subjective affective reaction: acceptance or rejection, e.g. "it is horrible". An emotional response may also be exhibited by the participant, such as returning a participant to a time in their life, e.g. childhood [135]. Thus, given the complex multi-faceted nature of this experience, it is of critical importance that the sensory researcher addresses the following:

1. Clearly define outcome measures.
2. The methodology must be designed to reduce the impact of subjectivity and bias on measurements while also minimising the burden on the participants, e.g. time.
3. Robust statistical interpretation of the data [130].

Therefore, when designing a sensory test, one must always consider the 5 S's: subjects, site, samples, statistical analysis and sensory method.

When considering the subjects, it is necessary to choose between trained or untrained subjects; the choice of which is largely dependent on the methodology being utilised. Where a descriptive analysis is sought, trained participants are necessary, while a binary assessment of like/dislike does not usually require such trained participants. Of course, the subject demographics, such as ethnicity or gender, must also be considered as such characteristics may affect a participant's sensory world [135].

The site of sensory assessment is also of great importance. For example, in consumer testing, it must be determined whether or not it is necessary for assessment to occur in the normal location in which the product is used, e.g. the home, or whether such conditions can be adequately replicated in the laboratory [135]. However, in the context of sensory pharmaceutics, it is considered that a space devoid of any unwanted sensory input is sufficient, rather than replicating an area in which a patient may administer a medicine, which is highly variable.

The sample, and the way it is presented may also affect the participant's response. In consumer testing, this is tightly controlled. For example, when assessing the taste of instant coffee, an extensive multi-stage process is adhered to, in which all aspects of the coffee-making process are controlled - from weighing of the coffee granules to the heating of the mug to a standardised temperature – in order to mitigate any differences that might affect a participant's sensory experience [135]. When assessing medicines, there are of course strict regulations regarding sample preparation, e.g. they must be prepared extemporaneously under the supervision of a pharmacist, but confounding factors must also be considered in order to minimise variability. Further, the sample stability must also be considered, which will inform when the sample is prepared and the container in which the sample is stored and administered.

As with any experiment, data analysis and statistics are key to reaching the correct conclusion from the obtained data. Data from sensory experiments is often complex and may not follow Gaussian distribution, thus necessitating the use of non-parametric statistical tests. Of course, an adequately large sample size is key to ensuring robust and valid statistical analysis.

Lastly, the sensory method is of course critical and can often be hugely complicated to decide on given the many considerations, such as participant age, understanding and outcome measure assessed. The methodological toolkit available to the sensory researcher is however extensive, thus allowing for the aforementioned factors to be accounted for.

There is a wealth of guidance pertaining to the 5 S's when evaluating food in consumer research [136,137], however in the nascent area of pharmaceutical sensory evaluation, there is a dearth of such guidance. Although lessons learnt from food research may be used to guide developments in pharmaceutical sensory research, this must be done so tentatively given the vast differences in these research areas. While food research focuses on the maximisation of pleasure, pharmaceutical sensory research focuses on the minimisation of aversiveness towards neutrality rather than pleasure. In addition, given that pharmaceutical sensory evaluation involves drugs, there are additional considerations such as safety, toxicity and principles of good manufacturing practice (GMP) among others. Indeed, this complexity is further compounded when assessing paediatric medicines.

This chapter will focus on two of the five S's: the subjects and the statistics; by questioning how many participants and which participants are required for a human gustatory panel. It is currently not known how many participants are necessary to maximise statistical significance, while minimising participant burden when assessing varying levels of bitterness. Indeed, a search of the literature reveals a complete lack of agreement in the number of participants required for a human taste panel. A search was conducted on 13th May 2019 using PubMed Central, using the following search terms: "human", "taste", "gustatory", "test", "panel", and the Boolean operators: "and", "or" with the search restricted to abstracts; a total of 380 publications were returned. Limiting the aforementioned search to those papers published in the last 5 years returned 165 publications, whose abstracts were assessed for relevance based on whether or not the paper described a human taste panel. A total of 33 papers were found to be relevant, from which the sample sizes used in human taste panels were extracted and plotted as per Figure 2-1.

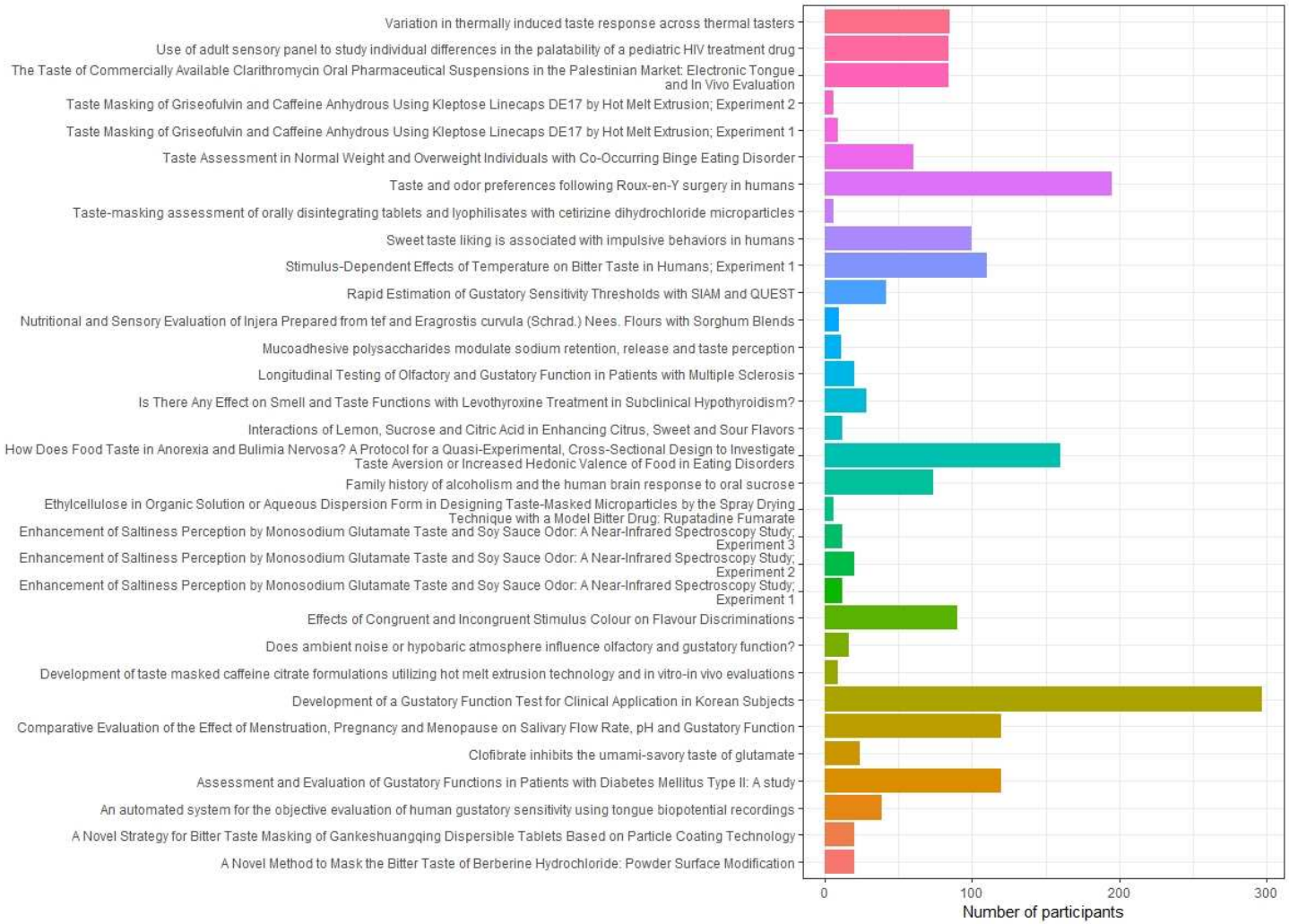


Figure 2-1 The number of participants used in human taste panels in a 5-year search of the literature (search conducted on 13th May 2019).

Figure 2-1 reveals the stark differences in sample sizes used in human taste panels, with the minimum sample size observed to be 6 and the maximum being 297. The mean and median sample sizes were found to be 57.61 and 28 respectively. Indeed, 5 studies used less than 10 participants, 16 studies used less than 25 participants and 20 studies less than 50, with 13 studies using more than 50 participants. If those studies only pertaining to the assessment of bitter drugs are extracted from the initial literature search, 10 publications are returned, with the results shown in Figure 2-2.

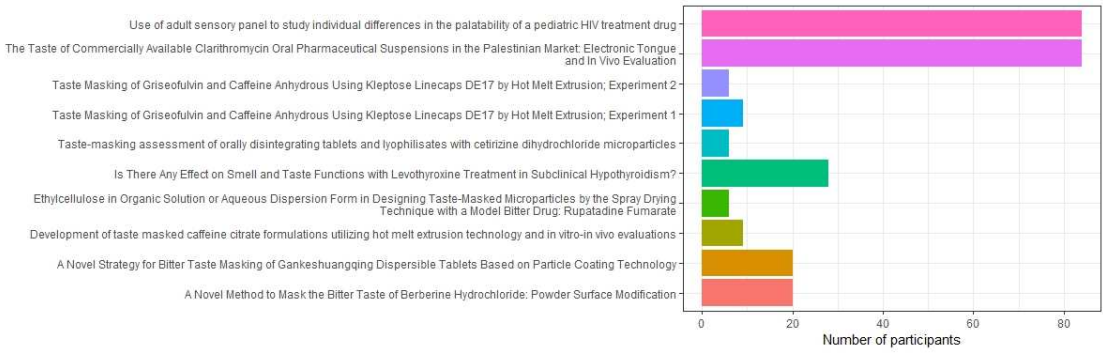


Figure 2-2 The number of participants used in human taste panels assessing bitter drugs in a 5-year search of the literature (search conducted on 13th May 2019).

A similar lack of concordance is thus observed among researchers using human taste panels to assess bitter drug compounds, with a minimum sample size of 6 and a maximum of 84, with mean and median sample sizes of 27.2 and 14.5, ,respectively. Therefore, for the sensory scientist entering the field or the formulation scientist hoping to understand the bitterness of a drug compound using a human taste panel, there is a real need for some clarity as to the number of participants necessary, thus demonstrating a real need for this figure to be sought.

A further consideration for the sensory researcher is which participants to use in the proposed human taste panel. Should those participants be selected based on sensitivity to the assessed sample, or perhaps a genetic trait conferring sensitivity to a given taste modality?

The obvious genetic trait to use to select participants is the ability – or not – to taste 6-n-propylthiouracil (PROP) or phenylthiocarbamide (PTC) (both containing the thiourea (N-

C=S) moiety responsible for the bitterness perceived), and if tasters, to what extent are they sensitive. This trait is conferred by the widely studied *TAS2R38* gene [138]. Indeed, 75 % of the human population are capable of tasting this compound, with varying bitterness levels identified, while the remaining 25 % are unable to detect any taste at all, thus "tasters" and "non-tasters", respectively [139,140]. The taster phenotype can be further stratified to "medium tasters" and "super tasters", with the latter perceiving the taste of PROP/PTC to be extremely bitter [141]. The distribution within the human population of non-tasters, medium tasters and super tasters has been found to be 25 %, 50 % and 25 %, respectively [141]. Isothiocyanates, present in *Brassica* vegetables, share the thiourea moiety found in PTC/PROP, thus PROP tasters have been shown in multiple studies to lead to an avoidance of such vegetables [142–147]. However, numerous studies have demonstrated that super tasters also dislike bitter foods that do not share the thiourea moiety, and foods that are not bitter but elicit a strong sensation within the mouth, such as sweets, spice and alcohol [143–146,148–150]. Thus, given the enhanced sensitivity of PROP tasters to bitter and strong oral sensations, PROP phenotyping may serve as a means to select the most sensitive participants for the assessment of bitter pharmaceuticals.

Of those publications discussed above, those using participant selection to gain the most sensitive participants and those simply using broad inclusion and exclusion criteria not based on taste sensitivity were compared. The majority of publications (66.66 %) did not use participant selection methodologies to acquire the most taste-sensitive participants, instead selecting based on simple inclusion and exclusion criteria, such as gender and lack of infirmity. If only those publications pertaining to bitter drugs are assessed, 60 % did not use participant selection methodologies. Therefore, the literature suggests such participant selection may not be necessary, but there is no strict guidance on this for the formulation scientist, thus this chapter will address *how* participants may be selected for human taste panels.

2.2 Aims

1. Elucidate the number of participants necessary for a human taste panel
2. Investigate how participants may be selected for human pharmaceutical taste panels.

2.3 Objectives

1.
    a. Conduct human taste panel assessing quinine hydrochloride
    b. Build a model to take varying samples from the total dataset and assess statistical significance thus informing the minimum number of participants necessary for distinguishing between varying levels of bitterness.
2.
    a. Identify potential ways of selecting participants for human taste panels
    b. Stratify participants using selected methodologies
    c. Assess different responses of stratified participants to a range of APIs identifying which methodology selects the most sensitive participants.
    d. Confirm findings using a model drug of unknown bitterness

2.4 Materials and Methods

2.4.1 Materials

Quinine hydrochloride, propylthiouracil, sodium chloride, ibuprofen sodium, telbivudine and ranitidine hydrochloride were purchased from Fagron (Newcastle-upon-Tyne, England).

2.4.2 Methods

2.4.2.1 Sample size

In order to inform sample size requirements of future human taste panels, a human panel consisting a range of concentrations of quinine hydrochloride (QHCl) was performed for the purpose of eventual multiple bootstrap sampling (see section 2.4.2.1.2) and statistical analysis of samples of increasing size to establish a power plot.

2.4.2.1.1 Taste Panel Procedure

Fifty-four volunteers between the ages of 18 and 38 years old (median 22 years old) were enrolled in a randomised single-blind study. Participants were recruited by internal advertising at UCL School of Pharmacy, and included university staff and students. All participants provided written consent to participate in the study. The study was conducted in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by the Research Ethics Committee at UCL School of Pharmacy (REC ID: 4612/012).

All taste studies described in this thesis were conducted within a designated room at UCL School of Pharmacy devoid of any unwanted distractions or unnecessary sensory input.

The 'swirl and spit' methodology as described in [122] was employed, whereby the participants were presented with 10 mL of the following QHCl concentrations: 0.0097, 0.097 and 0.32 mg/mL representing low, medium (EC_{50}[151]) and high bitterness levels respectively, prepared extemporaneously under the supervision of a UK-registered pharmacist. The participants were instructed to swirl each sample around their mouths 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 2-3).

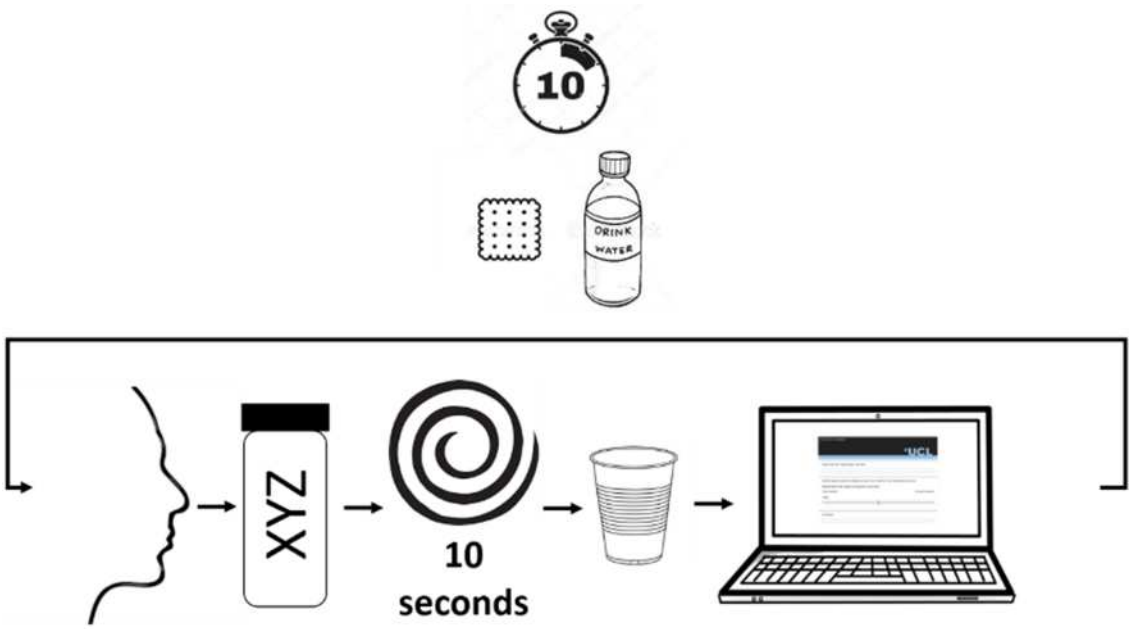


Figure 2-3 Flow diagram representing the 'swirl and spit' methodology steps in a human taste panel

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' (Figure 2-4).

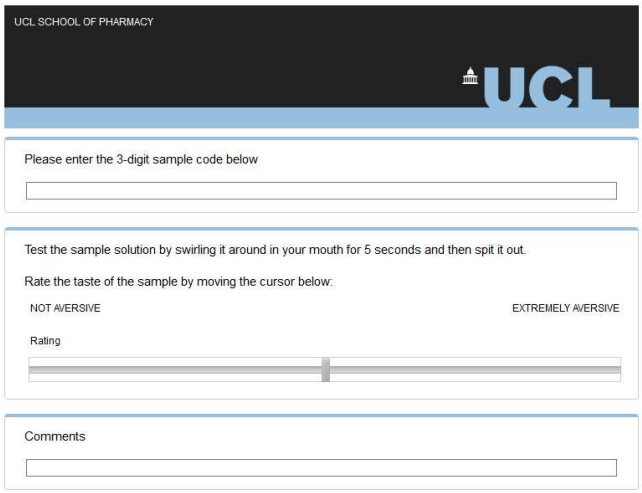


Figure 2-4 The participants entered the 3-digit sample code prior to rating the sample on the VAS

2.4.2.1.2 Sample sizing model

Using R (open source), a model was built – see Figure 2-5 – which enabled bootstrap sampling of varying sample sizes – from 1 to 50 participants – from the initial dataset. Bootstrapping simply refers to the sampling, with replacement, of smaller proportions of the total dataset. The Kolmogrov-Smirnov non-parametric test was used to assess the statistical difference between bootstrapped samples. Figure 2-5 provides a graphical representation of this process; it shows two complete datasets from which smaller proportions of samples are taken (see coloured sections) and compared against each other using the Kolmogrov-Smirnov test. Bootstrapping was performed 2000 times for each sampling proportion. The p-value resulting from each of the 2,000 bootstrapped samples at the same sample size was automatically pasted into a specified database, which was subsequently combined with other databases corresponding to sample sizes at differing levels (1 to 50 participants) to form a final database of 28, 000 p-values. ggplot2 was used to transform said database into a plot showing the power as a function of sampling proportion.

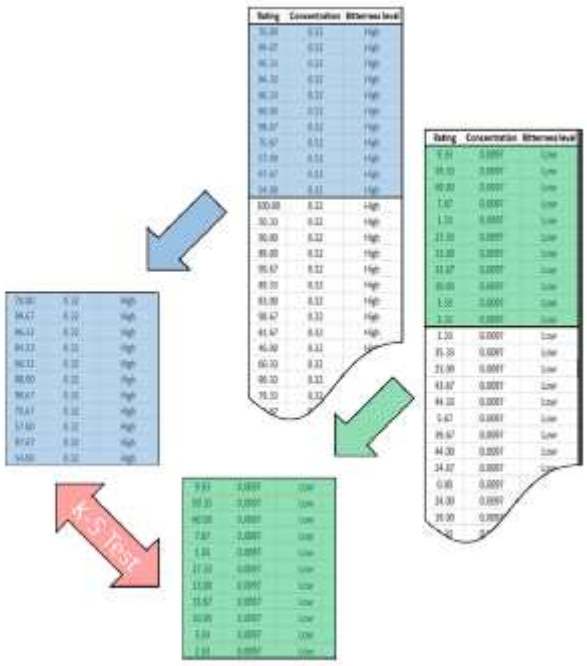


Figure 2-5 Graphical representation of the model: showing random sampling and subsequent K-S test.

2.4.2.2 Participant selection methodologies

Four participant selection methodologies were explored in this study: propylthiouracil (PROP) taste phenotype analysis, sensitivity to the API under investigation, QHCl sensitivity and QHCl precision. The latter two utilise taste analysis of QHCl as a surrogate measure of a participant's response to aversive APIs. The effectiveness of these methodologies was assessed by identifying how the respective strata respond to other APIs of varying aversiveness. The aforementioned methodologies were explored in turn.

2.4.2.2.1 PROP Phenotype Analysis

Critically, to establish different responses to PROP, a standard that is perceived as equally intense to all subjects regardless of phenotype must be chosen against which ratings of PROP can be compared. Failure to do this would lead to all subjects being deemed medium tasters. As such, NaCl (sodium chloride) was chosen as this compound is not tasted in a systematically different way among different PROP phenotypes [141].

Therefore, each participant was given three samples of PROP and NaCl, respectively, at concentrations specified in Table 2-1.

Table 2-1 Concentrations of PROP and NaCl used in the determination of PROP phenotype

Compound	Concentrations assessed (% w/v)	Sample number
PROP	0.0005	1
	0.005	2
	0.05	3
NaCl	0.0584	1
	0.584	2
	5.84	3

Sixty volunteers between the ages of 18 and 47 years old (median 22 years old; 14 males and 46 females; 11 ethnicities (7 white British, 11 Asian/Asian British other, 1 white Irish, 4 mixed other, 10 white other, 4 Arab, 15 Chinese, 2 African/Caribbean other, 2 Pakistani and 3 Indian) were enrolled in a randomised single-blind study. The protocol was approved by the Research Ethics Committee at UCL School of Pharmacy (REC IDs: 4612/010 & 4612/012).

The 'swirl and spit' methodology was employed in this study, as explained in 2.2.2.1.1. However, participant assessment differed slightly. Participant assessment of each sample was achieved using the online survey software Qualtrics®, which calls on the participant to rate a given sample's intensity on a 100 mm visual analogue scale (VAS) from 'barely detectable' to 'strongest imaginable' (Figure 2-7). Participants were first asked to read a statement – as shown in Figure 2-6 – that clearly outlines what was meant by intensity of taste and thus how to use the online VAS.

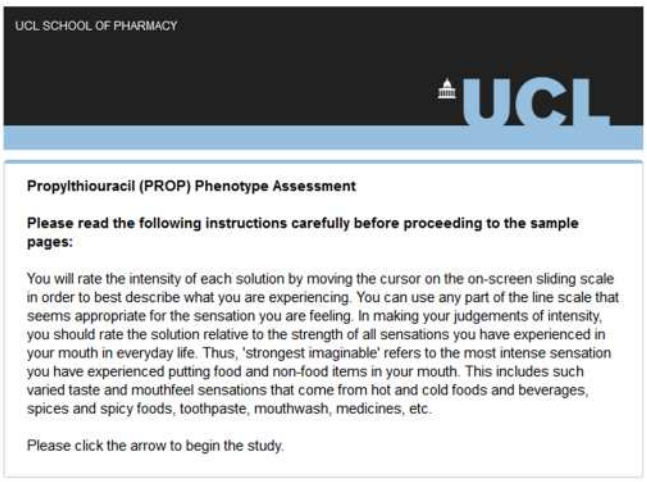


Figure 2-6 The participants were explained how to use the online VAS, with a clear description of what 'strongest imaginable' denotes in terms of taste sensation.

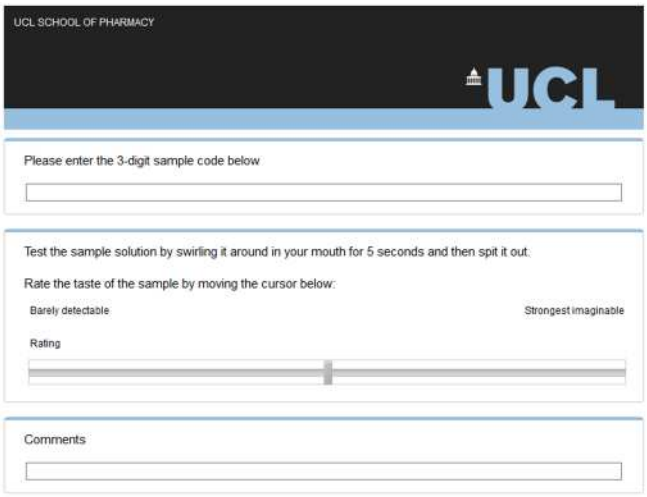


Figure 2-7 The VAS used by the participants to rate each presented sample, with additional comments section should the participant wish to add further comments

Assignment of phenotype was achieved by assessing response to samples 2 and 3 of PROP and NaCl relatively. If the participant's rating of samples 2 and 3 of NaCl were not significantly different to that of samples 2 and 3 of PROP respectively, the participant was assigned the medium taster phenotype (Figure 2-8). If, however, the participant rated samples 2 and 3 of PROP significantly higher than samples 2 and 3 of NaCl respectively, the participant was assigned the supertaster phenotype (Figure 2-8). Lastly, if the participant rated samples 2 and 3 of PROP significantly lower than samples 2 and 3 of

NaCl respectively, the participant was assigned the non-taster phenotype (Figure 2-8). Statistical significance was determined by performing the Wilcoxon signed-rank test.

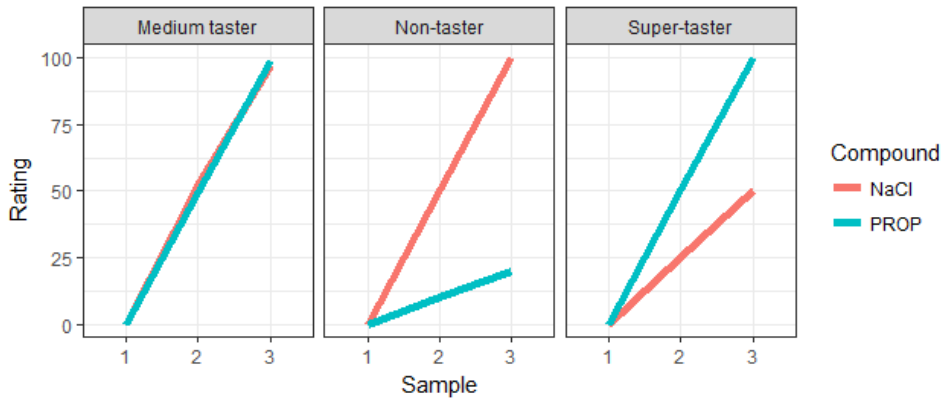


Figure 2-8 A graphical representation of a medium taster, non-taster and supertaster.

2.4.2.2.2 API Sensitivity

Initially, participant sensitivity to three APIs – namely ibuprofen sodium, ranitidine hydrochloride and telbivudine – was established. Subsequent studies involving an anonymised compound (due to the compound being proprietary) of unknown bitterness – compound X – were carried out to further test the established hypothesis. Sensitivity was established by presenting each compound at a low concentration relative to its bitterness taste threshold – see Table 2-2.

Table 2-2 Concentrations of APIs assessed by participants to determine API sensitivity

Compound	Concentrations assessed (mg/mL)
Ibuprofen sodium	1
Ranitidine hydrochloride	0.25
Telbivudine	1
Compound X	0.01

Thirty one volunteers between the ages of 18 and 38 years old (median 22 years old; 8 males and 23 females; 10 ethnicities (2 white British, 10 Asian/Asian British other, 1 white Irish, 2 mixed other, 3 white other, 2 Arab, 8 Chinese, 1 African/Caribbean other, 1 Pakistani and 1 Indian)) were enrolled in a randomised single-blind study. The protocol was approved by the Research Ethics Committee at UCL School of Pharmacy (REC ID: 4612/012).

The methodology employed was identical to that which is outlined in section 2.2.2.1.1

2.4.2.2.2.1 Determining API sensitivity

A participant was deemed sensitive to a given API if their response on the online VAS exceeded 25 at the concentrations specified in Table 2-2. The threshold of 25 was chosen as the lower limit above which the response was deemed sensitive given the low concentrations administered. Figure 2-9 demonstrates a hypothetical example of sensitive and non-sensitive response at an arbitrary concentration of 1 AU.

All participants were stratified as per Figure 2-9 and their response to a wider concentration range was assessed to determine the effectiveness of this selection methodology. The Wilcoxon signed rank test was used as the statistical method to assess the difference between responses between all stratified populations detailed in the following methods.

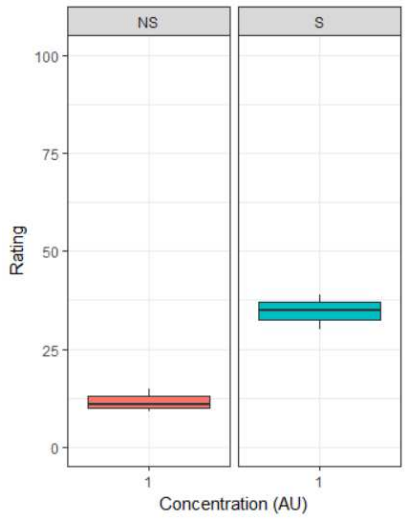


Figure 2-9 A demonstrative example of the output observed for non-sensitive (NS) (left) and sensitive (S) (right) participants in response to a hypothetical API at a low concentration.

2.4.2.2.3 QHCl sensitivity

Sensitivity to QHCl was determined in an identical way to 'API sensitivity' mentioned previously, using identical participants and Qualtrics® survey. However, here QHCl was used as a model bitter drug. This study was also carried out in accordance with REC 4612/012.

2.4.2.2.4 QHCl precision

QHCl precision was determined by assessing both the magnitude and range of responses to both low and high concentrations of QHCl relative to the EC_{50}. A participant was deemed precise if they rated their lowest sample below 25, their highest sample above 75 and the range between ratings of replicates of the same concentration did not exceed 50. The upper and lower limits were chosen as they represent the lower and upper quartile of the scale, which was predicted to be selected when the participant was presented with a low and high strength sample, respectively. See Figure 2-10 for example participants and their assignment based on the aforementioned principles.

All participants were stratified as per Figure 2-10 and their response to a wider concentration range was assessed to determine the effectiveness of this selection methodology.

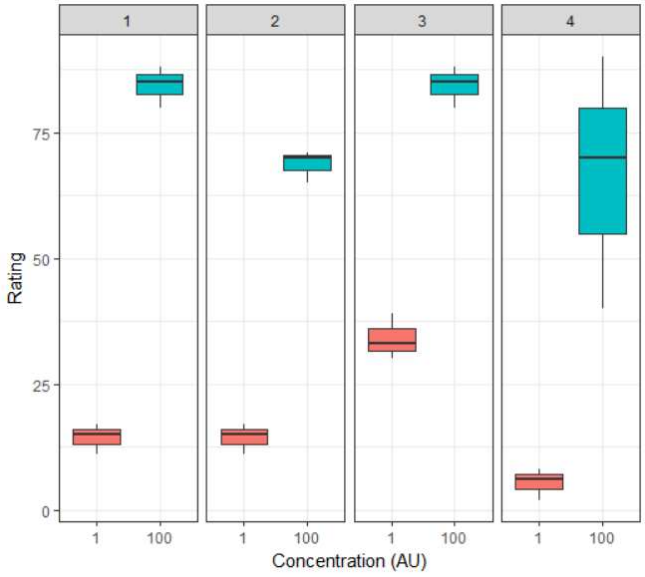


Figure 2-10 A demonstrative example of the output observed for 4 hypothetical participants. Participant 1 would be deemed QHCl precise given the lowest concentration is rates <25, the highest >25 and the range between ratings at the same concentration <50. Particip

2.4.2.2.5 Assessing the effectiveness of each participant selection methodology

The populations were stratified according to the aforementioned participant selection methodologies prior to assessing the responses of the various strata to a concentration range of APIs of varying aversiveness levels. Solutions of ibuprofen sodium, ranitidine hydrochloride, telbivudine and later compound X – for the purpose of hypothesis testing

– at concentrations specified in Table 2-3 were assessed by the stratified populations, and responses compared.

Table 2-3 Concentrations of APIs assessed by participants to determine API sensitivity

Compound	Concentrations assessed (mg/mL)
Ibuprofen sodium	1, 5, 20, 50
Ranitidine hydrochloride	0.25, 0.5, 1, 1.5
Telbivudine	1, 5, 10, 20
Compound X	0.01, 0.1, 0.6, 1.2

Thirty one volunteers between the ages of 18 and 38 years old (median 22 years old; 8 males and 23 females; 10 ethnicities (2 white British, 10 Asian/Asian British other, 1 white Irish, 2 mixed other, 3 white other, 2 Arab, 8 Chinese, 1 African/Caribbean other, 1 Pakistani and 1 Indian)) were enrolled in a randomised single-blind study. The protocol was approved by the Research Ethics Committee at UCL School of Pharmacy (REC ID: 4612/012).

The methodology employed was identical to that which is specified in fig. 7 of section 2.2.2.1.1.

2.4.2.2.5.1 Graphical representation of the data

The data relating to aversiveness of each API were presented in 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 – see Figure 2-11. Outliers, shown as black dots outside of the boxplot, are determined as such if the observation is greater or less than 1.5 times the interquartile range from 25th and 75th percentile respectively, i.e. outside of the whiskers. The aversiveness ratings were plotted as a function of increasing concentration.

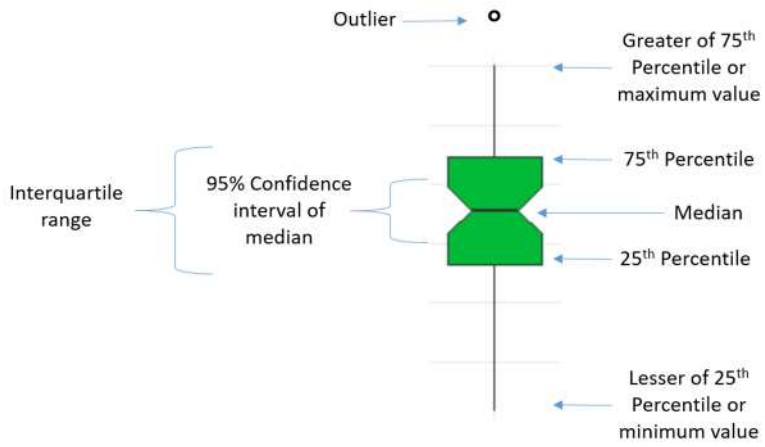


Figure 2-11 The elements of a notched boxplot explained

The EC_{50} – concentration of the drug that produces half of the maximal rating (100) in the human taste panels – was also determined for each API, however these are merely indicative of aversiveness given the small concentration range assessed [152]. The EC_{50} and its calculation is very well explained in a paper by Soto *et al.* [151].

Analysis and plotting was performed using R software (open source) and non-linear mixed effects (NONMEM) tool (version 7.3, ICON Development Solutions, Dublin, Ireland).

2.5 Results

2.5.1 Sample size

28,000 p-values following bootstrap sampling to varying extents and subsequent statistical testing as mentioned above were combined, and the results plotted to inform the number of participants required to distinguish between low to medium, medium to high and low to high concentrations of QHCl (Figure 2-12).

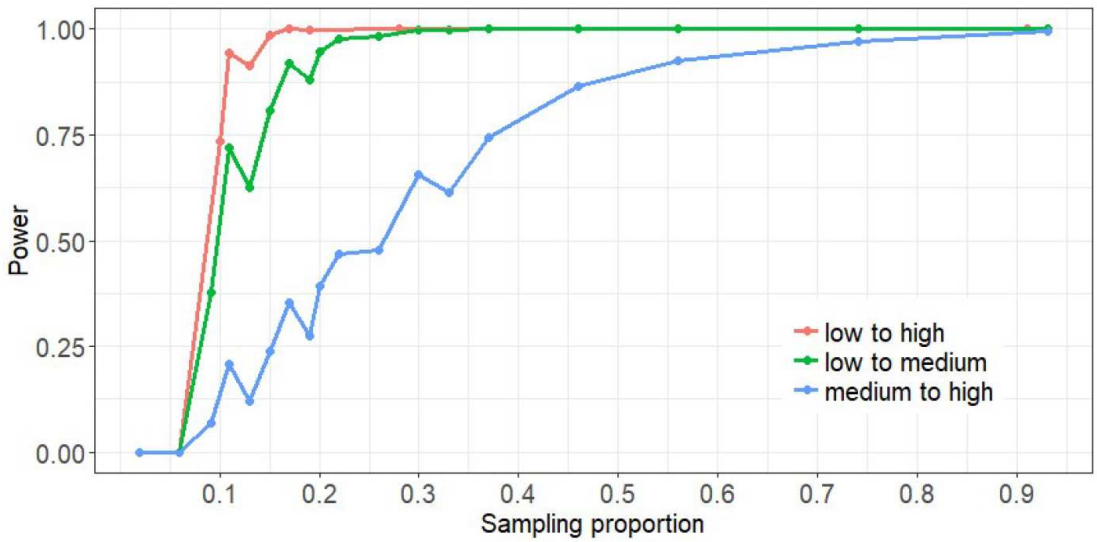


Figure 2-12 Assessing the sampling proportion necessary to achieve the power of the full dataset (54 participants) for differentiation between differing levels of bitterness

Figure 2-12 demonstrates that a different sample size is necessary to distinguish between different levels of bitterness. The results stand to reason, with the smallest number of participants necessary when distinguishing between extremes of bitterness (low and high), but increasing number of participants necessary when distinguishing between less extreme levels of bitterness (low and medium, medium and high), see Figure 2-13).

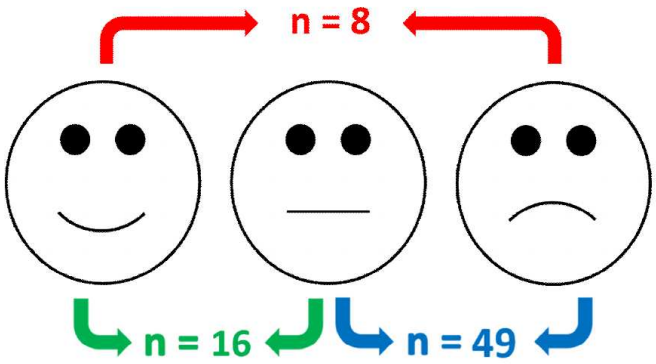


Figure 2-13 Sample sizes required for differentiation between low and high (red), low and medium (green), and medium and high (blue) bitterness levels.

Figure 2-12 demonstrates that a sampling proportion of 0.3 (16 participants) achieves the statistical power of 1, i.e. 16 participants are sufficient to achieve the same statistical power as 54 participants when assessing low and medium levels of bitterness, 0.0097 and 0.097 mg/mL QHCl respectively.

The assessment of a statistically significant difference between samples of medium (0.097 mg/mL QHCl (EC50)) and high (0.32 mg/mL QHCl) level bitterness requires a larger dataset relative to the above. To achieve the same statistical power as the total dataset of 54 participants, a sampling proportion of 0.9 is necessary; that is to say that 49 participants are required to achieve the same level of statistical significance when differentiating between middle and high level bitterness samples as 54 participants (Figure 2-13).

Figure 2-12 also demonstrates the number of individuals required to differentiate between low and high level bitterness samples to a statistically significant level. As expected, the sampling proportion required to achieve statistical significance between low and high levels of bitterness was the lowest of all bitterness levels investigated. Indeed, Figure 2-13 demonstrates that 8 participants are necessary to achieve the same statistical significance as 54 participants.

2.5.2 Participant Selection Methodologies

2.5.2.1 Participant Stratification

2.5.2.2 PROP phenotype analysis

The participants were successfully stratified into non-taster, medium taster and super taster populations by performing the Wilcoxon signed-rank test. Extremely small p values were attained for the super and non-taster phenotypes, indicative of significant difference, while the medium tasters show no statistical difference.

The proportion of individuals belonging to each phenotype is shown in Figure 2-14. The majority of participants were non-tasters, followed by medium tasters, with the super-tasters forming the smallest sub-population.

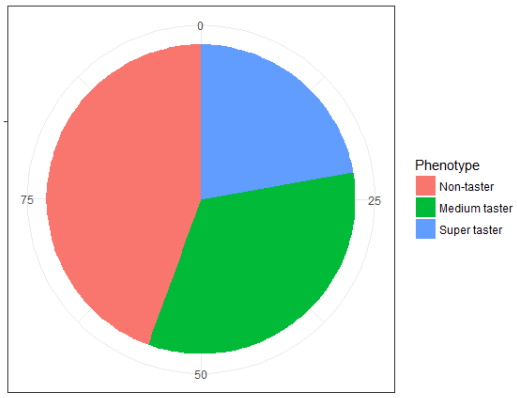


Figure 2-14 Proportion of medium tasters, non-tasters and super tasters. Those participants who were unassignable were excluded.

Both males and females demonstrated the same population order for each phenotype, as per Figure 2-15.

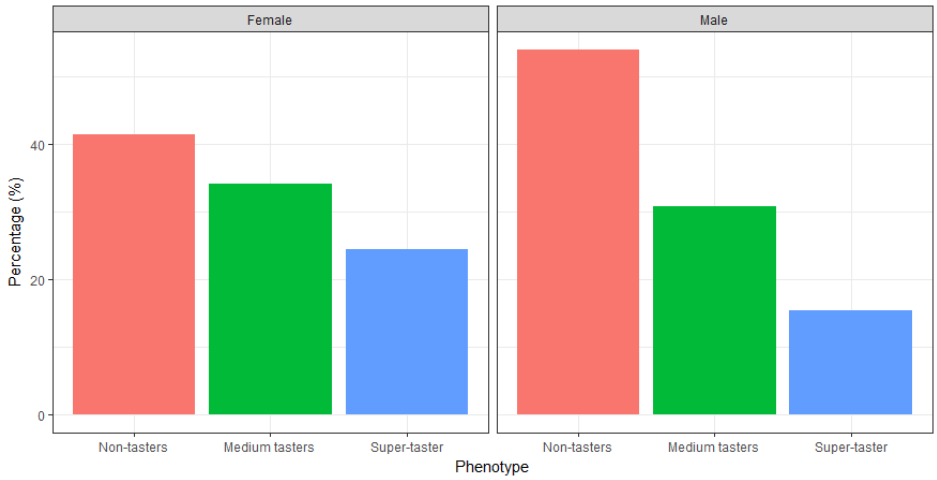


Figure 2-15 Proportion of non-tasters, medium tasters and super tasters in male (n = 14) and female (n = 46) populations

2.5.2.3 API Sensitivity

Participants were successfully stratified into sensitive and non-sensitive strata based on their recorded responses to low concentrations (relative to the respective IC/EC_{50}s) using the parameters stated above. For both ibuprofen sodium and telbivudine, the proportion of non-sensitive participants exceeded the sensitive participants. By contrast, with ranitidine hydrochloride, the majority of participants were sensitive – see Figure 2-16.

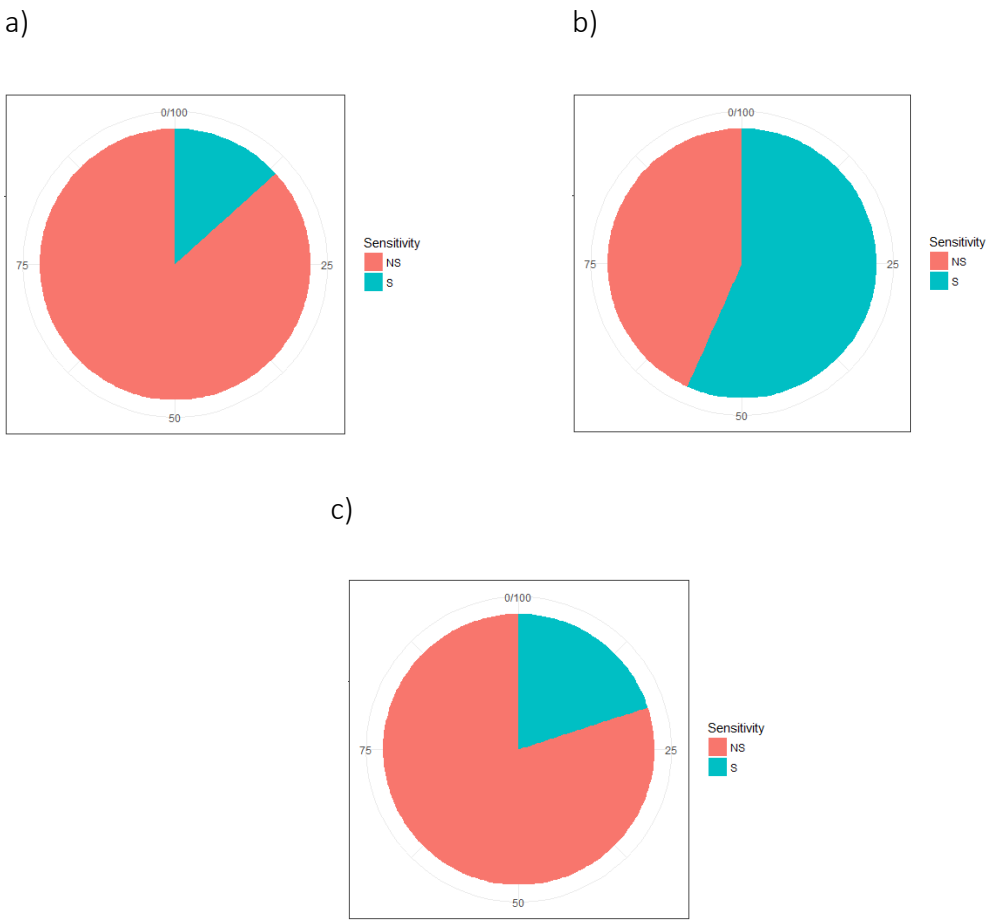


Figure 2-16 Proportion of sensitive and non-sensitive participants for a) ibuprofen sodium b) ranitidine hydrochloride and c) telbivudine

2.5.2.4 QHCl sensitivity

Participant response to the lowest concentration of QHCl was used to stratify the population by QHCl sensitivity. Figure 2-17 demonstrates the way in which the population was split, with the majority of participants assigned non-sensitive.

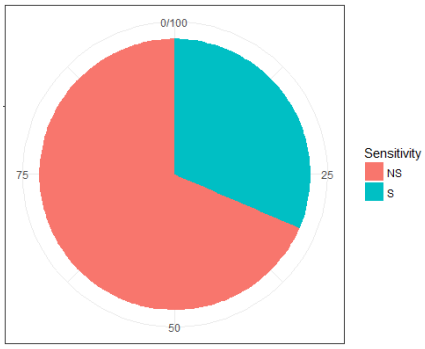


Figure 2-17 Proportions of sensitive (n = 21) and non-sensitive (n = 37) participants by QHCl sensitivity

2.5.2.5 QHCl precision

The population were successfully split by assessing the response to the lowest and highest concentrations of QHCl.

The majority of the participants were deemed imprecise based on the above criteria: see Figure 2-18.

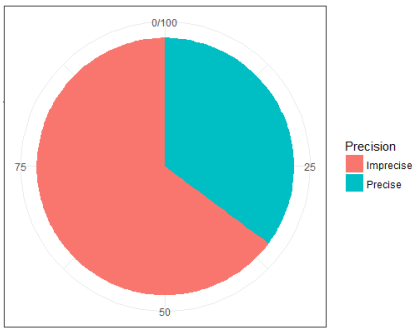


Figure 2-18 Proportions of imprecise and precise individuals following stratification

2.5.2.6 Drug x

In order to further assess the aforementioned participant selection methodologies for their respective abilities to stratify participants into subgroups of varying sensitivities to aversiveness, a separate study was performed, and the same methodologies explored for the purpose of hypothesis testing. However, PROP phenotyping was not performed in this section.

2.5.2.6.1 API sensitivity

The population were successful split in accordance with the ratings of the lowest concentration of drug x.

The majority of participants were assigned non-sensitive, as per Figure 2-19.

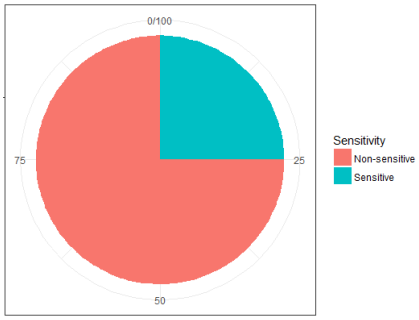


Figure 2-19 Proportions of non-sensitive and sensitive individuals following stratification

2.5.2.6.2 QHCl sensitivity

The population was successfully stratified into sensitive and non-sensitive based on QHCl.

The majority of participants were assigned non-sensitive as per Figure 2-20.

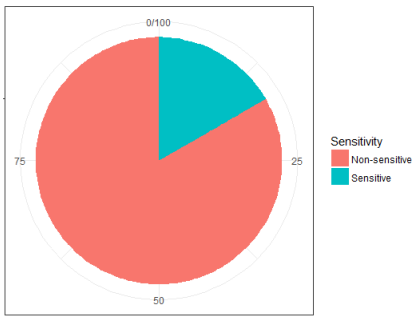


Figure 2-20 Proportions of sensitive and non-sensitive individuals following stratification

2.5.2.6.3 QHCl precision

The population was successfully stratified into precise and imprecise tasters, based on responses to QHCl at low and high concentrations.

The participants were split fairly evenly, with 56.25% being deemed imprecise and 43.75% being deemed precise: see Figure 2-21.

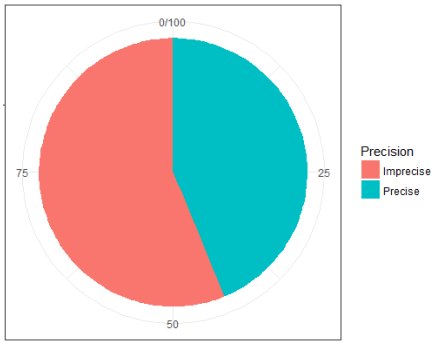


Figure 2-21 Proportion of imprecise and precise individuals following stratification

2.5.2.7 Assessing Participant Response Following Stratification

The above participant selection methodologies were tested by assessing responses of stratified populations to four APIs: ibuprofen sodium, ranitidine hydrochloride and telbivudine. Compound x was then utilised to assess whether the same hypothesis was reached. Each will now be explored in turn.

2.5.2.7.1 Ibuprofen Sodium

2.5.2.7.1.1 Response according to PROP phenotype

Stratification of the population by PROP taster phenotype showed an inability to identify more sensitive participants to ibuprofen sodium: see Figure 2-22.

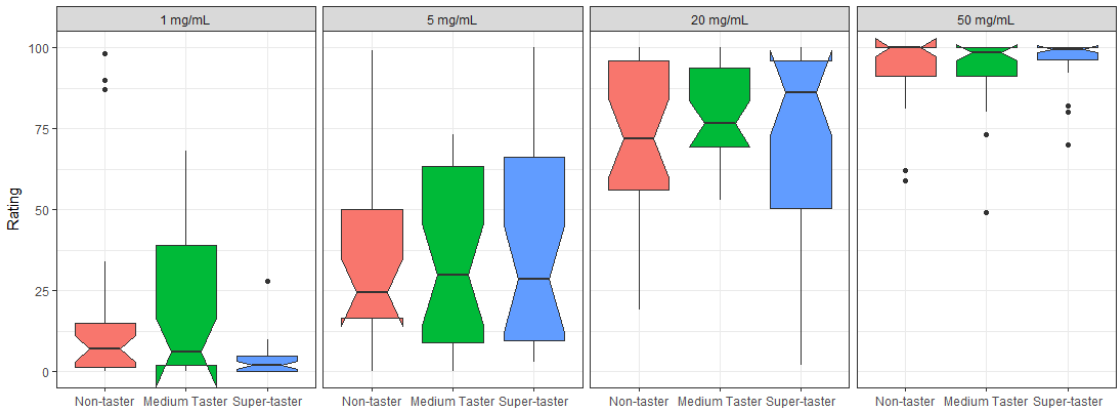


Figure 2-22 Figure 38 Responses of PROP non-tasters, medium tasters and super tasters to increasing concentrations of ibuprofen sodium.

At all concentrations of ibuprofen sodium, there was no significant difference ($p > 0.05$) between non-tasters, medium tasters and super tasters as indicated by an overlapping of notches. Identification of more sensitive participants through assessing PROP phenotype has not been achieved for this API.

2.5.2.7.1.2 Response according to 1mg/mL ibuprofen sodium sensitivity

Participants were stratified according to their response to 1mg/mL ibuprofen as a means to identify sensitivity to higher concentrations. Figure 2-23 demonstrates the varying responses observed between sensitive and non-sensitive participants.

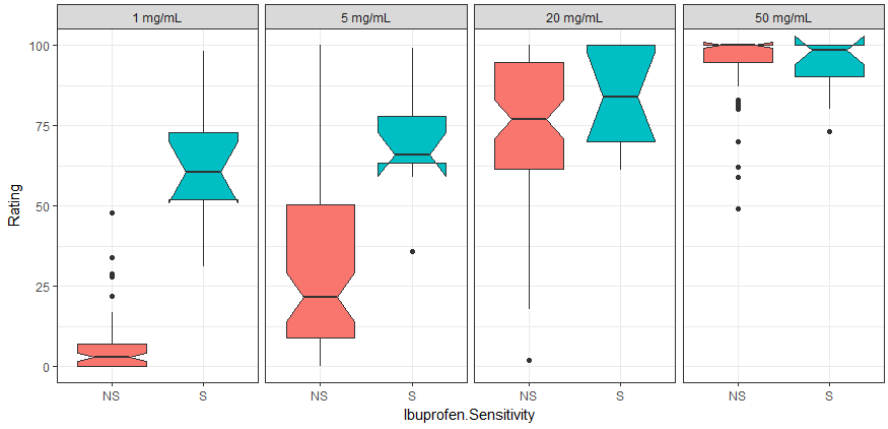


Figure 2-23 Response of participants to increasing concentrations of ibuprofen sodium following stratification by sensitivity to 1mg/mL ibuprofen sodium in water.

Naturally, at 1mg/mL, a significant difference ($p < 0.05$) between strata is observed given that stratification was carried out using this sample. At 5 mg/mL, a similar pattern is observed with no overlap of notches, and a significantly different ($p < 0.05$) response by sensitive and non-sensitive participants. As the concentration increases to 50 mg/mL, the marked difference between sensitive and non-sensitive participants becomes diluted, with overlapping of notches, and no significant differences between strata at 20 and 50 mg/mL ($p > 0.05$).

2.5.2.7.1.3 Response according to QHCl sensitivity

At 5 mg/mL ibuprofen sodium, a significant difference ($p < 0.05$) was observed between QHCl sensitive and non-sensitive participants, as indicated by the lack of overlap between the notches: see Figure 2-24. However, at 1, 20 and 50 mg/mL ibuprofen sodium, QHCl sensitive and non-sensitive participants showed no significant difference ($p > 0.05$) between ratings.

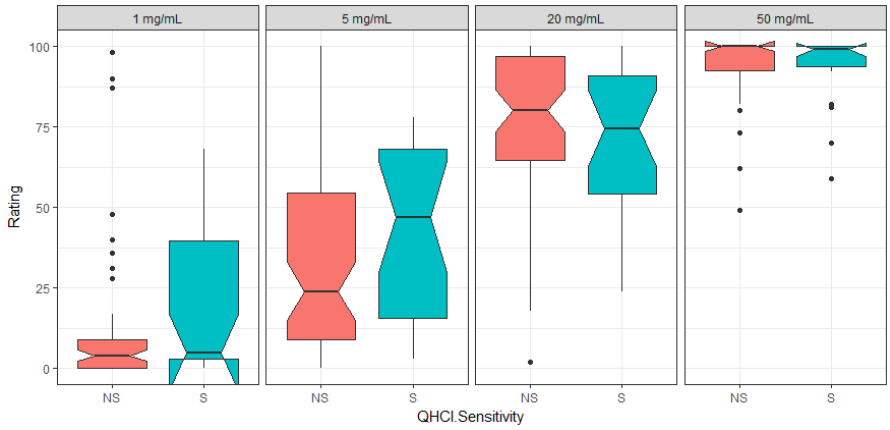


Figure 2-24 Response of participants to increasing concentrations of ibuprofen sodium following stratification by sensitivity to QHCl

2.5.2.7.1.4 Response according to QHCl precision

Responses to 1, 5 and 50 mg/mL ibuprofen sodium showed no significant differences ($p > 0.05$) between QHCl imprecise (IMP) and precise (P) individuals, indicated by an overlapping of notches shown in fig. 40. At 20 mg/mL, P individuals showed greater aversiveness rating relative to the IMP individuals, although this was not found to be significant ($p > 0.05$): see Figure 2-25.

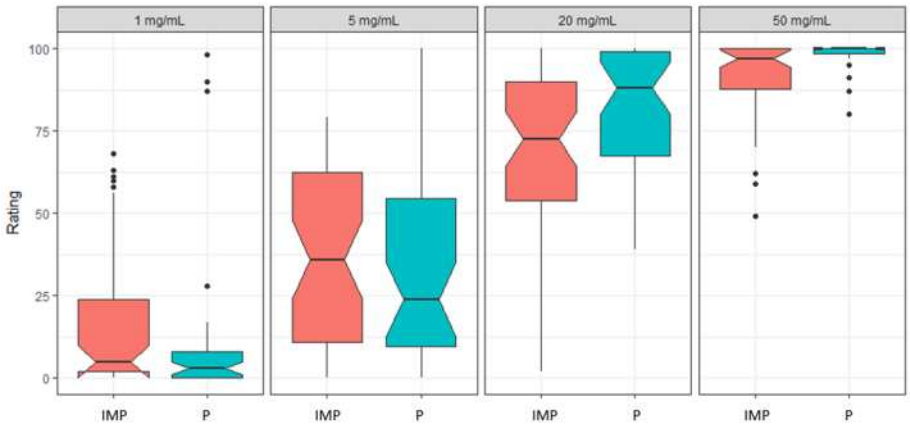


Figure 2-25 Response of participants to increasing concentrations of ibuprofen sodium following stratification by QHCl precision.

2.5.2.7.2 Ranitidine Hydrochloride

2.5.2.7.2.1 Response according to PROP phenotype

Phenotyping participants by PROP taste status was unable to identify those more sensitive to ranitidine hydrochloride: see Figure 2-26.

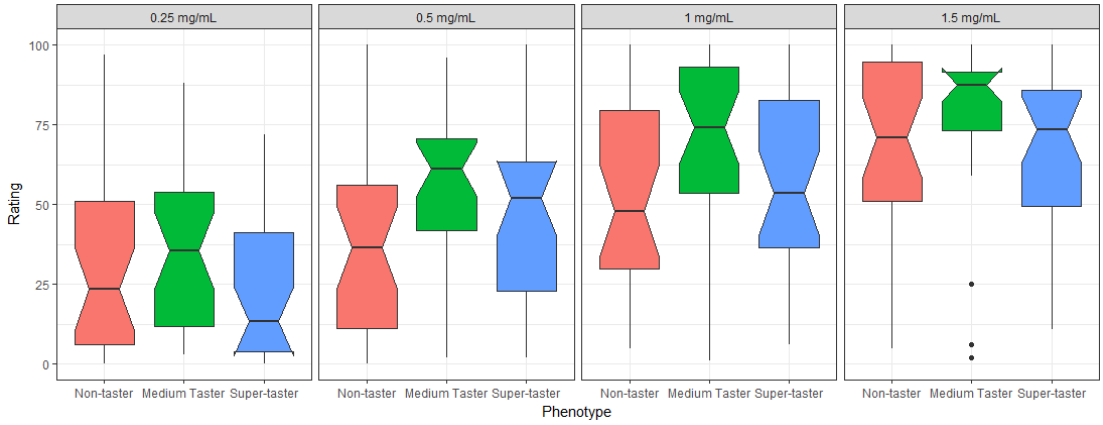


Figure 2-26 Response of participants to increasing concentrations of ranitidine hydrochloride following stratification by PROP phenotype

Across all concentrations, no significant differences ($p > 0.05$) between phenotypes was observed, as demonstrated by the overlap of notches seen in Figure 2-26. Thus PROP phenotyping does not adequately identify individuals sensitive to ranitidine hydrochloride.

2.5.2.7.2.2 Response according to 0.25 mg/mL ranitidine hydrochloride

Stratification of the participants by sensitivity to the lowest concentration (0.25 mg/mL) of ranitidine hydrochloride produced distinct populations with differing sensitivities: see Figure 2-27.

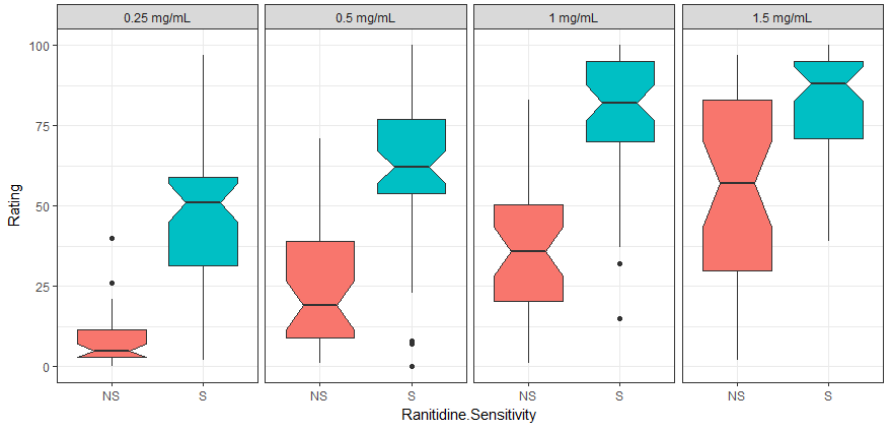


Figure 2-27 Responses of participants to increasing concentrations of ranitidine hydrochloride following stratification by sensitivity to 0.25 mg/mL ranitidine hydrochloride

Significant differences between non-sensitive and sensitive participants were observed across all concentrations of ranitidine hydrochloride assessed ($p < 0.05$), as indicated by

the lack of overlap between the notches. The magnitude to which the respective strata differed was approximately equal for the concentrations 0.25, 0.5 and 1 mg/mL, but reduced at the highest concentration of 1.5 mg/mL.

2.5.2.7.2.3 Response according to QHCl sensitivity

Stratifying the population according to sensitivity to QHCl, and subsequent assessment of the resultant sub-populations' sensitivities to ranitidine hydrochloride provided varied results: see Figure 2-28.

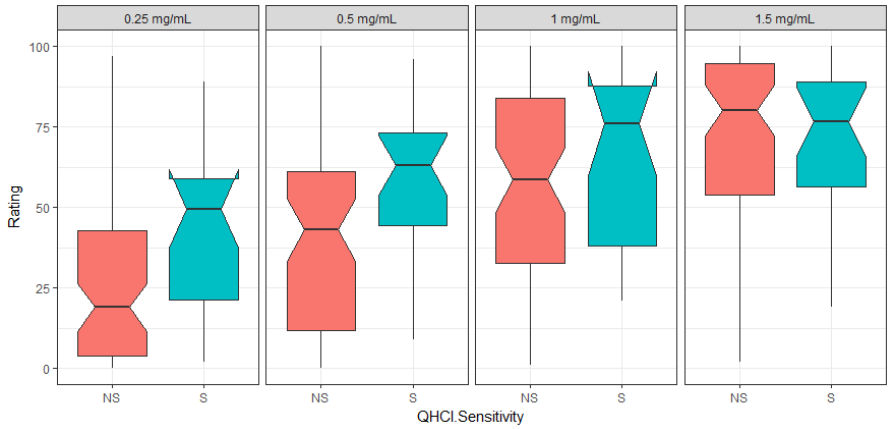


Figure 2-28 Responses of participants to increasing concentrations of ranitidine hydrochloride following stratification by QHCl sensitivity

At the lower concentrations of ranitidine hydrochloride – 0.25 and 0.5 mg/mL – significant differences ($p < 0.05$) were observed between the non-sensitive and sensitive individuals as indicated by the distance between the respective notches. However, at the higher concentrations – 1 and 1.5 mg/mL – no significant difference ($p > 0.05$) was observed between the sensitive and non-sensitive participants as represented by the overlapping notches in Figure 2-28.

2.5.2.7.2.4 Response according to QHCl precision

A varied response from each subpopulation was observed at each concentration of ranitidine hydrochloride: see Figure 2-29.

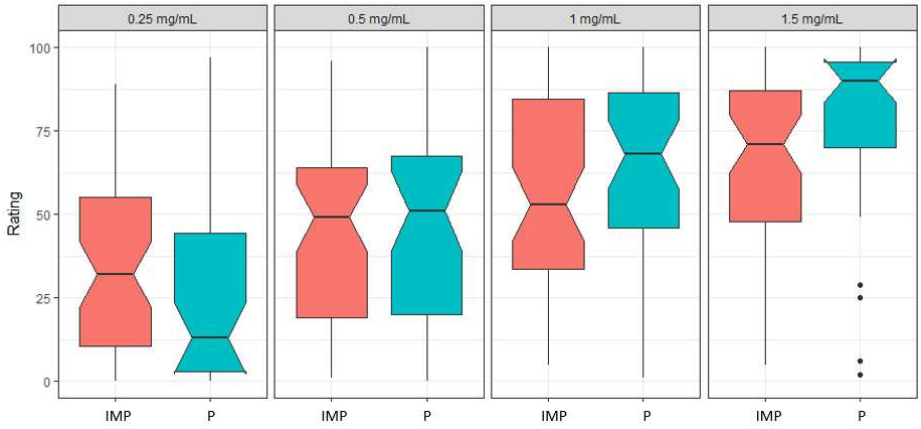


Figure 2-29 Responses of participants to increasing concentrations of ranitidine hydrochloride following stratification by QHCl precision

At the lowest concentration assessed – 0.25 mg/mL – the participants deemed P showed a reduced sensitivity compared to those deemed IMP, although not significant ($p > 0.05$). However, as the concentration increased, the difference between the subpopulations diminished further up to 1 mg/mL. At 1.5 mg/mL, the P subpopulation demonstrated significantly greater ($p < 0.05$) sensitivity than the IMP subpopulation, as demonstrated by the distance between the respective notches (Figure 2-29).

2.5.2.7.3 Telbivudine

2.5.2.7.3.1 Response according to PROP phenotype

The sensitivity observed by each phenotype varied depending on the concentration under investigation: see Figure 2-30.

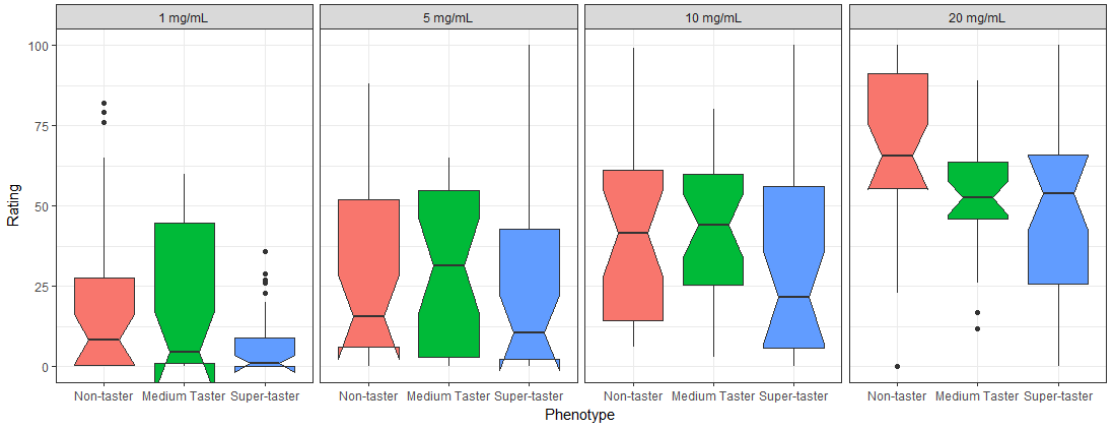


Figure 2-30 Responses of participants to increasing concentrations of telbivudine following stratification by PROP phenotype

At 1 and 5 mg/mL, no significant differences ($p > 0.05$) were observed between the subpopulations, as indicated by the overlapping notches (Figure 2-30). As the concentration increases, the super tasters demonstrate greater tolerance of the API relative to the non- and medium tasters, although this effect is not significantly different ($p > 0.05$). This effect is most marked at 20 mg/mL: see Figure 2-30.

2.5.2.7.3.2 Response according to 1 mg/mL telbivudine

Stratification of the population by assessing response to the lowest concentration of telbivudine (1 mg/mL) has resulted in distinct populations with significantly different responses to increasing concentrations of telbivudine: see Figure 2-31.

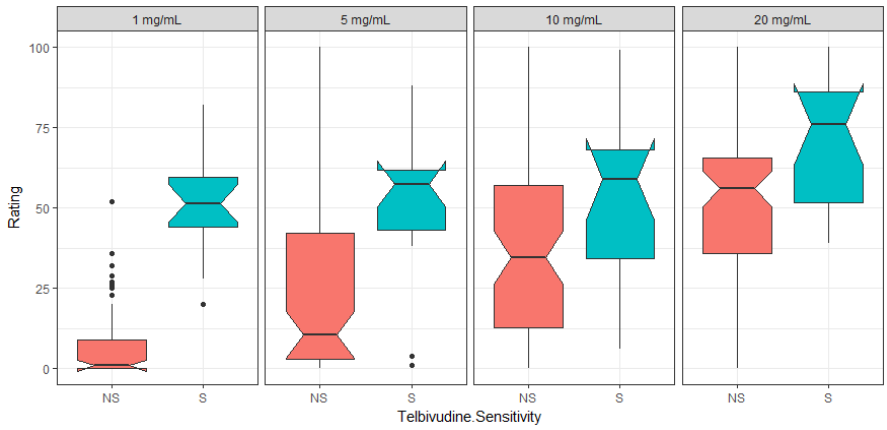


Figure 2-31 Responses of participants to increasing concentrations of telbivudine following stratification by sensitivity to telbivudine at 1 mg/mL.

Figure 2-31 demonstrates that at all concentrations of telbivudine, there was no overlap of notches observed between strata, indicative of significant differences, which was confirmed using the Wilcoxon signed rank test ($p < 0.05$). Importantly, no significant differences were observed among the sensitive subgroup across all concentrations ($p > 0.05$), while those deemed non-sensitive demonstrated significantly different ratings as the concentration was increased ($p < 0.05$).

2.5.2.7.3.3 Response according to QHCl sensitivity

Varied differences between the sensitive and non-sensitive subgroups were observed dependent on the concentration of telbivudine under investigation: see Figure 2-32.

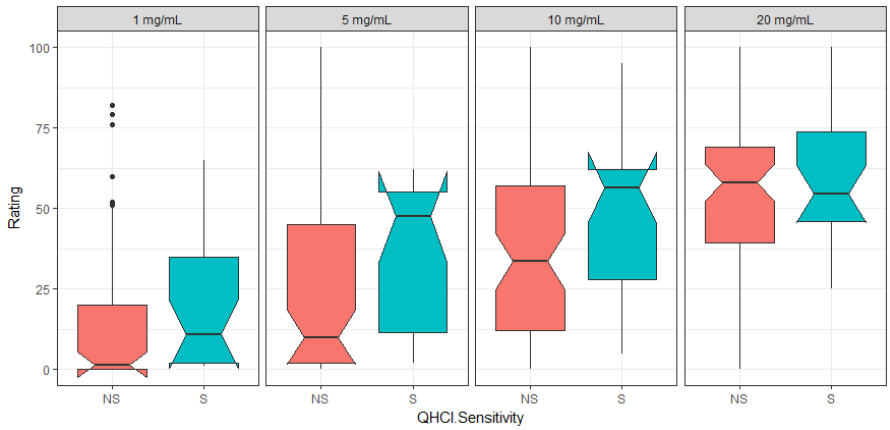


Figure 2-32 Responses of participants to increasing concentrations of telbivudine following stratification by QHCl sensitivity

At both 1 and 20 mg/mL no significant difference was observed between sensitive and non-sensitive participants ($p > 0.05$), as indicated by the overlap of notches as seen in

Figure 2-32. By contrast, at concentrations 5 and 10 mg/mL telbivudine, sensitive participants rated the samples significantly ($p < 0.05$) more aversive than the non-sensitive participants.

2.5.2.7.3.4 Response according to QHCl precision

Stratifying by QHCl precision resulted in highly varied responses between the resulting subgroups to increasing concentrations of telbivudine: see Figure 2-33.

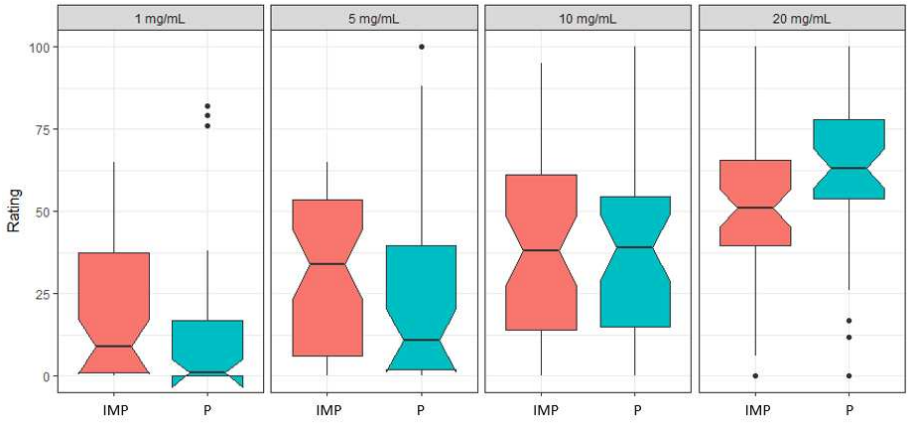


Figure 2-33 Responses of participants to increasing concentrations of telbivudine following stratification by QHCl precision

At 1 and 10 mg/mL, Figure 2-33 demonstrates no significant difference ($p > 0.05$) between IMP and P individuals as indicated by the overlapping of notches. At 5 mg/mL, the P assigned participants are less sensitive to the solutions administered relative to the IMP participants. This difference was significant ($p < 0.05$) as indicated by the distance between the respective notches in Figure 2-33. Conversely, at the highest telbivudine concentration, those participants designated P, perceived the samples to be significantly more aversive when compared to IMP participants ($p < 0.05$): see Figure 2-33 for the distance between the notches at concentration 20 mg/mL.

2.5.2.7.4 Drug X

2.5.2.7.4.1 Response according to drug X sensitivity

Stratification by sensitivity to drug X resulted in subgroups with differing responses at all concentrations: see Figure 2-34.

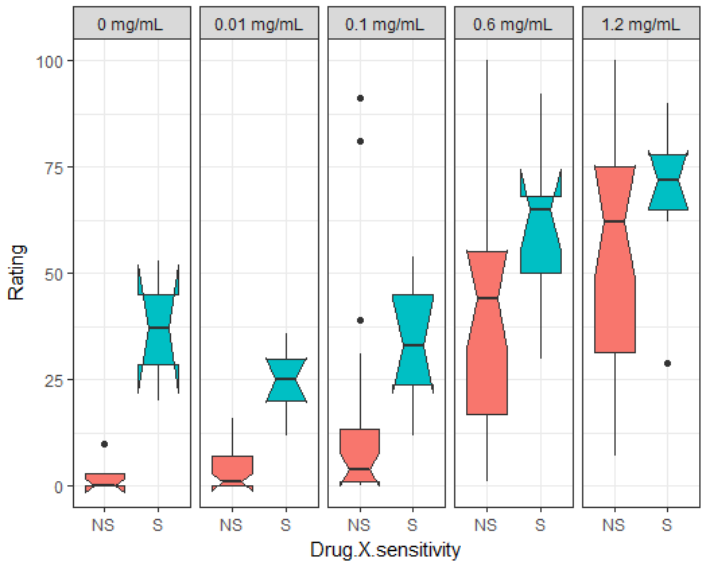


Figure 2-34 Responses of participants to increasing concentrations of drug X following stratification by drug X sensitivity

Interestingly Figure 2-34 shows that sensitive participants are capable of distinguishing between water (0 mg/mL) and the lowest concentration of drug X, and actually find water more aversive. Non-sensitive participants, however, do not rate 0, 0.01 or 0.1 mg/mL of drug X to a significantly different extent ($p > 0.05$), as indicated by the overlap of notches at said concentrations. Comparing non-sensitive and sensitive participants at all concentrations, it can be seen from Figure 2-34 that sensitive participants rate each concentration significantly more ($p < 0.05$) aversive than non-sensitive participants, with the exception of the highest concentration (1.2 mg/mL), where no significant difference was identified ($p > 0.05$).

2.5.2.7.4.2 Response according to QHCl sensitivity

Across all concentrations, there is no significant difference ($p > 0.05$) between QHCl sensitive and non-sensitive participants, thus both sub-populations rate the aversiveness of drug x to the same extent: see Figure 2-35. Further, both sensitive and non-sensitive strata do not significantly differentiate ($p > 0.05$) both the lower concentrations (0.01 and 0.1 mg/mL) and the higher concentrations (0.6 and 1.2 mg/mL).

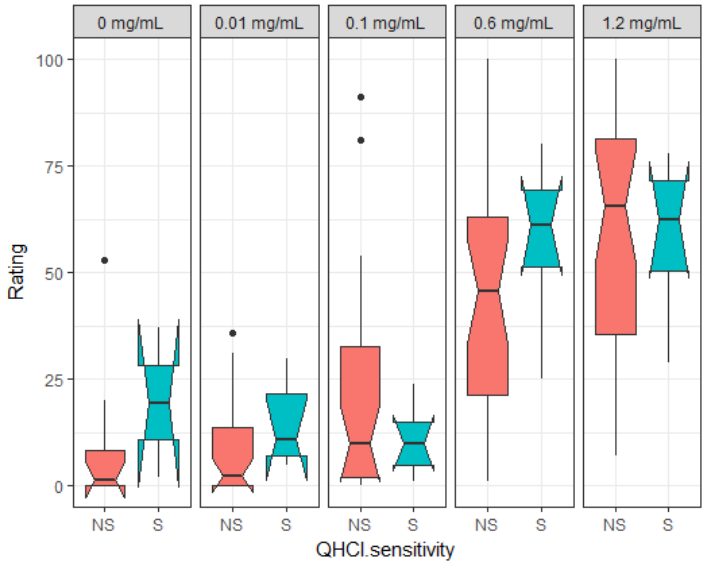


Figure 2-35 Responses of participants to increasing concentrations of drug X following stratification by QHCl sensitivity

2.5.2.7.4.3 Response according to QHCl precision

Stratification of the population by QHCl precision resulted sub-populations that do not rate drug x significantly different ($p > 0.05$) from each other. Indeed, Figure 2-36 shows that across all concentrations, the notches of the P and IMP participants consistently overlapped.

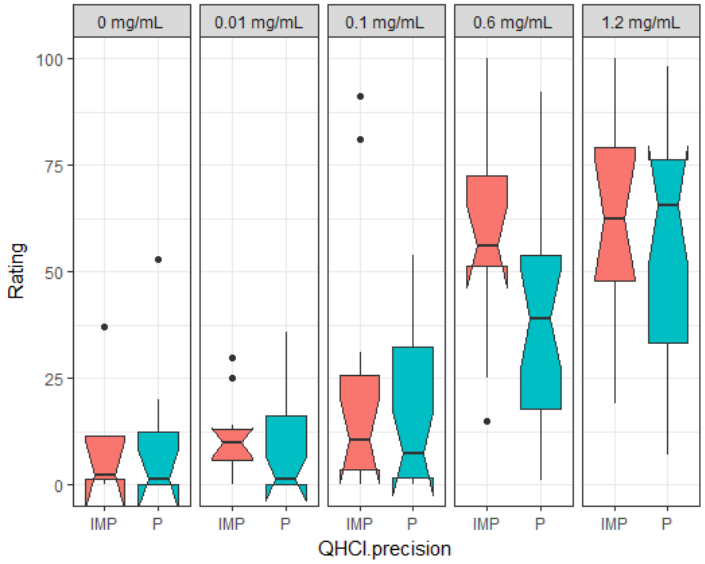


Figure 2-36 Responses of participants to increasing concentrations of drug X following stratification by QHCl precision

However, in terms of the ability of IMP and P subpopulations to differentiate between increasing concentrations of drug x, P individuals were capable of significantly

differentiating ($p < 0.05$) the uppermost concentrations (0.1, 0.6 and 1.2 mg/mL), while the IMP individuals are only capable of differentiating between the lowest concentrations (0, 0.01 and 0.1 mg/mL) and the highest concentrations (0.6 and 1.2 mg/mL) as can be gleaned from Figure 2-36.

2.6 Discussion

Two key parameters to human taste panels were successfully explored; namely the effect of participant selection was addressed with four distinct methodologies compared and the necessary sample sizes for comparing varying levels of bitterness identified.

2.6.1 Sample size

Bootstrap evaluation of an original dataset consisting responses to QHCl at low (0.0097 mg/mL), medium (0.097 mg/mL) and high (0.32 mg/mL) bitterness was successfully carried out, with evaluation of the number of participants necessary to distinguish between varying levels of bitterness: see Table 2-4.

Table 2-4 Number of participants required to distinguish between differing levels of bitterness, based on equalling the statistical power of a 54 participant sample size.

Bitterness distinction	Participants required
Low – medium	16
Medium – high	49
Low – high	8

As highlighted in section 2.1, there is a marked disagreement in the literature as to the number of participants necessary for a human taste panel. In a 5-year search of the literature (search conducted on 13th May 2019), it was found that for studies assessing the taste of bitter pharmaceutical compounds, there is a wide range of sample sizes employed, with a minimum sample size of 6 and a maximum of 84 with mean and median sample sizes of 27.2 and 14.5, respectively. In all of the identified papers, the sample sizes were not adequately justified. Moreover, even in the ISO guidance on sensory analysis methodologies (ISO 13299 [153]), there is real ambiguity and uncertainty as to the number of participants necessary, stating 'specific instructions regarding panel size are not appropriate because of the many factors that have to be considered'. This is a clear demonstration that the number of participants required for a human taste panel is simply

not known, and therefore needs to be addressed urgently. Thus, the present study provides, for the first time, justification for sample sizes necessary for assessing the taste of pharmaceutical compounds.

It was assumed that 54 participants was representative of the tasting population, thus the statistical power of increasing sample sizes was compared to this sample size. The lowest number of participants (8) are necessary to distinguish between low and high bitterness levels, followed by 16 to distinguish between low and medium bitterness levels and 49 to distinguish between medium and high levels of bitterness, based on achieving the identical statistical power as 54 participants. These conclusions can inform future taste panels, with knowledge of the taste threshold of a given compound, one can conclude the number of participants necessary to distinguish between increasing concentrations. It must, however, be noted that it is likely that 54 participants – the full dataset – was insufficiently large to meaningfully distinguish between medium and high levels of bitterness, thus a larger initial dataset is necessary to meaningfully establish the number of participants required to distinguish between these levels of bitterness. Importantly however, distinguishing between high and low concentrations of an API is what is required to determine an EC_{50} value, and thus characterise its aversiveness; therefore it can be concluded that a minimum of 8 participants are necessary to meaningfully characterise the aversiveness of an API in a human taste panel.

2.6.2 Participant Selection Methodologies

2.6.2.1 Participant Stratification

The participants were successfully stratified according to PROP phenotype, API sensitivity, QHCl sensitivity and QHCl precision.

The approximate distribution of non-tasters, medium tasters and super tasters within the population is reported to be 25%, 50% and 25% [141]. This study, however found that 44.44%, 33.33% and 22.22% were non-tasters, medium tasters and super tasters respectively. Indeed, the proportions of tasters is highly variable depending on ethnicity; for example a study assessing PROP phenotype and its impact on food preference of

populations along the silk road found distributions of PROP phenotypes as per Table 2-5 [154].

Table 2-5 Distribution of PROP phenotype by sex and population along the silk road (Robino et al., 2014). NT, MT and ST are non-taster, medium taster and super taster, respectively.

	PROP Phenotype		
	NT	MT	ST
All	37%	40%	23%
Male	44.2%	41.7%	14.1%
Female	32.1%	39.3%	28.6%
Georgia	50.9%	38.8%	10.3%
Azerbaijan	38.3%	46.8%	14.9%
Uzbekistan	40.7%	40.7%	18.6%
Kazakhstan	31.6%	50.9%	17.5%
Tajikistan	36.2%	32.5%	31.3%
Armenia	22.0%	39.0%	39.0%

The present study included 11 ethnicities (7 white British, 11 Asian/Asian British other, 1 white Irish, 4 mixed other, 10 white other, 4 Arab, 15 Chinese, 2 African/Caribbean other, 2 Pakistani and 3 Indian), and small sample sizes from each, thus great variability among ethnicities combined with insufficient sample sizes from each will serve to provide a misleading representation of the population as a whole.

Furthermore, it is important to note that genetic variations – the *TAS2R38* gene – account for only between 50 – 80% of the phenotype, with other genetic and non-genetic factors thought to play a role [154]. For example, the gustin (CA6) gene is implicated in taste bud growth and maintenance, with the major allele being associated with greater taste bud densities, a key characteristic of super tasters [155,156]. Therefore, given the multi-faceted complexity of this phenotype, it is difficult to truly ascertain the *true* distribution of PROP phenotype for comparison to results gained experimentally.

As per Figure 2-15, which assessed the sex effect, the same pattern of NT > MT > ST was observed in both males and females. However, assessing the absolute figures, females had a larger percentage of super tasters relative to males, and males a larger percentage of non-tasters relative to females, which is consistent with findings that females are generally more sensitive to bitterness than males [141]. This finding is also consistent with the findings of Robino *et al* [154].

Stratification of the population by QHCl sensitivity, QHCl precision and individual API sensitivity was successfully achieved. However, the key findings relate to how the participant response varies following participant stratification: which of the investigated methodologies best selects bitter-sensitive individuals?

2.6.2.2 Assessing Participant Response Following Stratification

PROP phenotyping was unable to identify more sensitive participants when assessing ibuprofen sodium, ranitidine hydrochloride and telbivudine. This was likely due to the lack of bitterness of the APIs under assessment; indeed telbivudine, ibuprofen sodium and ranitidine hydrochloride were deemed weakly aversive, weakly aversive and mildly aversive, respectively in rats: see Table 2-6.

Table 2-6 Taste assessment of APIs using rat BATA model, showing IC50 values and degree of aversiveness. Data produced as part of the SPAEDD-UK project (http://www.paediatricscienceuk.com).

API	IC_{50} (mM)	Aversiveness degree
Ibuprofen sodium	90	Weak
Ranitidine hydrochloride	4	Mild
Telbivudine	29.9	Weak

Indeed, there were anecdotal reports of a sour sensation of the ibuprofen sodium and PROP phenotyping, by its very nature, will only be capable of identifying participants sensitive to truly bitter tastants, thus if the taste profile is more complex involving other taste modalities, it may not be an effective method for participant stratification.

Utilising quinine hydrochloride as a model bitter drug for the purpose of identifying sensitive and/or precise individuals yielded varied success depending on the API assessed. While stratification by QHCl precision was unable to identify more sensitive participants to ibuprofen sodium, differences were identified with ranitidine hydrochloride and telbivudine. However, inconsistent results were acquired for ranitidine hydrochloride, with QHCl precise individuals perceiving reduced aversiveness relative to QHCl imprecise at 0.25 mg/ml, while at 1.5 mg/ml QHCl precise individuals perceived greater aversiveness relative to the QHCl imprecise individuals. For telbivudine, no significant differences were observed between QHCl imprecise and precise individuals at individual concentrations, however, between concentrations, QHCl precise individuals were capable of significantly distinguishing between increasing concentrations of telbivudine,

while QHCl imprecise individuals were not. Stratification by QHCl sensitivity yielded varied results with significant differences observed between the respective strata at only a single concentration of ibuprofen sodium (5 mg/ml), the lowest concentrations of ranitidine hydrochloride (0.25 and 0.5 mg/mL) and the middle concentrations of telbivudine (5 and 10 mg/mL), while all other differences observed were insignificant. However, stratification by sensitivity to the individual API under assessment provided significant differences between strata for all APIs and all concentrations. Thus, according to this dataset, stratification according to sensitivity to a low concentration of the API under scrutiny serves as the best means to select sensitive participants.

To establish if the same pattern was observed with another dataset, the established hypothesis – stratification by sensitivity to the individual API under scrutiny serves as the best means to identify sensitive individuals – was tested by performing the same analysis using an anonymised proprietary drug of unknown bitterness identified as drug X. PROP phenotyping was not performed for this dataset, but successful stratification by API sensitivity, QHCl precision and QHCl sensitivity was achieved. While QHCl precision and QHCl sensitivity was unable to stratify the population into strata with differing responses to drug X, API sensitivity was capable of achieving this, with sensitive individuals consistently perceiving all but the highest concentration (1.2 mg/mL) as more aversive than that perceived by non-sensitive individuals. Thus, the conclusion that stratification with a low concentration of the API under scrutiny, rather than a surrogate bitter drug, is the best approach to identify sensitive individuals is conserved. However, if this is not possible, stratification by QHCl precision provides the best alternative, given the differences between QHCl precise strata are greater than QHCl sensitive strata. By utilising the identified methodologies in selecting participants, the data yielded will be of greater quality, providing the best evaluation of the aversiveness of a given API. However, performing participant selection adds a further step to the experimental procedure and may require the screening of many participants to achieve the desired sample size, thus adding significant time and cost to the procedure. It is therefore important to balance the quality of the data necessary against the time and capital available to conduct the project.

2.7 Conclusion

A lack of consensus in the number of participants necessary for a human taste panel was observed in the literature, as well as whether or not to select participants and how that might be achieved. Indeed, of all published research articles assessing bitter APIs using human taste panels in five years previous to May 2019, the number of participants ranged from 6 to 84. Furthermore, of said publications, two-thirds did not select participants. Thus, this study sought to explore statistically the number of participants required for a human taste panel assessing bitter APIs. It also sought to assess proposed participant selection methodologies for efficacy in identifying the most sensitive participants.

To this end, a model was built using R to assess the minimum sample size required to distinguish significantly between varying levels of bitterness using quinine hydrochloride as a model bitter drug. It was found that different sample sizes were required when distinguishing between different levels of bitterness, with 8 participants necessary to significantly distinguish between low and high bitterness, 16 between low and medium bitterness and 49 participants necessary to significantly distinguish between medium and high levels of bitterness. Thus, it can be concluded that for a compound of unknown bitterness, e.g. a new chemical entity, a minimum of eight participants is necessary for the taste panel as the distinction between low and high levels of bitterness is necessary to characterise a full concentration range of a compound and thus yield a taste threshold.

Participant selection methodologies in human taste panels were explored by assessing four methodologies and comparing their ability to select sensitive participants for three known APIs, and the established hypothesis re-tested with a drug of unknown identity and bitterness: drug X. The tested methodologies were broad and consisted PROP phenotyping, sensitivity and precision in assessing the model bitter drug quinine hydrochloride, and sensitivity to the tested compound. It was found that the most sensitive participants were identified by assessing sensitivity to the tested drug, but where this is not possible, participants should be stratified by precision in rating of the model bitter drug: quinine hydrochloride.

Thus, the aforementioned studies have served to address two key gaps in taste research: sample size and the participant selection methodologies, providing the sensory

researcher with further confidence in methodological design in future human gustatory panels.

3 Assessing Gender Differences in the Rodent (Rat) Brief Access Taste Aversion (BATA) Model

3.1 Introduction

3.1.1 Establishment and optimisation of the BATA model

The work of Soto, J in her thesis entitled "Assessing the feasibility of using an animal model for *in vivo* taste assessment of pharmaceutical compounds and formulations" served to establish the BATA model as an *in vivo* pharmaceutical taste assessment methodology at the UCL school of pharmacy [157]. This chapter will summarise the methodological development performed by Soto, and discuss a key factor in taste perception that was not investigated during the early methodological development of the BATA model: gender.

3.1.1.1 BATA model recap

The BATA model, as highlighted in chapter 1, is a taste assessment methodology, which uses rats that have been water deprived for 22 hours prior to commencement of the experiment. The rat is then placed into a lickometer (Davis MS-160, DiLog instruments, Tallahassee, Florida, USA) where it will be presented with samples in sipper tubes from which it can lick and the number of licks are recorded: see Figure 3-1.

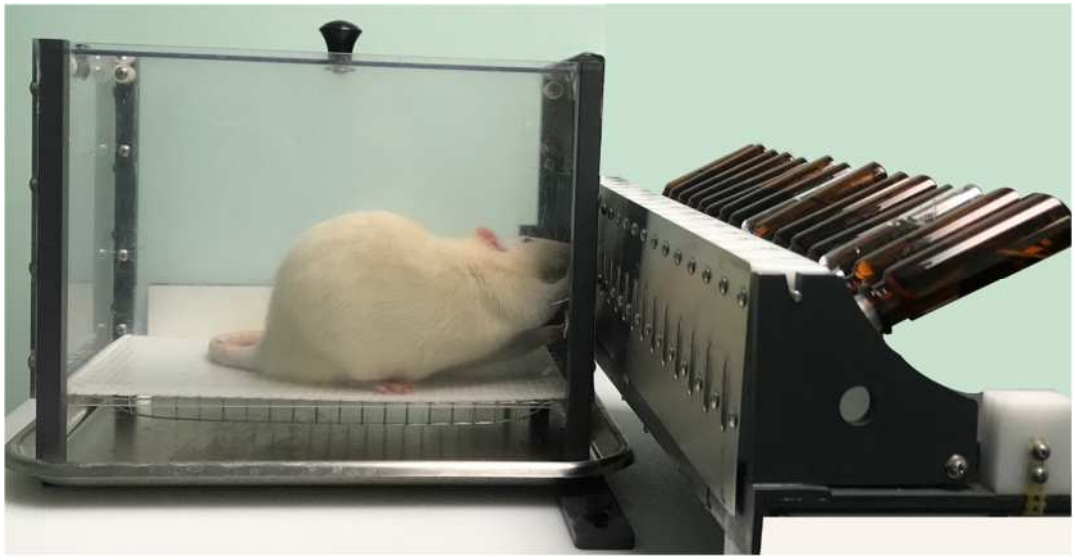


Figure 3-1 The lickometer, showing a rat licking from a sipper tube presented to it from rack of 16 possible sipper tubes

The sample tubes (up to 16) are placed on the rig, which moves laterally in order for the desired sample tube to be presented. The rat is only able to lick from the sipper tube for a designated amount of time, which is controlled by a shutter. The amount of time during which the rat is able to lick (8 s) only begins following the rat's first lick, before which a waiting time will be observed (20 s). If the rat fails to lick from a sample, it will be presented again, known as a retry, with the number of retries controlled (8 retries). Between each sample presentation, there is a designated amount of time, known as the inter-trial interval (5 s). The sample presentations are bracketed by water rinse presentations (usually between 2 – 4 s). Each sample tube will be presented twice during the experiment, with the exception of the water rinse tube, which is repeatedly presented as mentioned. Thus a total of 60 presentations occur during a single BATA experiment (15 samples presented in duplicate, in addition to 30 water rinse presentations).

The methodological development of the aforementioned BATA procedure will now be discussed before presenting a key variable in the BATA model that must be addressed.

3.1.1.2 Washout-period length

Initial studies by Soto found that there was a rightward shift in the concentration-response curve for QHCl, i.e. a lower IC_{50} and attenuation of taste aversion, when the rats received only a 48-hour washout period relative to naïve rats [157]. It was proposed that

this could be due to reduced neophobia as the rats became used to the bitter taste of the tastant. Thus, an increased washout period was investigated to ascertain if the rats could be returned to their taste-naïve state; 4-, 2- and 1-week washout periods were investigated and the results for QHCl compared to naïve rats. It was found that, for the majority of concentrations of QHCl, there was no significant difference ($p < 0.05$) between washout periods, demonstrating that a 1-week washout period is sufficient to return the animals to their naïve state.

3.1.1.3 Inter-trial interval

Initially, the inter-trial interval used in the BATA model was 10 s, but it was found that the rats were displaying impatience and attempting to open the shutter with their teeth and paws. Thus, the interval was reduced to 7 s and then to 5 s, and found that the compliance and willingness of the rats improved while also reducing the experiment time, thus a 5 s inter-trial interval was observed from then on [157].

3.1.1.4 Number of concentrations assessed per session

Initially, twelve concentrations were assessed during the early stages of BATA methodological development, as it was thought that such a high number of concentrations was necessary to achieve the full concentration-response curve. However, it was later found that reducing the number of concentrations to six, each a factor of 3 higher or lower than the adjacent concentration, enabled each sample to be presented four times and reduced the experiment time to 40 minutes, without preventing the acquisition of a full concentration-response curve. As such, the assessment of 6 concentrations became convention, although up to 14 different concentrations or samples can be assessed if necessary.

3.1.1.5 Number of presentations of the same solution per session

As mentioned, during the initial experiments, 12 concentrations were presented to the rats in triplicate, resulting in a total of 90 presentations when water and water rinse presentations are included. However, when the number of concentrations was reduced to 6 presented in duplicate, with each tube presented in duplicate, a total of four presentations were achieved for each concentration but with a reduced total number of

presentations of 60. The impact of this reduction in total number of presentations on maintaining the interest of the animal throughout the experiment was significant, with an average of 98.33 % of trials initiated on day 1 compared to 77.22 % of trials initiated on day 1 for 60 and 90 presentations, respectively. Thus, it became convention to limit presentations to 60 per session, with each session time not exceeding 40 minutes.

3.1.1.6 Number of rats

Soto investigated the effect of the number of rats on the outcome variable: IC_{50} using design of experiment (DOE) principles by utilising a GLM model in R. These analyses found the sample size not to have a significant impact on this outcome variable, but further analyses using the Wilcoxon signed-ranked test found that there was a significant difference between the IC_{50}s obtained for the two sample sizes assessed: 8 and 10, although the absolute difference was very small. Plotting the data, Soto found that the variability was visually smaller using 10 rats, thus it was decided that a cohort of 10 rats would be used in future experiments [157].

3.1.1.7 Number of testing days

Initial experiments by Soto found that one testing day was insufficient to mitigate the variability of the results and achieve statistically significant results, thus two or three testing days were proposed. Both the GLM and Wilcoxon signed-ranked test concluded no significant difference between the IC_{50}s yielded from two and three testing days, thus to reduce the experimental time and thus benefit the animals' welfare, two testing days became the convention [157]. However, if large discrepancies between days is observed, a three-day protocol is recommended.

3.1.1.8 Water-rinse length

The water rinse time is a key parameter in the BATA as it must be long enough to enable sufficient rinsing of the animals' mouths to mitigate carry-over effects, and short enough to minimise the experiment time for the welfare of the animal. Water rinse lengths of 2 and 4 s were investigated by Soto, and found to yield no significant differences in IC_{50} in both the Wilcoxon signed-ranked and GLM analyses. It was thus decided that the shorter

2 s water-rinse time would be used to not prolong the experiment unnecessarily for the welfare of the animal [157].

3.1.1.9 Number of retries at the same tube

If no licks are detected at the tube, the sample is re-presented in order to establish a lick number for the given sample, thus accounting for times when the animal loses interest in the experiment. Soto investigated the two options for this factor: 6 and 8 retries at the same tube, and found from both the Wilcoxon signed-ranked and GLM analyses that no significant difference between IC_{50}s was observed. However, reduced variability was observed from visual assessment of boxplots with 8 retries, thus, 8 retries at the same tube became convention.

3.1.1.10 Waiting time before first lick is detected

The sample presentation time of 8 s does not commence until the rat has taken its first lick, thus the time that each tube is presented to the rat before it takes its first lick must be controlled, or the experiment would continue *ad infinitum*. Soto investigated waiting times of 20 and 40 s. Wilcoxon signed-ranked and GLM analyses that no significant difference between IC_{50}s was observed for either waiting time, however visual assessment of the boxplots reveals lower variability when a waiting time of 20 s is observed, thus a 20 s waiting time was chosen for all future BATA experiments [157].

3.1.1.11 Age effect

Age-related changes in taste are known to occur, for example children are known to live in different sensory worlds to adults, with differing responses to certain tastes [158,159]. Indeed, children are more sensitive to bitter tastes – the modality commonly associated with APIs – therefore Soto explored whether or not this enhanced bitter sensitivity in children can be better replicated by juvenile rats [157,160]. Three APIs of varying bitterness were assessed: quinine hydrochloride (high bitterness), amlodipine besylate (medium bitterness) and caffeine citrate (low bitterness) with rats of varying ages; from post-weaning to aged. Soto found some age-related differences in taste perception, but the differences were compound-specific. Thus, no age-effect on sensitivity was observed for quinine hydrochloride from juvenile to adult rats. However, an age-dependent reduction

in sensitivity was observed for both amlodipine besylate and caffeine citrate, with a decrease in bitterness sensitivity to a plateau with age and post-weaning rats being more sensitive than other age groups, respectively. Soto concluded from these findings that rats neither post-weaning nor geriatric should be used for BATA experiments [157].

3.1.1.12 Summary of BATA parameters

To summarise, the optimised parameters for the BATA model are shown in Table 3-1.

Table 3-1 Summary of BATA parameters

Factor	Setting
Training session (s)	- 2 (training sessions 1 and 2) if the rats are used for the first time. - 1 (training session 2) if the rats are "re-used".
Number of rats	10
Number of testing sessions	2
Water-rinse length	2 s
Number of retries at the same tube	8
Waiting time before the first lick is detected	20 s
Washout period	> 1 week
Number of concentrations assessed per session	6 unless more are required
Inter-trial interval	5 s
Number of presentations of the same tube per session	4 unless more than 7 samples are tested
Total number of presentations	60 unless more samples are assessed
Session length	40 minutes unless more samples are assessed

3.1.2 The forgotten variable: sex

In all preceding experiments, Soto consistently used male rats. This decision was taken to mitigate the possible effect of hormonal changes on the taste sensitivity of the rats. However, is sex an important variable in taste?

There is currently no consensus on the effect of gender on taste in the literature. In humans, several studies have revealed no significant difference in taste perception between males and females, while others have found stark differences. For example, in

a study assessing the impact of threshold, age, gender, medication and health on taste acuity in the elderly, in which participants were given solutions corresponding to each taste modality and identify them as appropriate, no significant difference was identified for gender [161]. Another study, in which the emotional responses to salty, sour, sweet and bitter solutions was assessed in 17 males and 17 non-pregnant females, again no significant difference was identified between males and females [162]. Conversely, in a study assessing gender differences in PROP/PTC phenotype and how it relates to alcoholism in a sample size of 244 participants, significantly more female supertasters were identified relative to males: 39 % and 21 %, respectively [163]. The importance of PROP/PTC phenotype to the sensory world of a human has been demonstrated extensively in the literature (as highlighted in 2.1), thus it stands to reason that such a stark difference in phenotype would impact on bitterness sensitivity. Furthermore, a study of 477 men and 519 women in Japan, found that gender differences existed in taste recognition thresholds, but that such differences were age-specific, with women showing significantly lower thresholds than males for sour, salty and bitter tastes for the those participants between the ages of 69-71 and 79-80 [164]. Indeed, the differences in taste perception among different genders may also be reflected in their respective diets; in a study of 2295 participants assessing dietary taste patterns by gender as well as weight status, it was found that men consume relatively more energy from 'salt, umami and fat' and 'bitter' tasting foods than women, who consume more energy from 'sweet and fat' and 'sweet and sour' tasting foods [165]. Gender differences in taste in children have also been explored. Joseph *et al.* assessed sucrose thresholds in 235 children and looked at the effect of bitter-sensitive alleles on said threshold, while also assessing gender and age differences, finding that sensitivity was greatest in older females with two bitter-sensitive alleles [166]. Thus, the literature points to females being more sensitive than males.

In rats, some stark differences in taste perception have also been observed among males and females. Indeed, oestrogen seems to play a key role in attenuating sensitivity to tastants of varying taste modalities, certainly in Sprague-Dawley rats. One study demonstrated sex differences in electrophysiological and behavioural responses to NaCl taste by assessing the chorda tympani (CT) responses to solutions of varying NaCl concentration in males and ovariectomized females. The ovariectomized females were

given either oestrogen or placebo. Males were found to exhibit greater CT responses to NaCl than females and, among the females, those given placebo rather than oestrogen were more sensitive [167]. In a second study, which also used male and ovariectomized Sprague-Dawley rats, but focused on behavioural responses using the BATA model, found greater sensitivity to NaCl among males relative to females with or without estrogen [168]. However, NaCl elicits a salty taste rather than a bitter taste, thus enhanced sensitivity to saltiness may not necessarily confer enhanced sensitivity to APIs, which tend to be bitter. However, from all the aforementioned studies in rats and humans, it is clear that there is little consensus on the gender effect on taste, but the majority of studies find some effect. Given that rats are used in the BATA model as an analytical tool, it is obvious that the most sensitive tool is desired. Therefore, if gender affects taste sensitivity, it is critical that the most sensitive gender is used. This must therefore be investigated.

3.1.3 Female rats in research

A further reason for exploring the use of females in the BATA model, is their absence from the majority of animal research. In a 1-year period from 16th May 2018 (search conducted on PubMed identifying studies using male or female rats either exclusively or in combination published during the 1 year period from 16th May 2018 to 16th May 2019 inclusive), 8838 studies included only male rats, while only 2872 included female rats, often in combination with males.

Given that both male and female rats are bred for research, the lack of use of female rats contravenes the 3Rs given that female rats may be culled needlessly as they are not bought for research. Thus, if only males are used, and females culled, the number of animals required for experimentation is effectively doubled. Thus, it is in line with both the principles of reduction and refinement of the 3Rs to explore the use of females in the BATA model.

3.2 Aims

- To explore the gender effect in the rat BATA model.

3.3 Objectives

- Explore the differences in PROP phenotype between male and female rats.
- Select APIs of varying levels of bitterness – low, medium and high – and assess male and female differences.
- Link PROP phenotype to rat response to selected APIs.
- Ascertain the most sensitive gender to use in future rat BATA experiments.

3.4 Materials and Methods

3.4.1 Materials

Propylthiouracil, sodium chloride, quinine hydrochloride, ranitidine hydrochloride and caffeine citrate were purchased from Sigma-Aldrich (St Louis, Missouri, USA).

3.4.2 Methods

3.4.2.1 Gender differences in PROP Taste Phenotype

In order to first explore the sensory worlds of male and female rats, ten male and ten female rats were assessed for their respective PROP phenotype, which is a key phenotype affecting bitter taste perception as highlighted in 2.1.

In much the same way as previously explored in humans, to establish different responses to PROP, a standard that is perceived as equally intense to all rats regardless of phenotype must be chosen against which ratings of PROP can be compared. Failure to do this would lead to all subjects being deemed medium tasters. As such, NaCl was chosen as this compound is not tasted in a systematically different way among different PROP phenotypes.

Therefore, each rat was given three samples of PROP and NaCl, respectively, at concentrations specified in Table 3-2.

Table 3-2 Concentrations of PROP and NaCl used in the determination of PROP phenotype

Compound	Concentrations assessed (% w/v)	Sample number
PROP	0.0005	1
	0.005	2
	0.05	3
NaCl	0.0584	1
	0.584	2
	5.84	3

Ten male and ten female Sprague-Dawley rats (Charles River, Kent, UK), aged 24 weeks underwent PROP phenotype assessment.

During the BATA procedure, ten male and ten female Sprague-Dawley 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 samples were presented in triplicate and at random. During the testing days, the samples were presented to the rat for 8 s after the initial lick, followed by a 2 s water rinse presentation. Between each presentation, a 5 s inter-presentation interval was observed [122]. All the procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668) by Alexander Keeley (Personal Licence: I1826CBD0).

In a subtly different way to human PROP phenotype assessment, assignment of phenotype was achieved by assessing response to samples 2 and 3 of PROP and NaCl relatively. If the rat's lick numbers of samples 2 and 3 of NaCl were not significantly different to those of samples 2 and 3 of PROP respectively, the rat was assigned the medium taster phenotype (Figure 3-2). If, however, the rat's lick numbers for samples 2 and 3 of PROP were significantly higher than samples 2 and 3 of NaCl respectively, the rat was assigned the non-taster phenotype (Figure 3-2). Lastly, if the rat's lick numbers for samples 2 and 3 of PROP were significantly lower than samples 2 and 3 of NaCl respectively, the rat was assigned the non-taster phenotype (Figure 3-2).

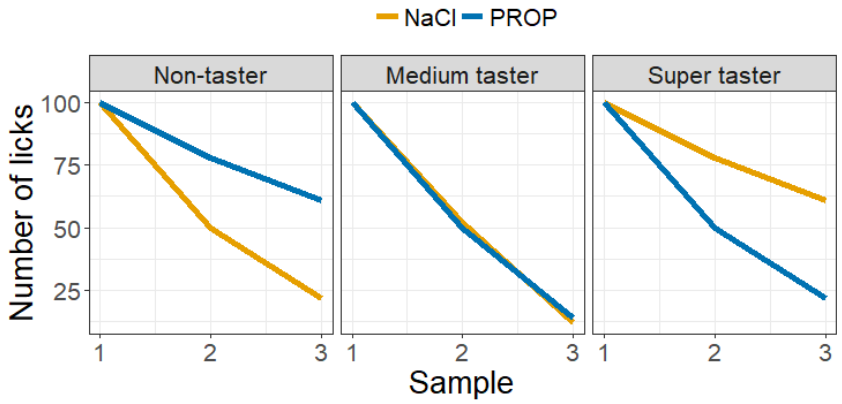


Figure 3-2 A graphical representation of a hypothetical rat medium taster, non-taster and supertaster

3.4.2.2 Gender differences in taste sensitivity to a range of APIs

Following phenotyping, sensitivity of male and female rats to APIs of varying bitterness levels was assessed.

3.4.2.2.1 BATA procedure

The rodent brief-access taste aversion model was utilised to assess the rat response to each solution; as described above.

3.4.2.2.2 Data analysis

Data were visualised as notched box-plots as described in Figure 2-11. The distribution of the data was assessed using the Shapiro-Wilk test: 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 [169]. 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) [152].

3.4.2.2.3 Taste solutions

To provide a true picture of the gender differences in taste sensitivity in rats, compounds with a broad range of IC_{50}s were chosen; from extremely aversive to mildly aversive; namely quinine hydrochloride, ranitidine hydrochloride and caffeine citrate: (Table 3-3).

Table 3-3 Selected APIs and their corresponding concentrations for BATA assessment

Compound	IC50 (mM) and corresponding 95 % CI	Concentrations assessed (mM)
High bitterness Quinine hydrochloride	0.08 (0.01-0.16)	0.01 0.03 0.1 0.3 1 3
Medium Bitterness Ranitidine hydrochloride	4.02 (2.82-5.22)	0.17 0.36 0.71 1.43 2.85 4.28
Low bitterness Caffeine citrate	7.76 (5.62-9.90)	0.3 1 3 10 30 100

3.5 Results

3.5.1 Gender differences in PROP taste phenotype

PROP phenotyping of all 20 male and female rats was carried out with stark differences observed between males and females (see Figure 3-3, Figure 3-4 and Figure 3-5).

As described in 3.2.2.2, PROP phenotype was determined by assessing taste response to PROP relative to NaCl; if the rat perceives PROP to be more intense than NaCl, it is determined a super taster; while if the contrary is observed, the rat is deemed a non-taster; while if there is no significant difference observed between PROP and NaCl, the rat is deemed a medium taster.

Figure 3-3 demonstrates the female response to PROP relative to NaCl. Some stark differences in rat response were observed, with different phenotypes found among the ten rats. Rats 3, 7 and 9 show a significantly more intense response to PROP than NaCl for samples 2 and 3, thus indicative of the super taster phenotype. All other seven rats

show no significant difference between samples 2 and 3 of PROP and NaCl respectively, thus they were deemed medium tasters.

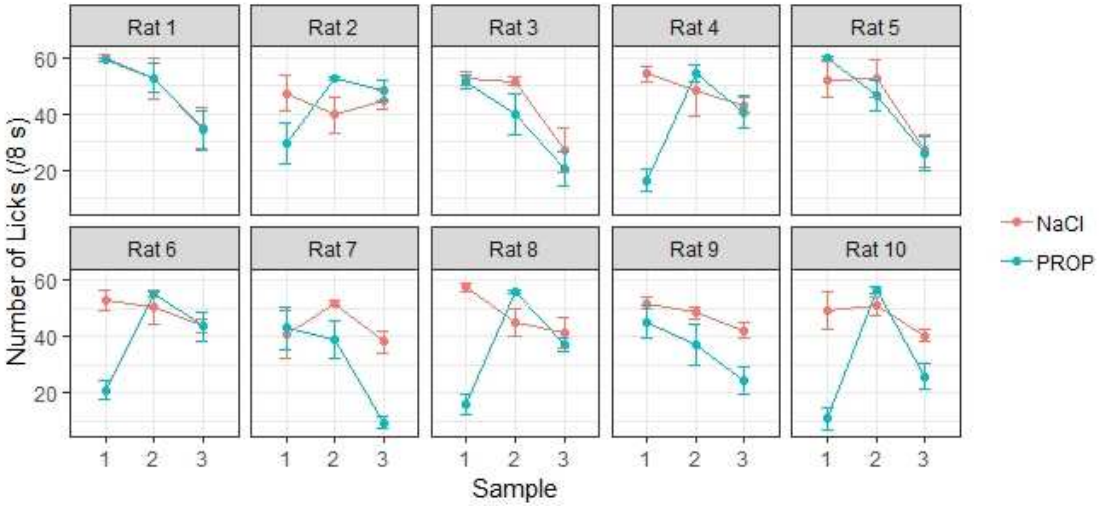


Figure 3-3 Female rat taste responses to NaCl and PROP showing mean lick number from day 1 and 2 +/- SEM

The male rats displayed a marked difference in PROP phenotype (Figure 3-4). Rats 1, 3 and 5 show no significant difference between lick numbers for samples 2 and 3 of PROP and NaCl, and were thus determined to possess the medium taster phenotype. The remaining seven rats, perceived the PROP to be significantly more aversive than NaCl for samples 2 and 3 as indicated by the significantly lower lick number for PROP relative to NaCl, and were thus deemed to be super tasters.

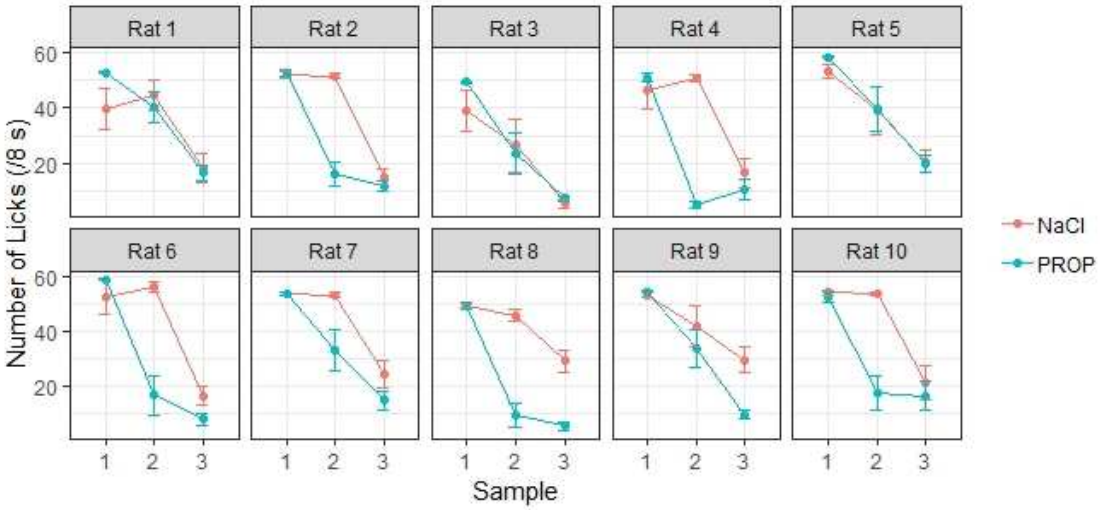


Figure 3-4 Male rat taste responses to NaCl and PROP showing mean lick number from day 1 and 2 +/- SEM

Therefore the taste worlds of the male and female rats were found to be significantly different, with the majority of males possessing the super taster phenotype, while the females demonstrated a predominance of the medium taster phenotype (Figure 3-5).

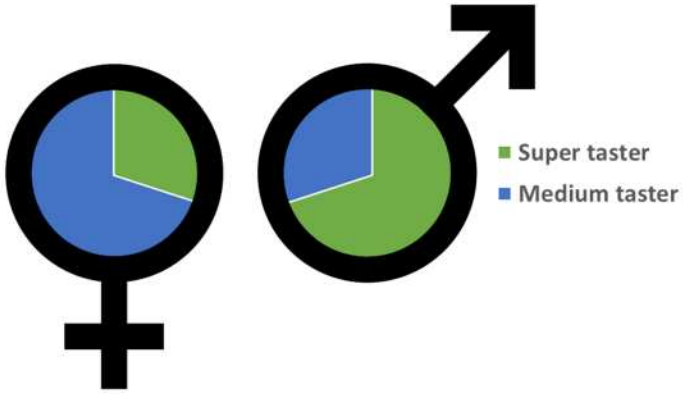


Figure 3-5 Proportion of rat PROP phenotypes by gender

3.5.2 Gender differences in taste sensitivity to a range of APIs

3.5.2.1 Low bitterness: Caffeine citrate

Taste assessment of aqueous solutions of caffeine citrate was successfully carried out in male and female rats.

Analysis of all rat data – both male and female – revealed that responses to all concentrations of caffeine citrate were significantly different to water (Figure 3-8). No

significant difference was observed between concentrations 0.3-3 mM, but significant differences were identified at 10 and 30 mM (Figure 3-6).

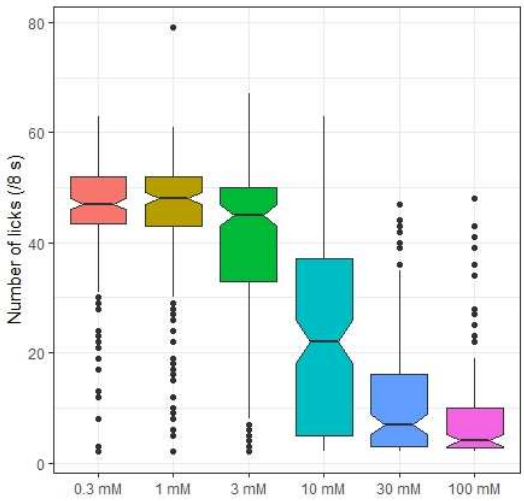


Figure 3-6 All rat taste response to increasing concentrations of caffeine citrate

Figure 3-7 and Figure 3-8 demonstrate the gender differences observed for caffeine citrate. Overall, significant differences between male and female rats were observed, however there was some variation depending on the concentration assessed. Indeed, male responses to all concentrations were found to be significantly lower than females ($p < 0.05$) for all concentrations with the exception of 10 and 100 mM. Thus, overall males demonstrated a greater sensitivity or aversion to caffeine citrate than did females.

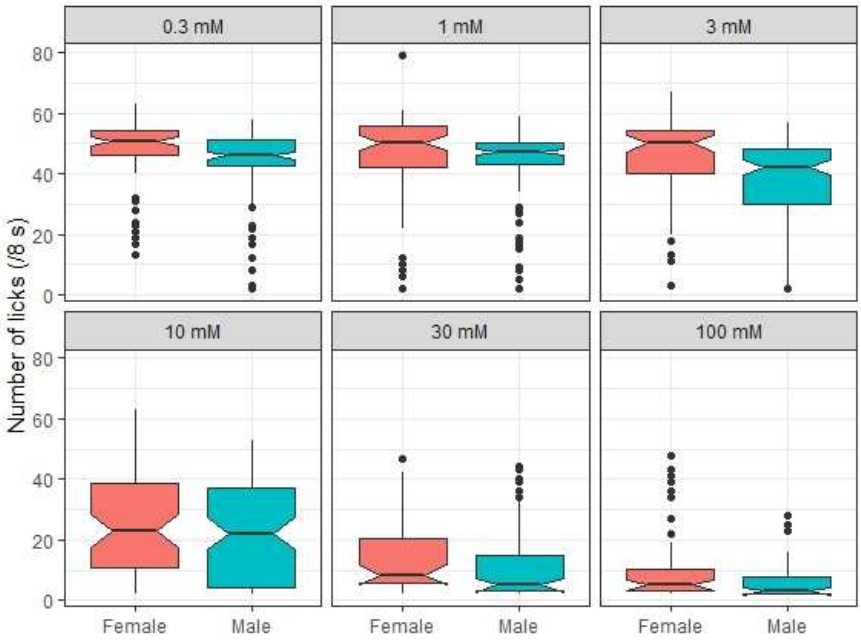


Figure 3-7 Female Vs. male taste response to each concentration of caffeine citrate

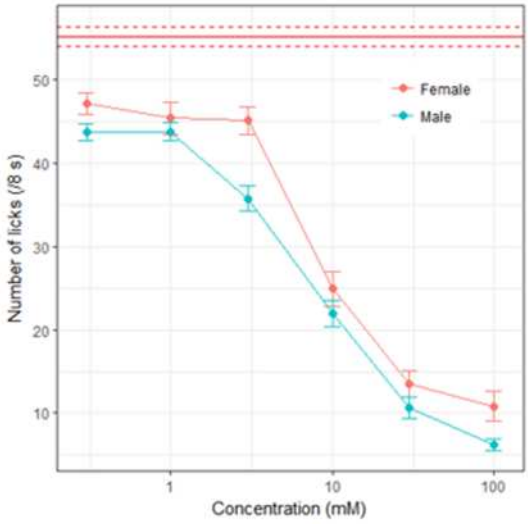


Figure 3-8 Female Vs. Male response to increasing concentrations of caffeine citrate showing the mean lick number +/- standard error of the mean (SEM)

3.5.2.2 Medium bitterness: Ranitidine hydrochloride

Taste assessment of aqueous solutions of ranitidine hydrochloride was successfully carried out in male and female rats

The data from male and female rats were pooled to assess the overall response to ranitidine hydrochloride. The lick number from all concentrations of ranitidine hydrochloride was found to be significantly different to that of water (Figure 3-9). No significant difference was observed for concentrations 0.17-1.43 mM, however 2.85 and 4.28 mM were found to be significantly different from each other and all other concentrations assessed (Figure 3-9).

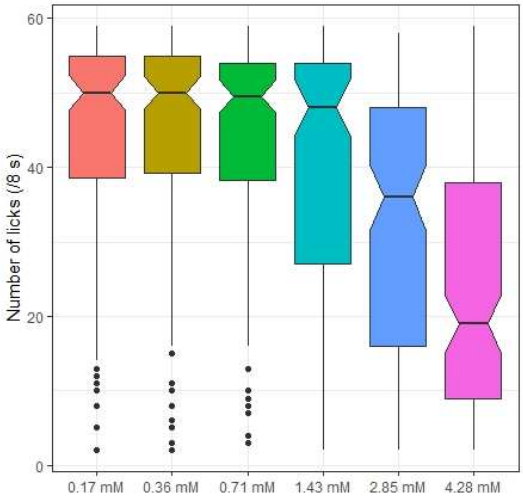


Figure 3-9 All rat taste response to increasing concentrations of ranitidine hydrochloride

When the data were split by gender, some differences were identified, with males being generally more sensitive than females by exhibiting a lower lick number (Figure 3-10 and Figure 3-11). However, not all observed differences were statistically significant. Indeed, the differences observed concentrations 0.17-1.43 mM were not statistically significant ($p > 0.05$), while at 2.85 and 4.28 mM, males were found to be significantly more sensitive than females at higher concentrations ($p < 0.05$) (Figure 3-10 and Figure 3-11).

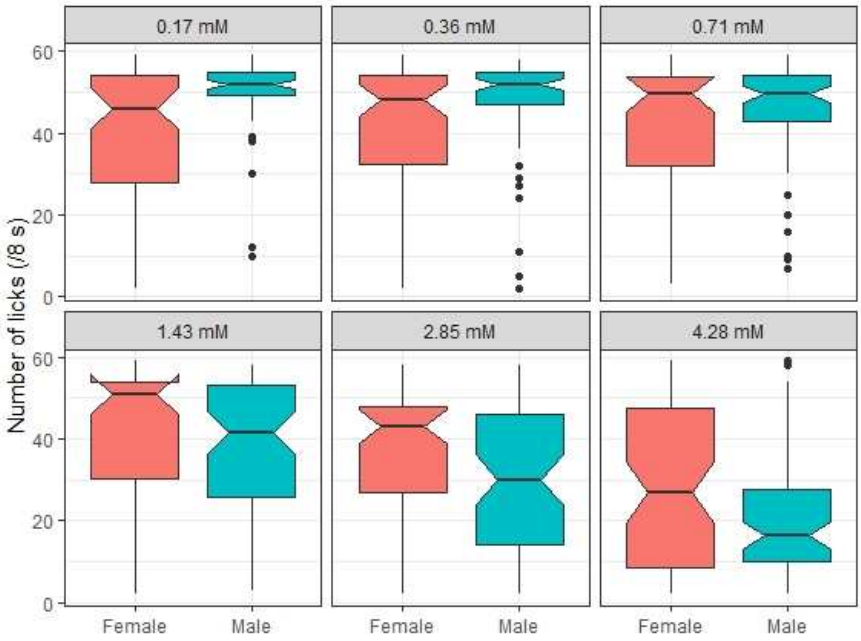


Figure 3-10 Female Vs. male response to each concentration of ranitidine hydrochloride

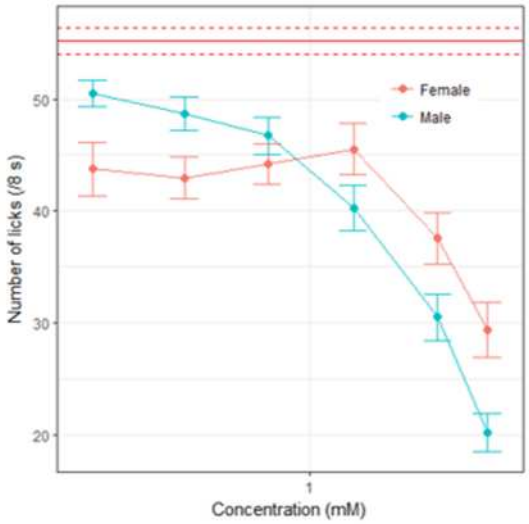


Figure 3-11 Female Vs. Male response to increasing concentrations of ranitidine hydrochloride showing the mean lick number +/- SEM

3.5.2.3 High bitterness: Quinine hydrochloride

Taste assessment of aqueous solutions of quinine hydrochloride was successfully carried out in male and female rats.

Both male and female rats responded significantly differentiated between water and all concentrations of quinine hydrochloride (Figure 3-14).

Figure 3-12 demonstrates the pooled taste responses of male and female rats. Overall, it can be seen from the lack of overlapping notches that significant differences were identified between lick number from all samples presented.

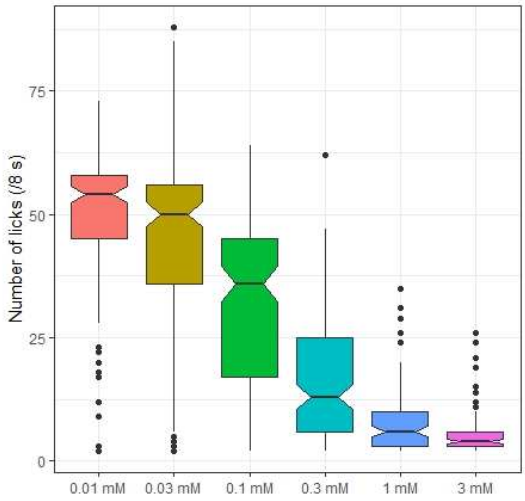


Figure 3-12 All rat taste response to increasing concentrations of quinine hydrochloride

Significant gender differences in taste response from solutions of quinine hydrochloride, however the results are less consistent than those observed for caffeine citrate (see Figure 3-13 and Figure 3-14). Females were found to be significantly more sensitive to quinine hydrochloride at 0.01 and 0.03 mM, with significantly lower lick number observed. However, at all other concentrations males were found to be more sensitive, however the differences were not consistently significant; at 0.1 mM, the gender differences were significant ($p < 0.05$), while at 0.3, 1 and 3 mM the observed differences were not found to be statistically significant.

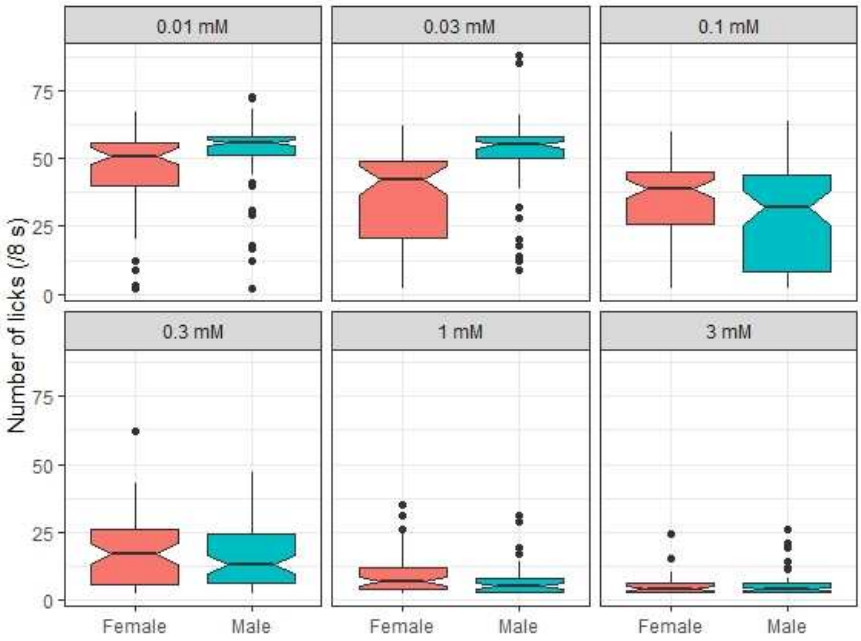


Figure 3-13 Female Vs. male response to each concentration of quinine hydrochloride

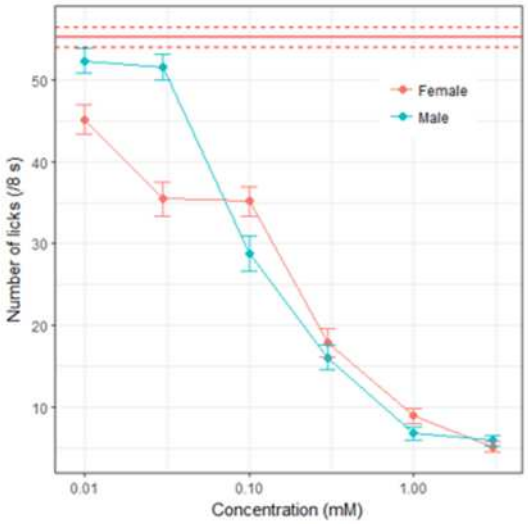


Figure 3-14 Female Vs. Male response to increasing concentrations of quinine hydrochloride showing the mean lick number +/- SEM

3.6 Discussion

In order to elucidate the importance of gender in the BATA model, male and female rats were assessed for their PROP phenotype; a key genetic trait conferring sensitivity to bitter substances. This study was the first such study to explore PROP phenotype in rats using the BATA model, and correlate this to gender and sensitivity to bitter APIs. It adapted a

method commonly used in humans [141] for PROP assessment to the BATA model and identified large differences in PROP phenotype between male and female Sprague-Dawley rats. Indeed, among males, a predominance (70 %) of the super taster phenotype was observed, while among females, a predominance (70 %) of the medium taster phenotype was observed. This finding provided real evidence that differences in taste sensitivity exist between male and female rats, which is a key finding for the BATA model, given its use as a taste assessment model.

The variation in PROP phenotype in humans is a function of sequence variations in the *TAS2R38* gene that result in differing sensitivities to PROP or the closely related PTC [154]. T2Rs, as well as T1Rs and ENaCs, are conserved among vertebrates, thus such differences in sensitivity to PROP/PTC should be seen in rats [170]. Indeed, different phenotypes were observed in this study, but these do need be confirmed with genetic sequencing.

To further elucidate the gender effect and how the differences in PROP phenotype may manifest themselves in sensitivity to bitter APIs, male and female responses to APIs eliciting varying levels of bitterness were assessed. Indeed, low, medium and high bitterness levels were explored using caffeine citrate, ranitidine hydrochloride and quinine hydrochloride, respectively.

Significant differences were identified between male and female rats, with males appearing more sensitive to all APIs as demonstrated by a generally lower lick number from males relative to females across the majority of concentrations of each API assessed. Given that the aversiveness to quinine hydrochloride, ranitidine hydrochloride and caffeine citrate is largely mediated by bitter taste receptors – T2Rs – it stands to reason that key differences in genes conferring sensitivity to bitterness may affect taste response to the aforementioned compounds. Thus, the stark differences in PROP phenotype observed between male and female rats, with 70 % of the males being super tasters may explain why such differences in sensitivity to a range of bitter APIs was observed.

Conversely, in a recent study assessing the acceptability of formulations of memantine hydrochloride (Ebixa®), significant differences have been observed between males and females in both humans and rats, confounding the findings in the present study [171].

Indeed, in humans (1517 patients over 64 years old), females "negatively accepted" the oral solution compared to males who "positively accepted" the oral solution. And in rats, using the BATA model, it was found that females also found the oral solution significantly more aversive than the males at all concentrations [171]. However, the fully formulated oral solution of Ebixa®, and its dilutions, were used in this study, thus the rats were exposed to multiple excipients in addition to the memantine hydrochloride active ingredient, therefore sensitivity to the bitter API was not assessed in this study, but instead the Ebixa® formulation and its excipients. Indeed, the findings may point to gender differences in sensitivity to excipients, rather than to the bitter API, therefore the results of this study may not necessarily confound the findings of the present study. However, to ascertain this, memantine hydrochloride alone must be assessed in male and female rats using the BATA model

The real importance of this study however is how it affects gender choice for the rat BATA model as a pharmaceutical taste assessment methodology. Conventionally, male rats have always been used [122] but this study sought to challenge this convention and provide justification for this gender choice. It demonstrated that male rats are more sensitive to APIs possessing a range of aversiveness levels, and provided a possible explanation for this in the form of differing PROP phenotypes. Thus, being more sensitive to bitter taste, males may provide a much more challenging, worst-case scenario taste assessment of a given novel API. Therefore, being more sensitive, the data from the male model may correlate more strongly to children who are more sensitive to bitter taste than are adults. However, this requires further exploration. For now though, we know that male Sprague-Dawley rats are providing us with sensitive taste analysis of novel APIs.

3.7 Conclusion

During the development of the BATA model, a multitude of key parameters were optimised to yield the successful taste assessment methodology currently in existence [122,157]. However, during this development, gender was not explored, and although there is no clear consensus on the gender effect on taste, the literature does point to the possibility of there being some differences in male and female taste. This study thus explored this possibility by first assessing PROP phenotypes in male and female rats and

found stark differences with males being predominantly super tasters while the medium taster phenotype predominated in females. The identified differences were further explored by assessing the BATA response of male and female rats to a range of bitter APIs, with males showing greater sensitivity to all as indicated by the predominance of the super taster phenotype. Given that rats are used in the BATA model as 'analytical tools' for taste, it can be concluded that male rats are the most sensitive tool relative to females and thus should continue to be used in all future BATA experiments in order to achieve the most sensitive assessment of bitter APIs going forward.

4 Assessing the feasibility of solubilisation as a means to expand the BATA model to poorly soluble drug compounds

4.1 Introduction

It stands to reason that an API can only elicit a taste if it is in solution in the mouth and can thus interact with taste receptors [9]. Indeed, if an API is deemed to be aversive, a strategy available to the formulation scientist is to simply limit the solubility of said API, by ensuring precipitation out of solution by utilising the physicochemical properties of the free form or other solid forms such as salts, co-crystals or poorly soluble polymorphic forms [9]. Therefore, solubility and bitterness are inextricably related, or are they? Figure 4-1 demonstrates that the relationship between solubility and aversiveness/bitterness may not be as simple as it seems; it shows a combination of published [127] and unpublished EC_{50} values – the concentration eliciting half the maximum response by humans on a VAS from 'not aversive' to 'extremely aversive', thus the lower the concentration, the more aversive the API – for fifteen APIs as a function of respective solubilities. Clearly, there is no correlation whatsoever between solubility and aversiveness.

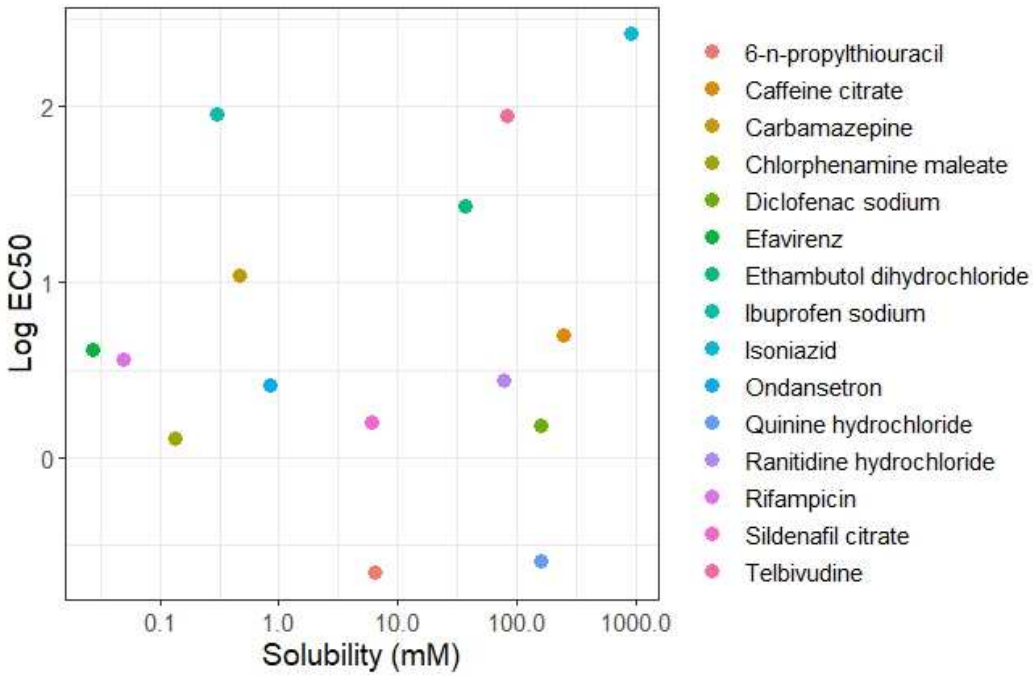


Figure 4-1 Aversiveness as a function of solubility. Data are a combination of published [127] and unpublished findings.

The complex relationship between solubility and bitterness/aversiveness can also been seen in the BitterDB, a database of over 1000 bitter molecules that have been reported

as tasting bitter [172]. It has evolved to include information on bitterness intensity, toxicity, bitter taste receptor (T2R) binding and also mouse, cat and chicken T2Rs. Analysis of the ALogP of the bitter molecules, as a surrogate measure of water solubility, contained within BitterDB reveals a huge range of values, from streptomycin at -8.2 to isoxantholupon at 7.1 [173]. Thus, even at minute solubilities, a compound may elicit an aversive taste.

Clearly, the link between solubility and taste is not so inextricable. Therefore, it is necessary to screen all compounds for taste aversion, regardless of solubility, to identify possible adherence issues going forward.

And so to a key limitation of the BATA taste assessment model: solubility. As described in chapters 1 and 3, it relies on the API under assessment being dissolved in water in order to be presented to rats in sipper tubes controlled by the lickometer. Thus, if the API in question is of low or non-existent water solubility, its taste assessment using the BATA model will be limited if not impossible. A key output of the BATA model is the IC_{50}, which is the concentration eliciting half the maximal lick response by the rats, and is very well explained in [152]. Its calculation relies on a sufficiently broad concentration range having been assessed in the BATA model, including low concentrations eliciting minimal lick inhibition and high concentrations eliciting maximum lick inhibition. Thus, poorly water-soluble APIs may not allow a sufficiently broad concentration range to be assessed, so impairing the calculation of the IC_{50} and hence preventing characterisation of the aversiveness of such APIs.

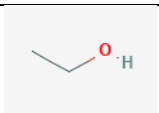
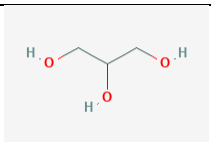
The same solubility-limiting issue is encountered in nonclinical *in vivo* safety assessments, in which an assessed compound is given at increasing doses in order to identify important safety and toxicity parameters such as the LD_{50} [174]. In order to administer a sufficiently large dose to enable calculation of say the LD_{50}, the compound may be administered in a vehicle, which maintains the stability of the active ingredient, but maximises its systemic bioavailability [174]. Such a practice could feasibly be employed in the BATA model to maximise the concentration of active ingredient administered orally. However, being a taste assessment experiment, it is critical that the employed vehicles do not themselves elicit a taste.

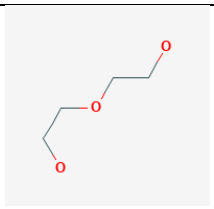
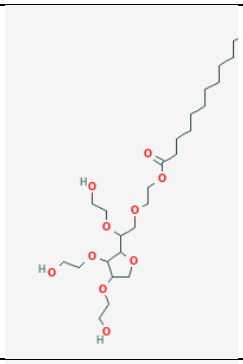
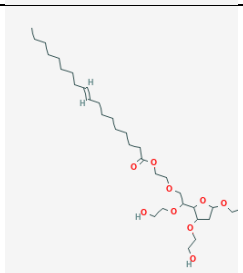
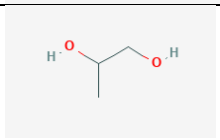
Co-solvents are frequently used in pharmaceutical formulations to enhance API solubility, particularly those APIs that do not contain ionisable groups and thus whose solubility cannot be increased by pH adjustment [175]. Co-solvents function by lowering the polarity of water, thus serving to enable the dissolution of non-polar drugs. However, most water-miscible organic liquids are toxic when taken orally, thus the toolkit of co-solvents available to the formulation scientist is limited. This chapter will look at this toolkit as a means to expand the range of compounds assessable in the BATA model to poorly soluble APIs. It is of course critical that said co-solvents do not, themselves, impart a taste and thus skew the output. Thus, the concentrations at which a given solvent is not distinguishable from water will be investigated and correlated, thus providing a toolkit for the sensory scientist to use when investigating the taste of a poorly soluble API.

The selected co-solvents for investigation are shown in Table 4-1. The co-solvents were chosen by reviewing those solubilising excipients used in oral formulations and selecting those that are most commonly encountered [176]. The concentrations were chosen by reviewing toxicological data on nonclinical vehicle use and ascertaining the maximum concentration safely assessable in the BATA model [174].

Table 4-1 demonstrates the range of co-solvents chosen for investigation. A range of tastes are described for these co-solvents in the Handbook of Pharmaceutical Excipients [177].

Table 4-1 Selected co-solvents for investigation. Information was taken from the Handbook of Pharmaceutical Excipients [177]

Compound	Structure	Concentration (%) in oral solutions	Description	Regulatory status
Ethanol		Variable	Clear, colourless, mobile, and volatile liquid with a slight, characteristic odour and burning taste.	Included in the FDA Inactive Ingredients Database
Glycerol		Variable	Clear, colourless, odourless, viscous, hygroscopic liquid; it has a sweet taste,	GRAS, Included in the FDA Inactive Ingredients Database

			approximately 0.6 times as sweet as sucrose.	
Polyethylene glycol (PEG) 400			Colourless or slightly yellow-coloured, viscous liquids. They have a slight but characteristic odour and a bitter, slightly burning taste.	Included in the FDA Inactive Ingredients Database
Polysorbate 20			viscous, oily liquids or waxy solids, and have a faint characteristic odour and a bitter taste.	Accepted as food additives in Europe, Included in the FDA Inactive Ingredients Database
Polysorbate 80			viscous, oily liquids or waxy solids, and have a faint characteristic odour and a bitter taste.	GRAS, accepted as food additives in Europe, Included in the FDA Inactive Ingredients Database
Propylene glycol		10-25 %	Clear, colourless, viscous, practically odourless liquid, with a sweet, slightly acrid taste resembling that of glycerin.	GRAS, accepted as food additives in Europe, Included in the FDA Inactive Ingredients Database

Of interest is the relatively similar chemical structures among the co-solvents but the vastly different tastes they elicit. For example, ethanol, glycerol and propylene glycol exhibit similar functional groups yet elicit burning, sweet and acrid tastes, respectively [177]. Of course to be used in their capacity to expand the APIs assessable in the BATA model to those that are poorly soluble, it is critical that the concentrations at which they are used are low enough so as to not themselves elicit a taste.

4.2 Aims & Objectives

4.2.1 Aims

Co-solvents are commonly used to enhance the solubility of poorly-soluble APIs. Poorly-soluble APIs are difficult to assess using the BATA model as it relies on the API being dissolved in water and presented to the rats in sipper tubes. It is therefore hypothesised that co-solvents may be used to expand the APIs assessable in the BATA model to those that are poorly soluble by utilising the solubilising power of co-solvents. However, co-solvents may not be inert in the sensory sense and may themselves elicit a taste; thus the concentrations at which selected co-solvents do not elicit a taste is sought, which can then be used to expand the APIs assessable to those that are poorly soluble.

4.2.2 Objectives

1. Assess full concentration ranges of selected co-solvents commonly used as solubility-enhancers in pharmaceutical oral formulations in the BATA model.
2. Assess the human response to the selected co-solvents.
3. Assess the human-rat correlation in taste-response to the selected co-solvents.

4.3 Materials and Methods

4.3.1 Materials

4.3.1.1 Rat BATA model l taste assessment

Polysorbate 20, polysorbate 80 (cell culture grade), propylene glycol (> 99.5%), were purchased from Sigma-Aldrich® (Dorset, UK). Glycerol and ethanol (99.8%) were purchased from Fischer Scientific. Polyethylene glycol (molecular weight 400) (PEG 400) was purchased from Scientific Laboratory Supplies (Nottingham, UK).

4.3.1.2 Human taste panel

Sodium chloride, caffeine, citric acid, sucrose, monosodium glutamate, glycerol, polyethylene glycol, polysorbate 20, polysorbate 80, propylene glycol, were purchased from Fagron (Newcastle-upon-Tyne, England); ethanol from Sigma-Aldrich (St. Louis, USA)

4.3.2 Methods

4.3.2.1 Rat BATA model taste assessment

4.3.2.1.1 Animals

Ten adult male Sprague–Dawley rats (Charles-River, Kent, UK) were used. Rats were housed in pairs in standard cages in a room that was maintained at 21±2 °C with 55±10% humidity and with a 12:12 h light/dark cycle. All training and testing occurred during the light phase of the cycle. Animals had free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a water-restriction schedule occurred (see procedure). Throughout the experiment, daily food and water consumption were monitored. As a safety and welfare measure it was checked 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).

4.3.2.1.2 Procedure

The experimental method described in this section was validated by *Soto. et al* [122]. The commercially available lickometer "Davis MS-160" from DiLog Instruments (Tallahassee, Florida, USA) was used for this experiment. Each rat was water-deprived for 22 h before each session (training and testing) and was then placed in the lickometer for a maximum session-length of 40 min. After each session, the rodents received tap water for rehydration. The initial days of the protocol were dedicated to training: on the first training day the shutter was continually open, presenting a single tube containing deionised water; on the second training day, sixteen tubes contained deionised water. The training was followed by two testing days during which each rat was presented with various sipper tubes containing either deionised water or one of the concentration of the solubilising agent. Each trial began when the rat took its first lick from the sipper tube, and ended eight seconds later when the shutter closed. A different sipper tube was then positioned behind the shutter in preparation for the next trial. Each trial was intercepted by a water rinse to minimise carry over effects from the previous solution tested. The order of presentation of the sipper tubes was randomised and each concentration was presented 4 times per session. The number of rats used was 10.

The solubilising agents were freshly prepared by serial dilution in deionised water at concentrations shown in Table 4-2.

Table 4-2 Solutions of co-solvents/excipients at specified concentrations assessed using the rat BATA model

Co-solvent/Excipient	Concentration (% w/v)				
Ethanol	0.1	0.5	1	3	10
Glycerol	1	10	20	30	50
PEG 400	0.1	0.5	1	3	10
Polysorbate 20	0.1	0.2	0.3		0.5
Polysorbate 80	0.1		0.3		0.5
Propylene glycol	0.1	0.5	1	3	10

4.3.2.1.3 Data analysis

The data relating to aversiveness of each co-solvent/excipient were presented in notched box-plots. The aversiveness ratings were plotted as a function of increasing concentration.

The IC_{50} – concentration of the compound that produces half of the maximal lick response– was also calculated for each co-solvent/excipient [151].

The distribution of the data was assessed using the Shapiro-Wilk test[178]: 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[169]. 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.

Analysis was performed using R software (open source) and non-linear mixed effects (NONMEM) tool (version 7.3, ICON Development Solutions, Dublin, Ireland).

4.3.2.2 Human taste panel

Given that it was predicted that the assessed co-solvents may elicit taste modalities other than merely bitterness, this taste panel was conducted in two stages: the first stage

familiarised the participants to the five taste modalities, while the second stage involved taste assessment of the co-solvents. The two stages are detailed below.

4.3.2.2.1 Tastant familiarisation

In this study, prior to sampling the co-solvents/excipients, participants were trained in the five tastes – sweet, salty, umami, bitter and sour – in accordance with the International Standard for Sensory Analysis – General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors (ISO 8586:2012(E)) under 'tests for detection of a stimulus' [153]. Participants were given five known solutions of tastants at supra-threshold concentrations, each indicative of a given tastant and labelled as such, as per Table 4-3 and instructed as per Figure 4-2.

Table 4-3 Solutions of tastants utilised in the training of participants as to the 5 tastes.

Tastant	Taste	Concentration (%w/v)
Caffeine	Bitter	0.02
Citric Acid	Sour	0.02
Sodium Chloride	Salty	0.13
Sucrose	Sweet	0.6
Monosodium glutamate	Umami	0.03

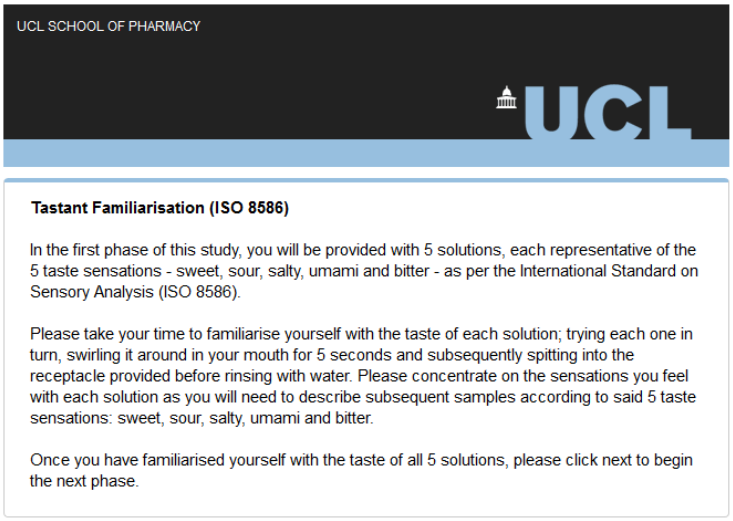


Figure 4-2 Participant instructions for tastant familiarisation

4.3.2.2.2 Measurement of aversiveness and taste characterisation using human adult volunteers

Solutions of varying concentrations (see Table 4-4) were prepared under the supervision of a UK registered pharmacist with sonication employed where necessary. The concentrations chosen were fewer than those assessed in the rat BATA in order to minimise participant burden.

Table 4-4 Solutions of co-solvents/excipients at specified concentrations assessed using a human taste panel

Co-solvent/Excipient	Concentrations assessed (% w/v)		
Ethanol	2.5	7.5	10
Glycerol	1	3	12.61
PEG 400	0.5	1	3
Polysorbate 20	0.3	0.5	0.75
Polysorbate 80	0.3	0.5	0.75
Propylene glycol	0.5	1	3

Thirty one volunteers between the ages of 18 and 38 years old (median 22 years old; 8 males and 23 females; 10 ethnicities (2 white British, 10 Asian/Asian British other, 1 white Irish, 2 mixed other, 3 white other, 2 Arab, 8 Chinese, 1 African/Caribbean other, 1 Pakistani and 1 Indian)) were enrolled in a randomised single-blind study. The protocol was approved by the Research Ethics Committee at The School of Pharmacy, University College London (REC ID: 4612/010).

As indicated above, each participant received self-directed training prior to commencing sampling. Training involved presentation with appropriately labelled tastants samples, each corresponding to the five taste sensations: sweet, salty, sour, bitter and umami. The 'swirl and spit' methodology was employed in this study, such that following the period of training, the participants were then presented with each of the concentrations of each co-solvent/excipient, which they were then instructed to swirl around their mouth for 10 seconds, before spitting into a receptacle provided. The solutions – each labelled with a random 3-digit code – were presented at random and in duplicate, with a 7.5-10 minute break between each presentation to allow for rinsing with and consumption of water. During this inter-presentation interval, participants were also encouraged to consume a plain cracker in order to neutralise the taste of the sample most recently tasted. Given

the number of samples, this assessment took place over two sessions of approximately 90 minutes with training at each phase.

Samples were assessed using Qualtrics® online survey software, which calls on the participant to rate a given sample's aversiveness using a 100mm visual analogue scale (VAS) from 'not aversive' to 'extremely aversive'. Additionally, the participant was also prompted to assign a taste sensation to each sample; either sweet, salty, sour, umami or bitter. A comments section was also provided for any further information the participant wished to share.

4.3.2.2.3 Graphical representation of the data

The data relating to aversiveness of each co-solvent/excipient were presented in notched box-plots. The aversiveness ratings were plotted as a function of increasing concentration.

Taste sensation data were plotted in stacked bar charts showing the 5 taste sensations as identified by the participants, and how their rating changes as a function of concentration.

The EC_{50} – concentration of the drug that produces half of the maximal rating (100) in the human taste panels – was also assessed for each co-solvent/excipient as described previously [151], however these are merely indicative of aversiveness given the small concentration range assessed.

The distribution of the data was assessed using the Shapiro-Wilk test[178]: 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[169]. 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.

Analysis was performed using R software (open source) and non-linear mixed effects (NONMEM) tool (version 7.3, ICON Development Solutions, Dublin, Ireland).

4.3.2.3 Human Vs. Rat Correlation

The correlation between humans and rats was assessed by evaluating the taste thresholds – IC_{50} and EC_{50} for rats and humans respectively – for each co-solvent where possible and comparing them against each other. The ranking of the aversiveness of the co-solvents was also compared between rats and humans.

4.4 Results

4.4.1 Rat BATA model

Six solubilisers were assessed in the BATA model at a range of concentrations dependent on toxicity, with each concentration of each solubiliser compared to water to establish the concentrations from which rats cannot distinguish water, thus informing the concentrations that may be used in future BATA experiments to solubilise poorly soluble APIs (Figure 4-3). A range of responses were observed, and will be discussed in turn in the following pages.

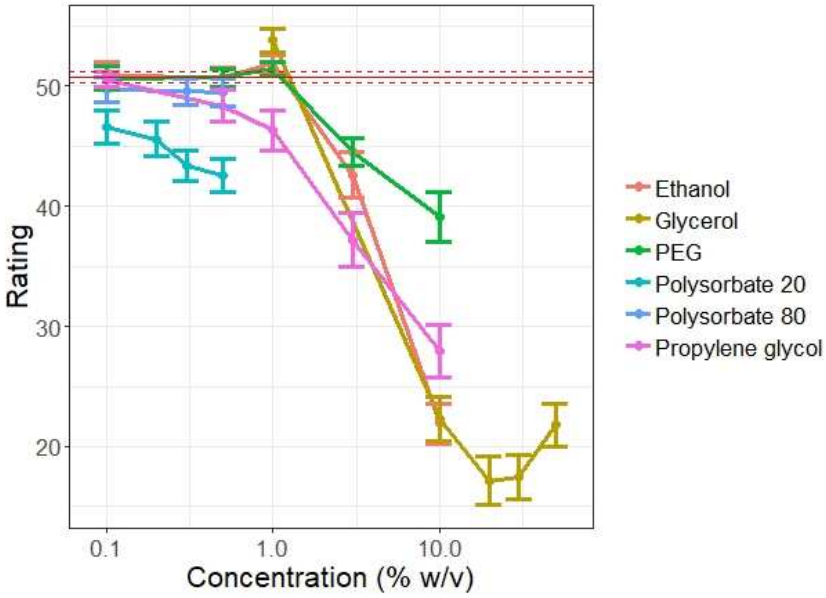


Figure 4-3 BATA assessment of selected solubilisers showing the mean lick number +/- SEM. For reference the lick number for the water control is also indicated as the mean +/- SEM as solid and dashed red horizontal lines respectively.

4.4.1.1 Ethanol

Ethanol was very well tolerated by the rats, as demonstrated by in Figure 4-4 where it can be seen that the boxplots overlap, and in some cases exceed the number of licks of water indicating an inability of the rats to distinguish ethanol from water up to but not including concentrations of 10 % w/v. Indeed, this finding was confirmed by statistical analysis; concentration was found to have a significant ($p < 0.05$) impact on lick number. Gao's posthoc analysis provided further insight confirming the lick numbers for 0.1-3 % w/v were not significantly different to water ($p > 0.05$), while rats found 10 % w/v ethanol

significantly more aversive than water ($p < 0.05$) with a mean lick number of 21.9/ 8 s (+/- 1.63). The calculated IC50 for ethanol was found to be 8.47 % w/v (5.75-11.19 % e/v), however this value may be somewhat misleading given that a full sigmoidal curve was not acquired.

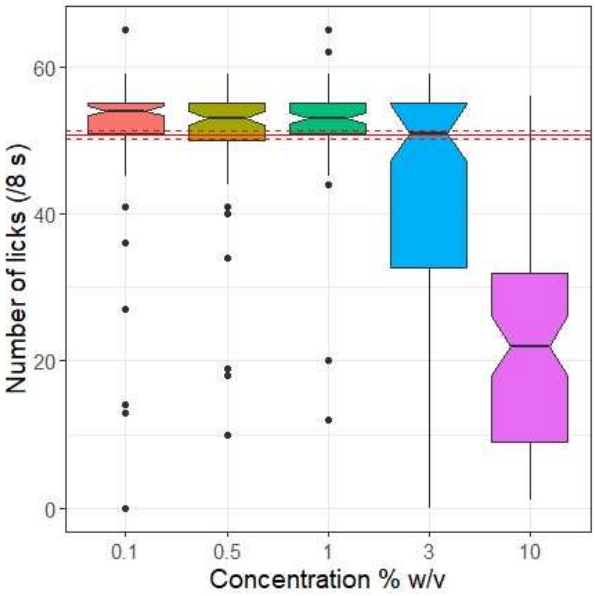


Figure 4-4 BATA assessment of a range of concentrations of ethanol showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.1.2 Glycerol

Glycerol was poorly tolerated by the rats as shown in Figure 4-5 in which it can be seen that there is no overlap between the boxplots and the indicated mean lick number of water. Indeed, confirmation was provided by statistical analysis, which revealed that all concentrations of glycerol assessed had a lick number significantly different from water ($p < 0.05$). However, it must be noted that 1 % w/v glycerol had a lick number significantly higher than that of water (53.79 +/- 0.96 licks/8 s) suggesting an elevated tolerance, while those concentrations exceeding 1 % w/v glycerol were found to be significantly more aversive than water, i.e. a lower lick number. The calculated IC50 was 9.94 % w/v (6.09-13.77 % w/v).

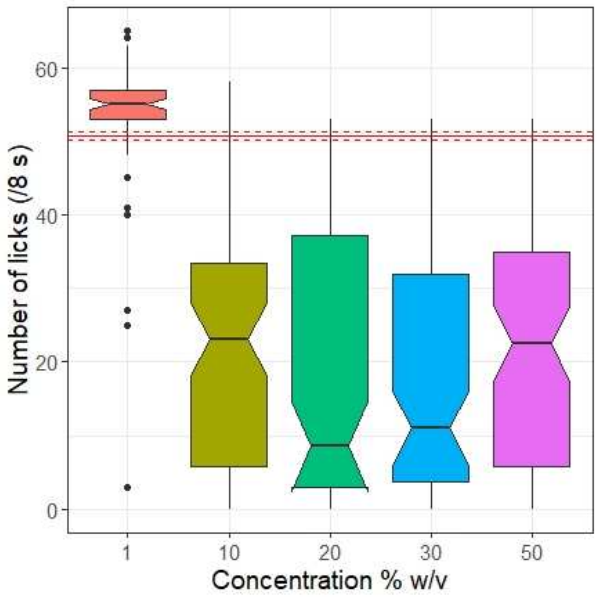


Figure 4-5 BATA assessment of a range of concentrations of glycerol showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.1.3 Polysorbate 20

Figure 4-6 appears to demonstrate that Polysorbate 20 is reasonably well tolerated by the rats, as the boxplots appear to overlap the mean water lick number, most notably at concentrations of 0.1 and 0.2 % w/v. However, statistical analysis reveals that at all concentrations of Polysorbate 20 assessed, the lick number was significantly different from water ($p < 0.05$). Although the differences appear small with mean lick numbers of 46.58 (+/- 1.34) and 45.58 (+/- 1.44) at 0.1 %w/v and 0.2 % w/v Polysorbate 20 relative to water's 50.64 (+/- 0.69), it must be concluded that the rats are capable of distinguishing between solutions of Polysorbate 20 and water, thus questioning its use as a solubiliser in the BATA model. However, despite being significantly different from water, polysorbate 20 only induced mild lick inhibition even at the maximum concentration assessed, thus an IC_{50} could not be calculated as the response curve was flat.

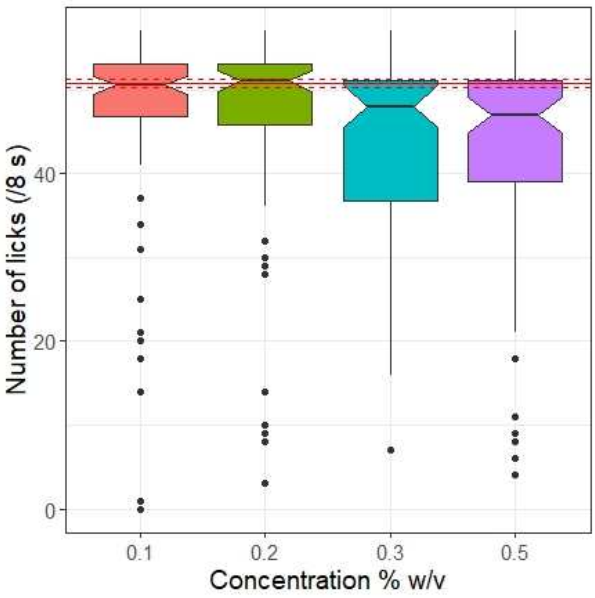


Figure 4-6 BATA assessment of a range of concentrations of polysorbate 20 showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.1.4 Polysorbate 80

In contrast to Polysorbate 20, Polysorbate 80 was very well tolerated in the rat BATA experiments, as shown in Figure 4-7, where the overlap of the mean water lick number and boxplots can be seen. Indeed, Kruskal-Wallis and Gao's posthoc analysis confirmed that all concentrations of Polysorbate 80 did not elicit a lick number significantly different from water ($p > 0.05$). The mild lick inhibition by all solutions of polysorbate 80 portended an inability to calculate the IC_{50}.

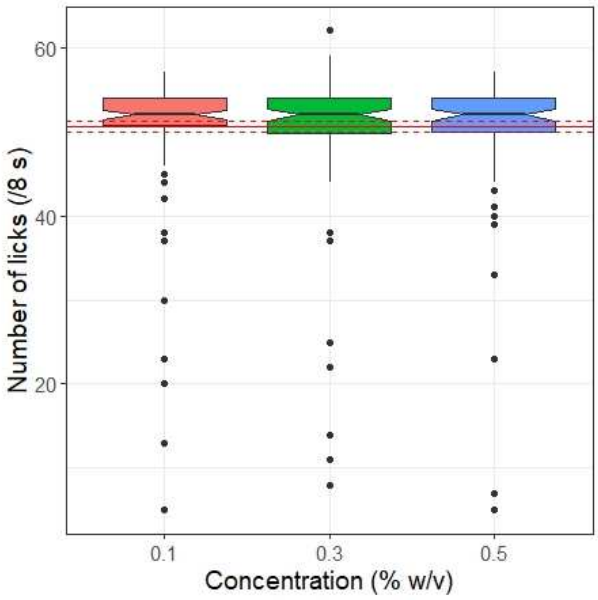


Figure 4-7 BATA assessment of a range of concentrations of polysorbate 80 showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.1.5 Polyethylene glycol (PEG) 400

A varied response to PEG 400 was observed in the rat BATA model, with some concentrations eliciting a response different to that of water, while others were indistinguishable (Figure 4-8). At concentrations 0.1 – 1 % w/v PEG 400, the rats produced a lick number that did not differ significantly from water ($p > 0.05$), while at concentrations exceeding 3 % w/v PEG 400, a significantly different lick number was observed ($p < 0.05$), with mean lick numbers of 44.48 (+/- 1.14) and 39.10 (+/- 2.08) for 3 and 10 % w/v PEG 400, respectively. An IC_{50} could not be calculated as only mild lick inhibition was observed.

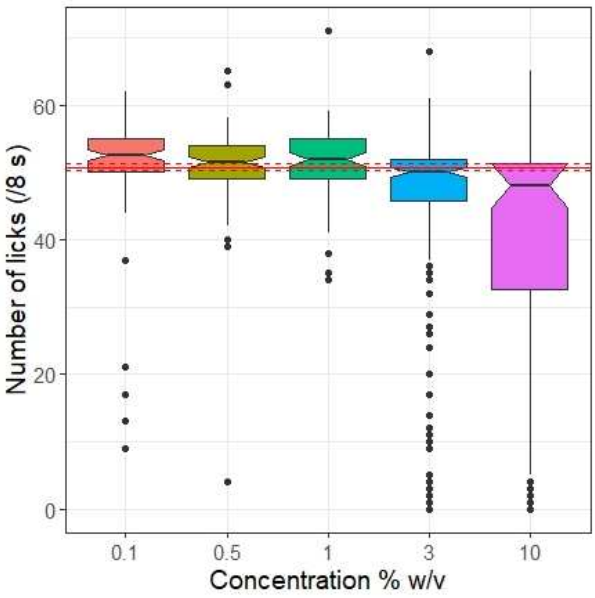


Figure 4-8 BATA assessment of a range of concentrations of PEG 400 showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.1.6 Propylene glycol

Figure 4-9 demonstrates the varied response that was observed to increasing concentrations of propylene glycol. Indeed, the response was similar to that seen for polyethylene glycol; at concentrations below 1 % w/v propylene glycol inclusive, the rats found the solutions indistinguishable from water in terms of lick number, as shown by the overlapping of the respective boxplots and the mean water lick number and confirmed with Kruskal-Wallis and Gao's posthoc analysis ($p > 0.05$). By contrast, at concentrations above 3 % w/v propylene glycol inclusive, a significantly different lick number was observed ($p < 0.05$) indicating that the rats were able to distinguish said concentrations from water, perceiving them to be more aversive. The calculated IC50 was found to be 12.50 % w/v (3.19-21.81 % w/v) in rats.

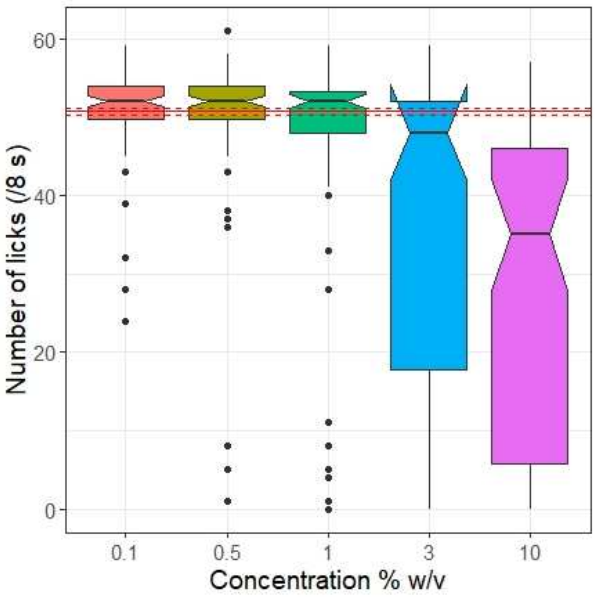


Figure 4-9 BATA assessment of a range of concentrations of propylene glycol showing, for reference, the lick number for the water control as the mean +/- SEM as solid and dashed red horizontal lines respectively

4.4.2 Human taste panel

All excipients assessed in this study demonstrated increasing aversiveness as a function of increasing concentration: see Figure 4-10. The solubilisers were perceived differently, with each eliciting differing aversiveness responses at varying concentrations. Each excipient will be explored in turn, with an assessment of the concentration response observed and the taste sensation perceived.

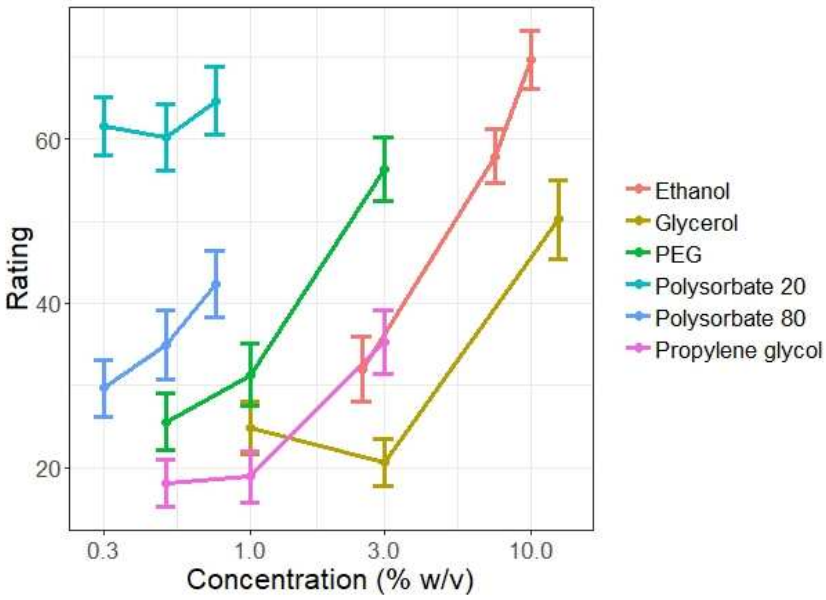


Figure 4-10 Human taste assessment of selected solubilisers showing the mean rating on a VAS +/- SEM.

By comparing the EC50 of each co-solvent, it is possible to establish the magnitude of aversiveness: see Figure 4-11.

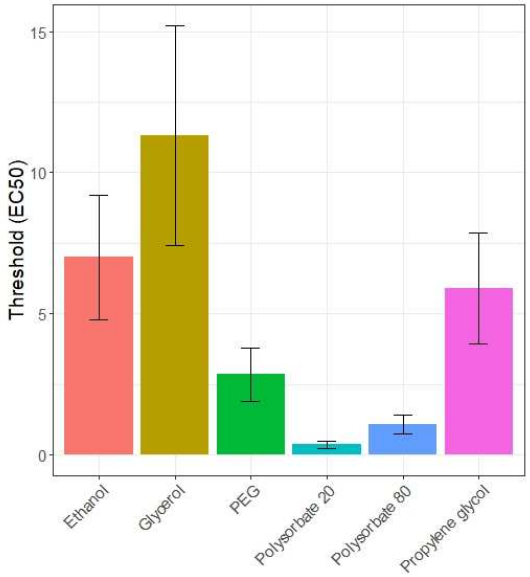


Figure 4-11 Comparing the EC50s obtained for each excipient assessed. Error bars are indicative of the 95% CI

Figure 4-11 identifies polysorbate 20 as the most potently aversive compound assessed, given that the EC50 obtained was significantly lower when compared with all other excipients assessed. The closely related polysorbate 80 was significantly less aversive, which is interesting given the close chemical similarity. Ethanol, glycerol and propylene

glycol were significantly less aversive than all other compounds assessed, but showed no significant difference in EC50 between one another.

4.4.2.1 Ethanol

The human participants demonstrated an increasing aversion to ethanol as the concentration increased. The Kruskal-Wallis rank sum test identified concentration to be a significant ($p < 0.05$) variable to the rating on a VAS, and Gao's posthoc analysis revealed that each concentration was rating significantly different to each other ($p < 0.05$). Figure 4-12 provides a visual demonstration of this with the lack of overlap of the notches indicative of significant difference. The EC50 was calculated to be 7.00 % w/v (4.79-9.22 % w/v).

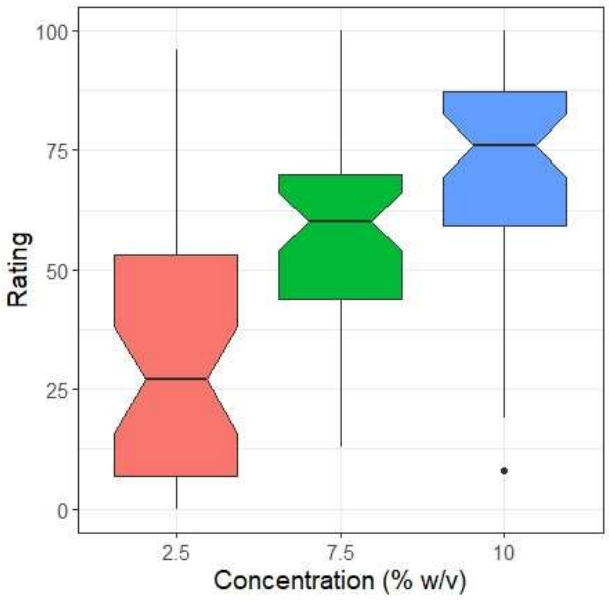


Figure 4-12 Human taste assessment of increasing concentrations of ethanol

As well as the changing aversiveness shown in Figure 4-12, the different concentrations of ethanol also elicited a changing taste sensation as described by the human panel. Figure 4-13 demonstrates the changing taste sensations that were observed in this study. As the concentration of ethanol is increased from 2.5 to 7.5 % w/v, there is a significant increase in the number of bitter responses and a reduction in the number of umami and sweet responses, which correspond to the increasing aversiveness. However, increasing the concentration of ethanol from 7.5 to 10 % w/v does not elicit a significant change in the

taste sensations reported. Also of note is the lack of significant change in sour perception as the concentration of ethanol is increased (Figure 4-13).

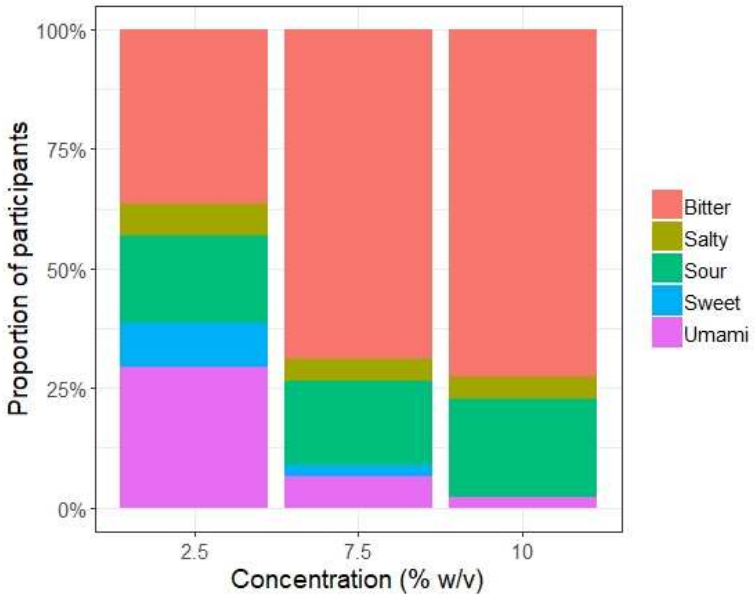


Figure 4-13 Taste sensations described by the participants as a function of increasing ethanol concentration

4.4.2.2 Glycerol

The human participants also demonstrated an increasing aversion to increasing concentrations of glycerol but only at the extremes of the concentrations assessed. As shown in Figure 4-14, there is no overlap between the notches for 1 and 12.61 % w/v glycerol indicative of significant difference which was confirmed using Kruskal-Wallis rank sum test and Gao's posthoc analysis ($p < 0.05$). The perceived aversiveness of glycerol reduced as the concentration increased from 1 to 3 % w/v as the mean rating on the VAS reduced from 24.79 (+/- 3.18) to 20.55 (+/- 2.85), respectively. However, the differences identified between 1 and 3 % w/v glycerol were not statistically significant ($p = 0.3235$). The EC50 was calculated to be 11.30 % w/v (7.40-15.20 % w/v).

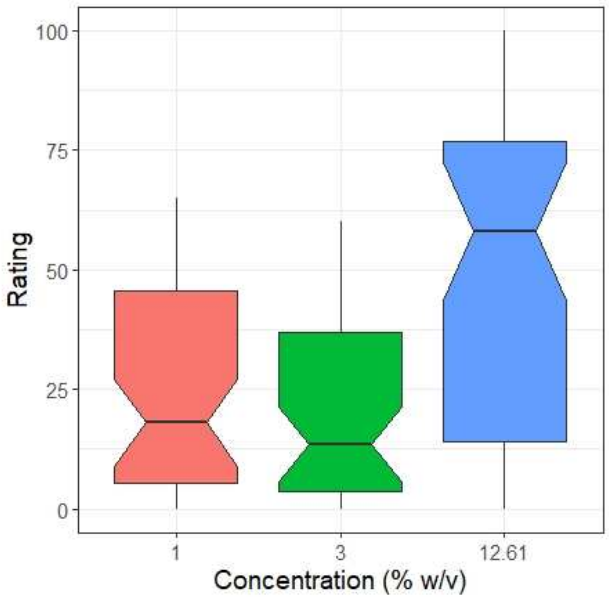


Figure 4-14 Human taste assessment of increasing concentrations of glycerol

As seen with ethanol in section 4.4.1.1, the differences noted in Figure 4-14 can be, at least partially, explained by the taste sensations reported by the participants as a function of glycerol concentration. As shown in Figure 4-15, there is a significant reduction in the sweet sensation reported by the participants as the concentration is increased from 1 to 3 % w/v glycerol and an increase in the umami sensation, which may explain why a reduced aversiveness was observed 3 % w/v relative to 1 % w/v as perhaps the participants found the solution to be *too* sweet. This finding is confirmed when the taste sensations reported for 12.61 % w/v glycerol are assessed; an overwhelming majority of the participants reported a sweet sensation, yet this solution was the most aversive, thus suggesting excessive-sweetness as the root of aversiveness in this case.

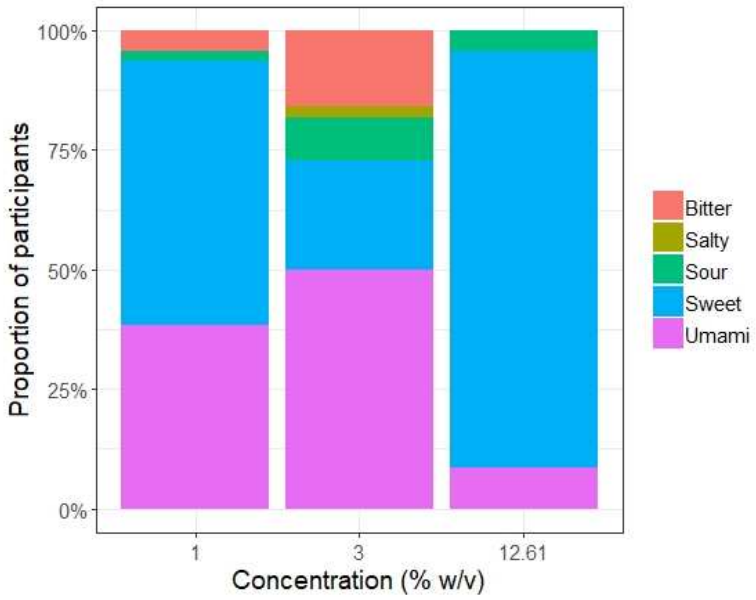


Figure 4-15 Taste sensations described by the participants as a function of increasing glycerol concentration

4.4.2.3 Polysorbate 20

Polysorbate 20 was perceived as reasonably aversive by the human taste panel with mean ratings on the VAS of 61.65 (+/- 3.53), 60.23 (+/- 3.98) and 64.70 (+/-4.06) for 0.3, 0.5 and 0.75 % w/v Polysorbate 20 respectively. The aforementioned differences were, however found to not be statistically significant (p = 0.621), thus the participants found all solutions aversive regardless of concentration. This finding is indicated by Figure 4-16, where overlapping of the notches is visible. The EC50 was calculated to be 0.35 % w/v (0.22-0.48 % w/v).

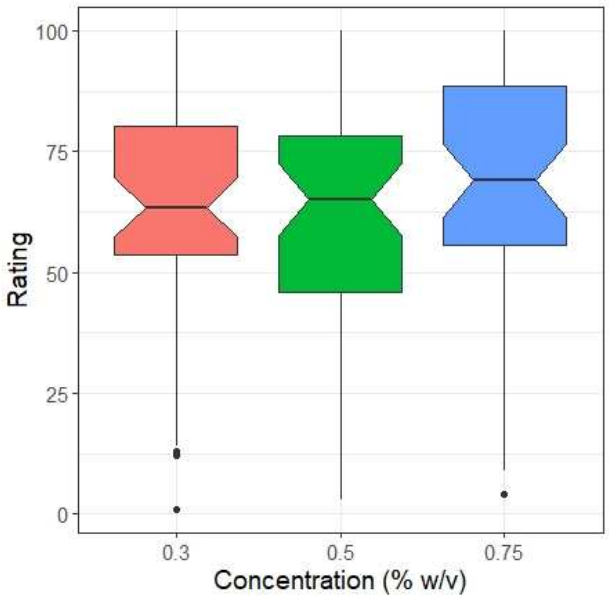


Figure 4-16 Human taste assessment of increasing concentrations of polysorbate 20

The aversiveness reported for Polysorbate 20 can be explained by the bitter sensation that it elicited among the vast majority of participants (Figure 4-17). Furthermore, as the concentration was increased, there was no change in the proportion of participants reporting a bitter sensation, thus explaining the aversion seen to all concentrations of Polysorbate 20.

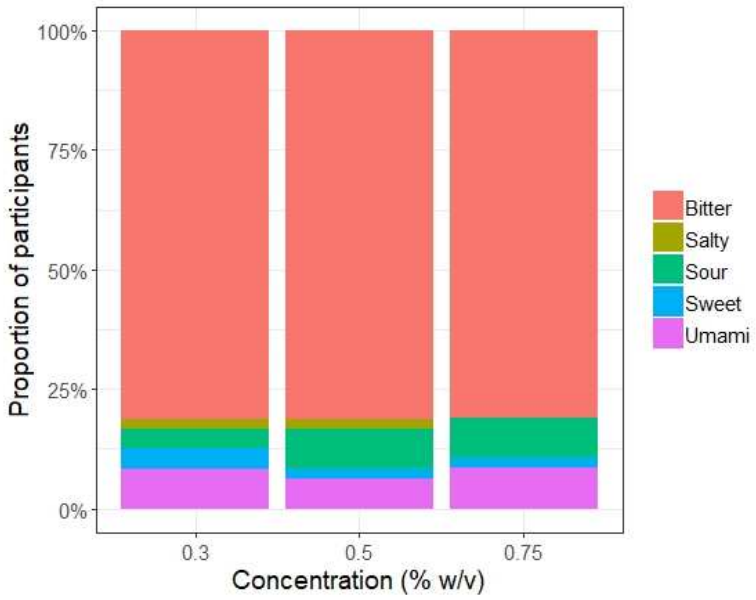


Figure 4-17 Taste sensations described by the participants as a function of increasing polysorbate 20 concentration

4.4.2.4 Polysorbate 80

The human response to Polysorbate 80 was markedly different to that observed for the chemically-related Polysorbate 20. The participants rated Polysorbate 80 as significantly less aversive than Polysorbate 20 with mean VAS ratings of 29.65 (+/- 3.46), 34.93 (+/- 4.15) and 42.38 (+/- 4.02) for 0.3, 0.5 and 0.75 % w/v, respectively. However, similarly to the response seen with Polysorbate 20, the participants were unable to distinguish between increasing concentrations of Polysorbate 80 as indicated by overlapping notches in Figure 4-18 and confirmed by Kruskal-Wallis rank sum test (p = 0.08566). The EC50 was calculated to be 1.07 % w/v (0.74-1.40 % w/v).

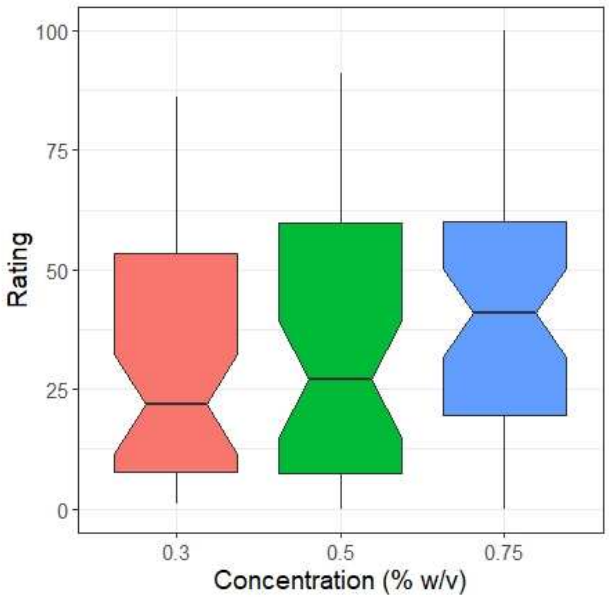


Figure 4-18 Human taste assessment of increasing concentrations of polysorbate 80

The reduced aversiveness of Polysorbate 80 relative to the chemically related Polysorbate 20 can be explained by the change in taste sensation observed. While, it was noted in 4.4.2.3 that Polysorbate 20 was perceived as bitter by a significant majority of the participants, Polysorbate 80 elicited much broader taste sensations among the participants Figure 4-19. There was no significant increase in the bitter sensation among the participants as the concentration increased, which may explain the lack of significant difference in aversiveness of the solutions with concentration. Other changes in taste sensations were, however, observed as the concentration changed. Indeed, the sensation of umami reduced from 0.3 to 0.5 % w/v Polysorbate 80, but increased again from 0.5 to 0.75 % w/v. While, the sensation of sourness increased from 0.3 to 0.5 % w/v and reduced again at 0.75 % w/v Figure 4-19.

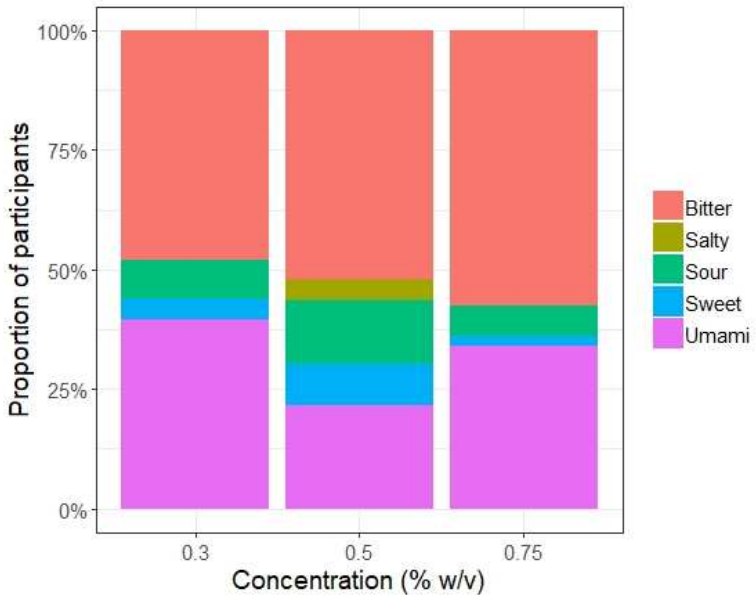


Figure 4-19 Taste sensations described by the participants as a function of increasing polysorbate 80 concentration

4.4.2.5 Polyethylene glycol (PEG) 400

A corresponding increase in aversiveness was observed with increasing concentration of PEG 400 with mean VAS ratings of 25.54 (+/- 3.51), 31.30 (+/- 3.82) and 56.35 (+/- 3.92) for 0.5, 1 and 3 % w/v PEG 400, respectively. Concentration was found to be a significant determinant of VAS rating ($p = 3.44e^{-7}$), but not all concentrations were found to be rated significantly differently from each other (Figure 4-20). Gao's posthoc analysis revealed significant differences between 0.5 and 3, and 1 and 3 % w/v PEG 400 ($p < 0.05$), but no significance was found between 0.5 and 1 % w/v PEG 400 ($p = 0.2111$). The EC50 was calculated to be 2.84 % w/v (1.89-3.79 % w/v).

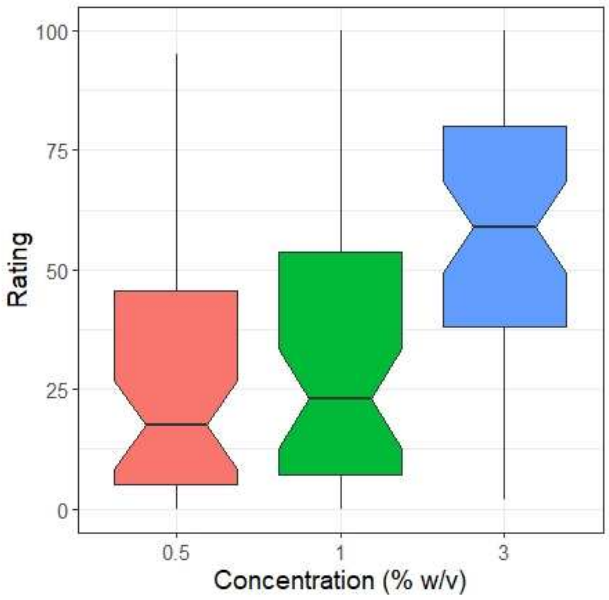


Figure 4-20 Human taste assessment of increasing concentrations of PEG 400

The corresponding increase in aversiveness with increasing PEG 400 concentration identified in Figure 4-20 can be explained by a change in taste sensation as the concentration is increased. Figure 4-21 demonstrates that the participants described the taste sensation of the highest concentration of PEG 400 as significantly more bitter than both 0.5 and 1 % w/v PEG 400. Furthermore, there is a complete disappearance of sweet sensation as the concentration is increased. A decline in umami sensation was also observed with increasing concentration.

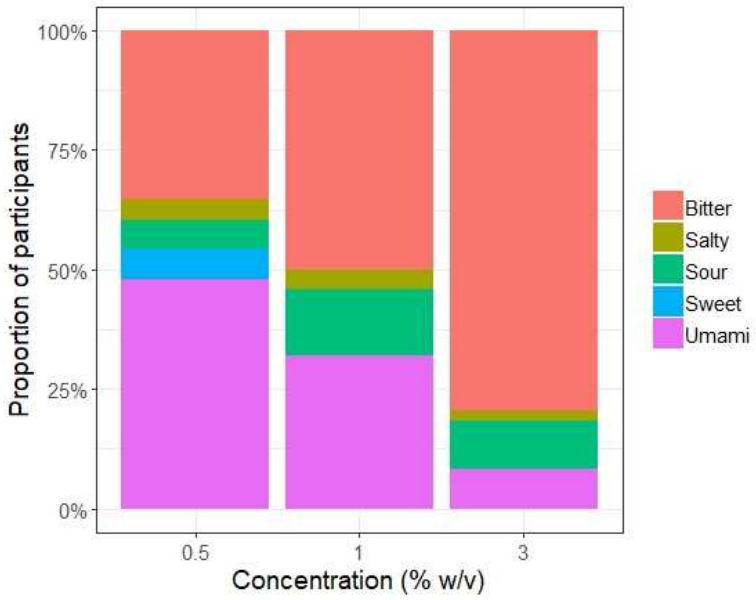


Figure 4-21 Taste sensations described by the participants as a function of increasing PEG 400 concentration

4.4.2.6 Propylene glycol

The human response to solutions of varying propylene glycol concentrations was varied with participants only capable of distinguishing between the extremes of the concentrations assessed as depicted in Figure 4-22. Participants rated 0.5 and 1 % w/v propylene glycol at 18.11 (+/- 2.87) and 18.90 (+/- 3.12) on the VAS scale, respectively: a very small difference despite a twofold increase in concentration, which was of course statistically insignificant ($p = 0.84$). However, participants were able to distinguish between both 0.5 and 1 % w/v and the uppermost concentration assessed: 3 % w/v ($p < 0.05$). However, with a VAS score of 35.23 (+/- 3.85), 3 % w/v propylene glycol was not rated as particularly aversive. The EC50 was calculated to be 5.90 % w/v (3.92-7.88 % w/v).

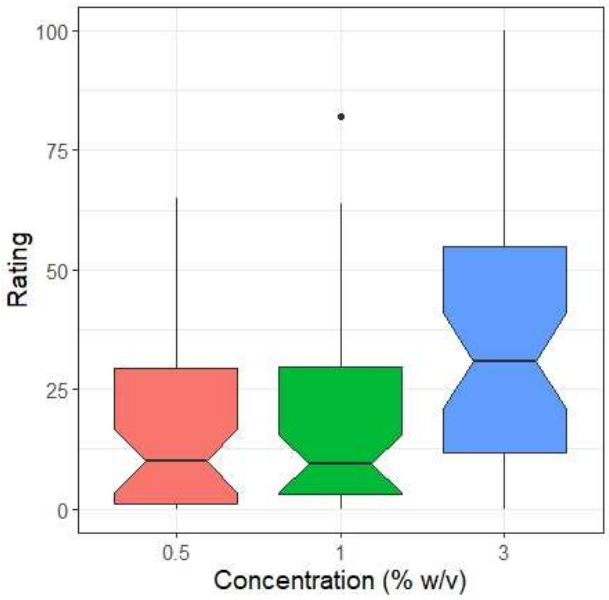


Figure 4-22 Human taste assessment of increasing concentrations of propylene glycol

The lack of aversiveness identified in solutions of propylene glycol can be attributed to a lack of bitterness and a predominance of sweet and umami taste sensations reported by the majority of the participants (Figure 4-23). A significant increase in bitter responses was however observed at 3 % w/v relative to both 0.5 and 1 % w/v, which may explain why the 3 % w/v was found to be significantly more aversive than the former concentrations (Figure 4-23).

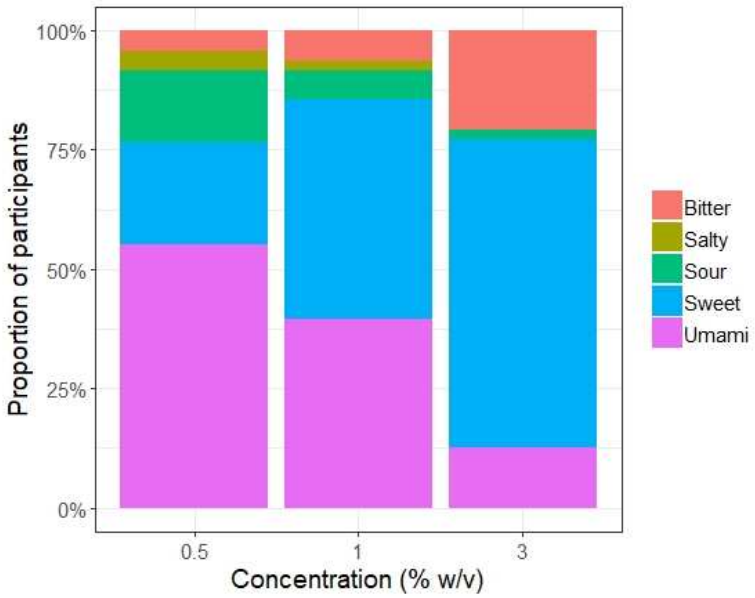


Figure 4-23 Taste sensations described by the participants as a function of increasing propylene glycol concentration

4.4.3 Human Vs. Rat Correlation

Comparison between human and rat response to the co-solvents was made difficult by the lack of aversiveness seen in certain co-solvents in rats. Thus, an IC_{50} value could only be calculated for ethanol, glycerol and propylene glycol; while PEG 400, polysorbate 20 and polysorbate 80 were not sufficiently more aversive than water, showing only mild lick inhibition, therefore the IC_{50} could not be calculated. The taste thresholds and corresponding error are summarised in Table 4-5.

Table 4-5 Human and rat taste thresholds for the assessed co-solvents showing the IC50 and EC50 and their respective 95% confidence intervals

Co-solvent	IC_{50} (95 % CI)	EC_{50} (95 % CI)
Ethanol	8.47 (5.75-11.19)	7.00 (4.79-9.22))
Glycerol	9.94 (6.09-13.77))	11.30 (7.40-15.20))
PEG 400	-	2.84 (1.89-3.79))
Polysorbate 20	-	0.35 (0.22-0.48))
Polysorbate 80	-	1.07 (0.74-1.40))
Propylene glycol	12.50 (3.19-21.81))	5.90 (3.92-7.88)

Figure 4-24 visually represents the correlation between human and rat taste thresholds for the co-solvents assessed. It can be seen that there is a good agreement between rats and humans for both ethanol and glycerol, but not for propylene glycol. However, the large variability observed in the rats for propylene glycol may have skewed the data making comparison difficult. It was not possible to calculate an IC_{50} for PEG 400, polysorbate 20 or polysorbate 80 given the mild lick inhibition observed in rats relative to water, thus a human-rat correlation could not be assessed for these co-solvents.

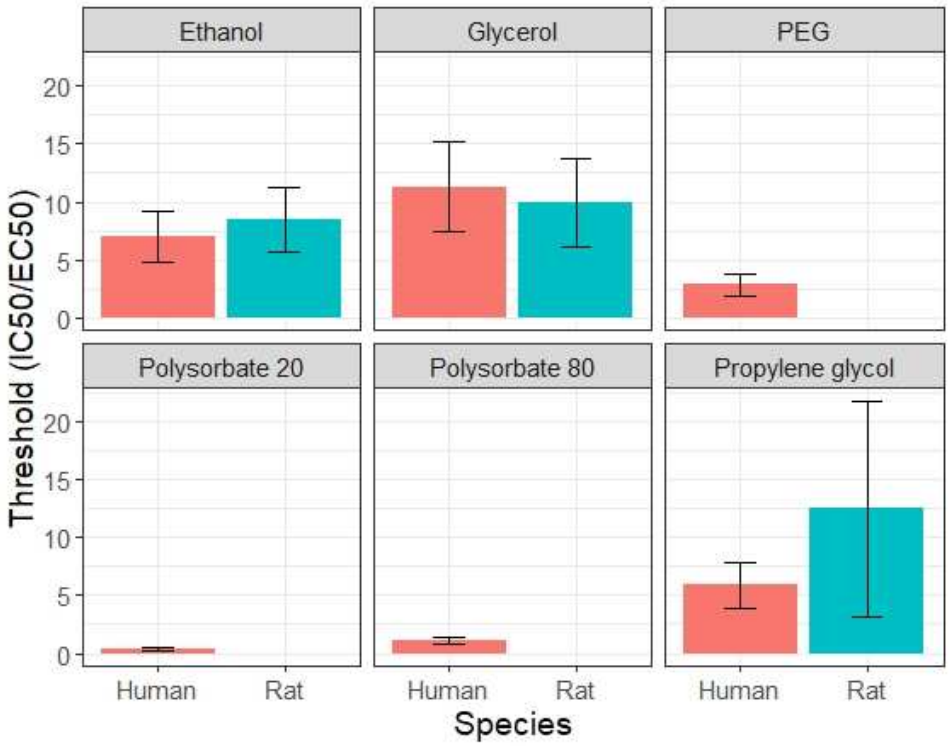


Figure 4-24 The correlation between human and rat taste thresholds for the assessed co-solvents

4.5 Discussion

As outlined above, solubility and bitterness/aversiveness may not be so inextricably linked given that even near insoluble compounds may elicit a bitter taste, e.g. isoxantholupon [179]. Thus, it is critical that all compounds, regardless of solubility are characterised for aversive taste. However, currently BATA assessment is restricted to only those APIs with sufficient solubility in water to enable a full concentration range to be assessed and an IC_{50} calculated. Thus, to overcome this problem and enable the assessment of a full concentration range of all APIs including those with low water solubility, a toolkit of co-solvents was sought that will serve to expand the APIs assessable in the BATA to those that are poorly soluble. Critically, however, such co-solvents must not themselves elicit an aversive taste.

BATA studies revealed that all co-solvents assessed, with the exception of polysorbate 20 were well tolerated by the rats, and at some concentrations were indistinguishable from water. Indeed, Table 4-6 summarises the findings, showing the toolkit now available to the sensory researcher for enhancing the solubility of poorly soluble APIs for BATA assessment, showing each co-solvent and its corresponding maximum concentration at which the rats were unable to distinguish it from water.

Table 4-6 Summary of co-solvent toolkit for enhancing the solubility of poorly soluble APIs for assessment using the BATA model

Co-solvent	Maximum concentration indistinguishable from water ($p > 0.05$) (% w/v)
Ethanol	3
Glycerol	1
PEG 400	1
Polysorbate 20	-
Polysorbate 80	> 0.5
Propylene glycol	1

There has been very limited investigation into the rat taste perception of the co-solvents discussed in this chapter in the literature. Indeed, no relevant studies were identified for glycerol, the polysorbates or PEG 400, although ethylene glycol was investigated in one study [180]. Sako et al. investigated the electrophysiological and behavioural responses of

rats to various alcohols; of note for this discussion was the study of ethylene glycol, propylene glycol and ethanol [180]. From the chorda tympani and glossopharyngeal bundle responses, it was concluded that alcohols have a taste similar to that of sucrose and quinine in rats, however the alcohols were each assessed at only a single concentration: 1M, corresponding to 4.61 % w/v. Sako et al. also found that alcohols with two or three hydroxyl groups elicited larger responses than other alcohols in both nerves [180]. Thus, this study suggests a possible aversion to the alcohols investigated here by rats, but the findings are inconclusive in terms of their proposed use here.

In another study, Loney et al. assessed the taste of a full concentration range of ethanol using the BATA model and correlated the response to quinine dihydrochloride, finding that response to ethanol and quinine dihydrochloride were correlated [181]. It was found that a significant reduction in lick number was observed at 5 % ethanol, compared to 10 % ethanol in this study. However, this may be in agreement to the findings presented in this chapter given that the penultimate concentration assessed was 3 % ethanol, thus it can be concluded that the water-indistinguishable limit lies between 3 and 10 % ethanol. The threshold value calculated by Loney et al. was approximately 17 % ethanol, thus in disagreement to the value presented in this chapter: 8.47 % (5.75-11.19 %), although it must be noted than Loney et al. used Long-Evans rats, while Sprague-Dawley rats were used here. Interestingly, Loney et al. found that when conditioned rats were used, a significant reduction in lick number was not observed until 20 % ethanol, thus suggesting the possibility of conditioning rats to co-solvents to increase the tolerable concentrations and thus possibly expand the model even further to ever more poorly soluble APIs, however caution must be advised so as to ensure the rats' bitter perception is not affected [181].

Kiefer et al., however presents findings that may warrant caution when using the ethanol as a dissolution enhancer in a taste experiment [182]. In this study, rats were infused intraorally with 3, 6, 9 and 12 % ethanol with their subsequent oral, facial and bodily responses assessed. While, ingestive responses were not affected by the concentrations assessed, aversive responses were noted and were greatest at 12 % ethanol [182]. Thus, while the BATA model demonstrates that no significant number of licks is observed at 3

% ethanol relative to water, Kiefer et al. suggests that the rat may still be aversive, thus questioning the use of ethanol as a co-solvent.

The human-rat correlation was also assessed in this study. However, the lack of aversiveness identified in the rat BATA model made correlating rat and human taste perception of the studied co-solvents difficult. Indeed, polysorbate 20, polysorbate 80 and PEG 400 showed only mild lick inhibition, thus it was not possible to calculate an IC_{50}. While there was good agreement between human and rat thresholds for ethanol and glycerol, there was very poor agreement observed for propylene glycol. Extensive variability was observed in the rats for the taste assessment of this co-solvent and as such there was great uncertainty in the IC_{50} as indicated by the 95 % CI: 3.19-21.81. Humans ranked the co-solvents in terms of increasing aversiveness as glycerol < ethanol < propylene glycol < PEG 400 < polysorbate 80 < polysorbate 20, while the rats ranked propylene glycol < glycerol < ethanol, with determination of where the polysorbates and PEG 400 lie difficult due to a lack of aversiveness at the concentrations assessed. Therefore, the only agreement observed between rats and humans was that ethanol was more aversive than glycerol. The lack of human-rat correlation may be due to the taste modalities elicited by the co-solvents. Ethanol and glycerol were evaluated by humans as tasting bitter and sweet, respectively. These are very much polar taste sensations – bad and good – which the rat can feasibly make a distinction between. However, the remaining co-solvents elicit much more complex tastes which included umami: polysorbate 80 and PEG 400 were described in the human taste panel as bitter/umami, while propylene glycol was described as sweet/umami. Indeed, it is likely that the umami nature of polysorbate 80, PEG 400 and propylene glycol resulted in preference, rather than aversion in the rats. Indeed, Sprague-Dawley rats are known to demonstrate preference of umami solutions over water [183]. In a study by Miura *et al.*, Sprague-Dawley rats were shown, in two-bottle preference tests, to significantly prefer 0.001 M 5′ -inosine monophosphate (IMP), 0.01 M monosodium glutamate (MSG), and binary mixtures of 0.001M IMP + 0.01 M MSG than deionized water [183]. This finding adds great complexity to the conclusions of this chapter given that co-solvents which do not themselves elicit a taste were sought to enable assessment of poorly soluble APIs in the BATA model. It was proposed that if the rat could not distinguish the co-solvent from

water, it must be neutral in taste, however this may not be the case; the co-solvent may be eliciting an umami taste, which the rat is actually showing preference for. This requires extensive investigation before the umami tasting-co-solvents may be included in the toolkit of BATA co-solvents.

It must also be noted that the concentrations listed in Table 4-6 are small questioning the usefulness of co-solvents at these concentrations as solubilisers. It is therefore necessary to assess more co-solvents in both rats and humans. However, the co-solvents used in human medicines for enhancing solubility are limited due to toxicity and regulatory limitations, thus the list of other co-solvents that can be used/assessed is itself limited. Therefore, a different approach may be required for the assessment of poorly water soluble APIs, such as a quinine hydrochloride equivalence, in which a single saturated solution of a poorly soluble API may be assessed in the BATA model and compared to the rat BATA response to a full concentration range of quinine hydrochloride, thus providing a concentration of quinine hydrochloride to which the bitterness of the assessed API is equivalent.

The discussed complexity of medicine aversion necessitates taste assessment and characterisation of APIs under a worst-case, highest exposure scenario, which the use of co-solvents may help to achieve. This chapter has provided a starting point for the development of a toolkit of co-solvents and corresponding concentrations, which can be used to expand the APIs assessable in the BATA model to those that are poorly soluble and thus may aid in assessing a greater range of APIs.

4.6 Conclusion

A key limitation of the BATA model was identified, that of solubility. Indeed, the BATA model is only capable of assessing APIs with some level of solubility. While it could be argued that solubility and taste are inextricably linked, with a lack of the former resulting in an absence of the latter, it is far more complicated than this with seemingly water insoluble compounds able to elicit a bitter taste [179]. Thus, the APIs assessable in the BATA model must be expanded to those that are poorly soluble. Co-solvents were proposed as a means to bridge this gap, as they are commonly used in pharmaceutical formulations to solubilise poorly soluble APIs. However, co-solvents could not be used in this capacity

if they themselves elicit an aversive taste, thus the concentrations below which they are indistinguishable from water by the rats were sought and correlated to human taste. Of the co-solvents assessed; ethanol, glycerol, PEG 400, polysorbate 80 and propylene glycol were found to be indistinguishable from water at a range of concentrations. However, the question of umami preference by Sprague-Dawley rats was also raised in this chapter thus questioning the use of co-solvents that may elicit a preferable taste. However, the foundations have been laid for a toolkit for the sensory scientist to expand the APIs assessable using the BATA model to those limited by water solubility.

5 Understanding the interplay between mouthfeel and taste in the BATA model: the combined effect of viscosity, grittiness and bitterness

5.1 Introduction

5.1.1 Mouthfeel and acceptability

This thesis has so far focused on taste as a barrier to acceptability, however there is more to acceptability than just taste, particularly where the paediatric population are concerned. In a study by Venables *et al.* 252 children (0-4 years, n = 92; 5-11 years, n = 93; 12-18 year, n = 67) or their parents/carers were interviewed on the formulation factors that affect oral medicines acceptability [184]. Taste was found to be the most commonly reported barrier to medicines administration affecting 35 % of all prescribed oral formulations, and associated with 64 % of formulations that were refused [184]. However, texture was also identified as a significant predictor of medicines refusal and was reported to have affected 8 % of all prescribed oral formulations. Volume or quantity, size and aversion to or difficulty with swallowing, and colour/appearance and smell were also found to be potential barriers to medicines acceptability, affecting 5 %, 5 % and 2 % of medicines prescribed, respectively [184]. This chapter will focus on mouthfeel as a barrier to acceptability.

As we saw in chapter 1, several platform technologies have been developed in order to circumvent acceptability issues for paediatric patients. However, neither of those discussed are a panacea, and frequently create an alternative acceptability issue in their attempt to solve another acceptability issue. For example multiparticulates, which can be easily taste-masked by polymer film coating may solve the problems of taste and dose flexibility, but create an issue with mouthfeel. Several studies have highlighted mouthfeel or grittiness of suspended multiparticulates to be problematic in terms of acceptability [45,185–187].

However, it is more complicated than just grittiness. More broadly, texture has been highlighted as a key area for improvement in the acceptability of medicines [188]. Allué *et al* assessed the acceptability of oral rehydration solutions, finding significant differences

with children preferring a 'gel' texture over a 'gelatine' texture [189]. Antiretroviral therapies (different dual nucleoside reverse transcriptase inhibitor therapy combinations with and without the protease inhibitor nelfinavir) have also been identified as suffering with issues of mouthfeel, although the specific causative parameter has not been investigated [29]. More generally, another study by Venables *et al.* assessing the barriers to medicines administration in children found texture to be the second-most important factor for refusal of medicines, with taste being the most important factor [190].

The rheology of the administered dosage form has also been shown to be of significant importance to patient acceptability, with the rheology of the vehicle affecting the palatability. It has been reported that as the consistency of a medicine increases, issues with organoleptic properties and thus acceptability arise [191,192].

5.1.2 Exploring the physiology of mouthfeel

Several definitions of mouthfeel or texture abound the literature, however most researchers now subscribe to that of Szczesniak [193,194]. Indeed, it is:

1. A sensory property of food that a human being can recognize and describe. Only certain properties of texture can be measured by physical techniques, and the results of such measurements require a sensory interpretation.
2. A multimodal property that depends on the food structure on several length scales, from the molecular to the macroscopic level.
3. A property that is detected by several senses, of which touch and pressure are the more important.

Looking more specifically at medicines, Batchelor defined mouthfeel as 'the sensation from the ingestion, mastication and swallowing of the medicine, all of which are influenced by the physical and chemical properties of the medicine being administered' [188]. The sensations perceived may range from hardness or softness to grittiness, creaminess or adhesiveness, to name but a few [188].

The sensation of mouthfeel is facilitated by sensory nerve endings that lie beneath the epithelium [195]. The human mouth as a sensory organ is a highly capable one, and may thus explain why mouthfeel of medicines can be so problematic in terms of acceptability.

Indeed, the upper surface of the tip of the tongue – thought to be the site at which the finest two-point discrimination is observed – can discriminate down to 1 mm [196]. The upper surface of the tongue consist small cone-shaped filliform papillae, which only have a mechanoreceptive function [197]. The aforementioned biology enables incredible tactile perception, with particles as small as 5 µm detectable [198]. However, it has been found that the shape and hardness of a given particle affects the size at which particles are perceived to be gritty, with soft and round particles detectable above 80 µm but with hard and irregular particles detectable below 22 µm [199]. Lubrication and friction also influence this sensory perception [200,201].

5.1.3 The need for mouthfeel testing

Therefore, given the highly sensitive nature of human oral mechanoreception, and the proven effect mouthfeel has on the acceptability of medicines, it is imperative that mouthfeel is assessed during formulation development, particularly in the wake of new platform technologies such as multiparticulates. In a search of the literature inspecting how mouthfeel is assessed, all studies utilised human taste panels with the exception of Batchelor *et al.* who utilised tribology to assess texture perception of oral liquid medicines [188]. During early drug development, such human taste panels cannot be used given the lack of safety data, thus necessitating the development and use of alternative methodologies. However, if point one of Szczesniak's definition of mouthfeel is recalled, physical techniques can only measure certain properties of mouthfeel, and *require sensory interpretation*, thus complicating measurement of mouthfeel *in vitro*. Rats, however, may provide the answer.

5.1.4 Rats as mouthfeel assessors

Maier *et al.* demonstrated that rats are capable of sensing mouthfeel, as well as odour and taste. By recording neural activity directly from the rat brain in response to multisensory flavour stimuli, it was found that information regarding mouthfeel, taste and odour converge on the primary taste and smell cortex [202]. Unfortunately, the study did not demonstrate exactly how mouthfeel was assessed in the rats. However, this is the only study of its kind to identify a mouthfeel aspect to the rat's organoleptic world and provides promising evidence that rats may be able to distinguish between samples of

varying mouthfeel and thus provide an insight into acceptability in terms of grittiness and mouthfeel, as well as taste. Thus, this study will assess rat responses to both taste and mouthfeel by administering suspensions of varying bitterness as well as viscosity and grittiness using the BATA model. Furthermore, if it is found that rats are capable of providing information on mouthfeel, in line with the principles of 3Rs as highlighted in the introduction, the data from the BATA model will be leveraged through the exploration of a predictive *in silico* model of rat response to samples of varying viscosity, grittiness and bitterness to aid formulation development with minimal animal use.

5.2 Aims

Mouthfeel is a critical formulation attribute to medicine acceptability, thus a methodology with which mouthfeel may be assessed without humans is sought, such that assessment may occur during early drug development. Rats have been shown in limited studies to respond differently to samples of varying mouthfeel Thus, the rat BATA model is proposed as a methodology by which mouthfeel, as well as taste, may be assessed.

5.3 Objectives

1. Assess rat response in the BATA model to samples of varying bitterness and mouthfeel, as governed by grittiness and viscosity.
2. Leverage the BATA data by modelling the complex interaction between taste and mouthfeel *in silico*.

5.4 Materials and methods

5.4.1 Materials

Quinine hydrochloride, Avicel PH 101 and Avicel PH 200 was provided by Sigma-Aldrich (Dorset, UK). Xanthan Gum was supplied by CP Kelco (Leatherhead, Surrey, UK).

5.4.2 Methods

To ascertain the interplay between taste and mouthfeel, a 3-level factorial design of experiment (DOE) was conducted to assess how viscosity, bitterness and grittiness of a formulation are perceived in the rodent BATA model, and to identify which of these dosage form characteristics has the greatest effect on acceptability.

Varying levels of viscosity, bitterness and grittiness were explored by combining three levels of xanthan gum, quinine hydrochloride (QHCl) and microcrystalline cellulose size (MCC) respectively, such that a total of 27 solutions/suspensions were presented to the rats as per Table 5-1. The particle sizes shown Table 5-1 are the median particle size (D50), however the D10 and D90 for Avicel PH 101 are 21.9 µm and 127.0 µm, respectively, and those for Avicel PH 200 are 86.3 µm and 369.0 µm, respectively (unpublished data).

Table 5-1 Levels of viscosity, grittiness and bitterness explored by varying [xanthan gum], MCC grade and [quinine hydrochloride], respectively.

	[Xanthan gum] (%w/v) (Viscosity)	Microcrystalline cellulose grade (Median particle size) (Grittiness)	[Quinine hydrochloride] (% w/v) (Bitterness)
Levels	0	-	0
	0.075	Avicel PH 101 60 µm	0.0028
	0.15	Avicel PH 200 205 µm	0.0361

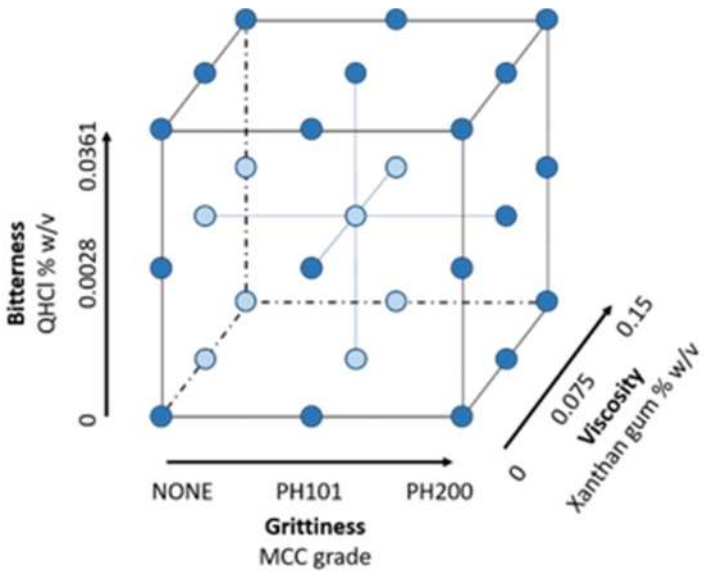


Figure 5-1 Experimental design space

The parameters specified in Table 5-1 and visualized in Figure 5-1 were chosen based on previous work. Xanthan gum was used as it is tasteless and widely used in pharmaceutical formulations. Lopez, F identified maximum levels of xanthan gum (unpublished work) and MCC size that can pass through the sipper tubes used in the BATA model unhindered. The QHCl concentrations were chosen based on the IC_{50}, with 0.0028 % w/v corresponding to this value, and 0.0361 % w/v chosen to provide an extreme level of bitterness relative to the IC_{50}.

For a detailed description of the rodent BATA model, please refer to section 3.1.1.1**.** The BATA model was however adjusted to allow for the assessment of suspensions, which require inversion prior to presentation to the rats. This was facilitated by fitting the lickometer with a platform and handle which enabled the inversion of the entire rig prior to presentation to the rats (Figure 5-2). Sample inversion occurred before each presentation, except water rinse.

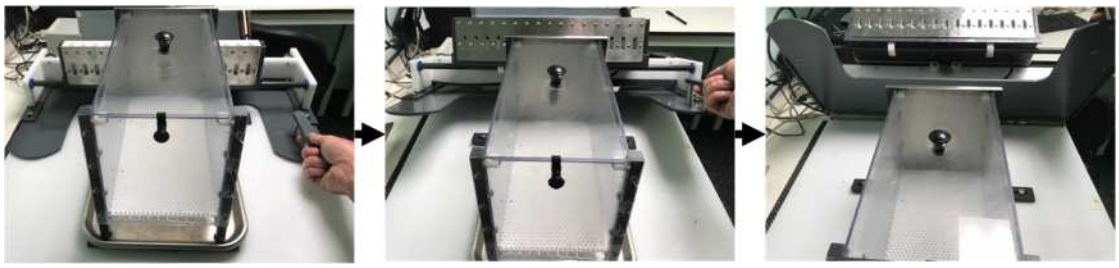


Figure 5-2 The modified BATA apparatus showing the platform and handle which enables the inversion of the entire rig prior to presentation to the rats.

The experimental setup and factorial regression was performed using Minitab® 17 Statistical Software (Minitab, Inc., State College, PA, version 17.1.0). Data were presented as notched boxplots and Pareto charts to demonstrate combined and individual effects of the aforementioned factors on the response: lick numbers. The elements of notched botch box plots have been described in previous chapters. A Pareto chart shows the contribution that altering a dependent variable (viscosity, grittiness or bitterness) by 1 has on the output variable, as indicated by 'standard effect' on the y axis. All graphs were produced using R statistical software (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.).

In addition, the data were compiled and utilised to build a prediction model in R, which predicts the lick number of a given sample based on the bitterness relative to QHCl, particle size and viscosity. Given that BATA model data are not normally distributed, may exhibit over-dispersion and consist an excess number of zeros, classical Poisson regression models did not suffice. Therefore, a variety of other models that account for such issues were explored using R statistical software, for example quasi-Poisson model, which has an additional dispersion parameter or the negative-binomial (NB) regression model. The data was modelled with three generalised linear models (GLM): Poisson, quasi-Poisson and negative binomial using the stats and MASS packages in R; in addition to a fourth zero-augmented hurdle negative binomial model using the pscl package in R. All of the aforementioned packages are freely available in the Comprehensive R Archive Network (CRAN) [203]. The models will be compared by first assessing the Akaike information criterion (AIC) and/or the Bayesian information criterion (BIC). Both the AIC and BIC provide information on how well the model-predicted outcome fits the actual data. The AIC was developed by Akaike, who linked the Kullback-Leibler measure – a measure that captures how much information is lost when approximating reality – with maximum liklihood estimation method [204]. The AIC is used as a relative measure, thus it is meaningless in itself, but is used to compare models, with the lowest AIC indicative of the model that best predicts reality. The BIC is similar to the AIC, but it imposes a greater penalty for the number of parameters used to build the model. Two superior models will be selected based on the AIC/BIC, which will then be visualised by running more than 1000 simulations varying the bitterness, grittiness and viscosity and plotting the predicted and experimental values using R statistical programming and assessing which model to select for *in silico* prediction of rat response based on the aforementioned parameters going forward.

5.5 Results

The BATA experiments assessing bitterness, viscosity and grittiness using design of experiment principles were successfully conducted. The data will now be discussed making a detailed assessment of the effect of each independent variable – bitterness, viscosity and grittiness – on the rat response and the interaction among the independent variables, before finally discussing model development.

5.5.1 Assessing the interplay between taste and mouthfeel

A general pattern is observed in Figure 5-3 whereby increasing viscosity leads to an increase in lick number, that is to say increasing viscosity is enhancing the acceptability of the formulation. In Figure 5-3, water is consistent with 0% w/v QHCl and 0% w/v XG, thus naturally this achieves the greatest lick number shown. A reduction in lick number is observed when the XG concentration is increased to 0.075% w/v at 0% w/v QHCl. However, at 0.15% w/v XG and 0% w/v QHCl, an increase in lick number is observed, such that no significant difference ($p > 0.05$) is observed from water, as indicated by the overlapping notches in . At all other concentrations of QHCl, an increase in lick number was observed as a result of increasing the viscosity, with the 0.15 % w/v XG consistently achieving significantly ($p < 0.05$) higher lick number relative to 0% w/v XG for both 0.0028 and 0.0361 % w/v QHCl: see overlapping notches in Figure 5-3. This demonstrates that there is a reduction in bitterness sensitivity with increasing viscosity.

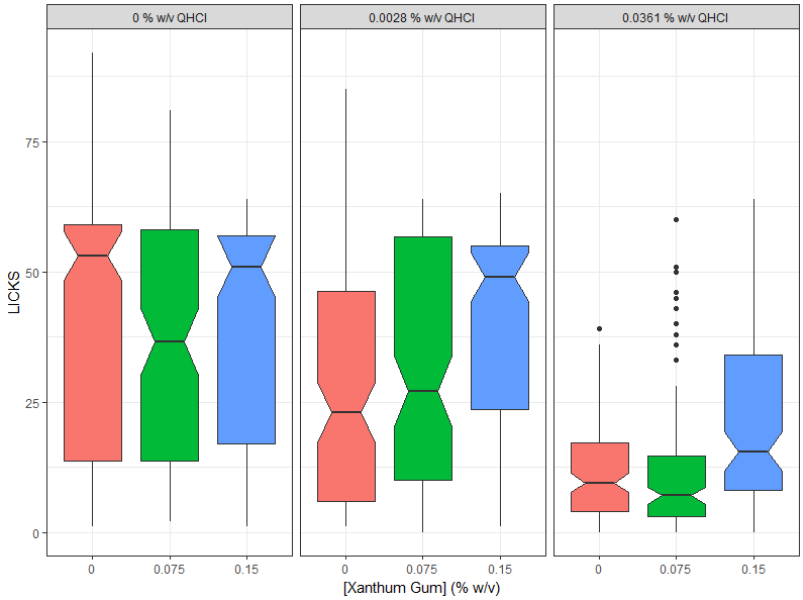


Figure 5-3 Lick number as a function of increasing xanthan gum concentration at increasing concentrations of QHCl

5.5.1.1 *Assessing aversiveness as a function of increasing grittiness at increasing levels of bitterness*

A pattern of reducing acceptability was noted as result of increasing the grittiness of the given formulations. This was observed at all levels of bitterness assessed – see Figure 5-4. A significant difference ($p < 0.05$) is observed between no MCC and Avicel PH200 at all levels of bitterness assessed, as can be gleaned from the distance between notches in Figure 5-4. No significant difference ($p > 0.05$) was observed between Avicel PH101 and Avicel PH200 at both 0.0028 and 0.0361 % w/v QHCl, identifying an inability of the rats to distinguish between different levels of grittiness in the presence of bitterness. However, in the absence of bitterness (0 % w/v QHCl), a significant difference ($p < 0.05$) was observed between the Avicel PH101 and Avicel PH200 – see Figure 5-4 – identifying the ability of the rats to distinguish between varying levels of grittiness in the absence of bitterness.

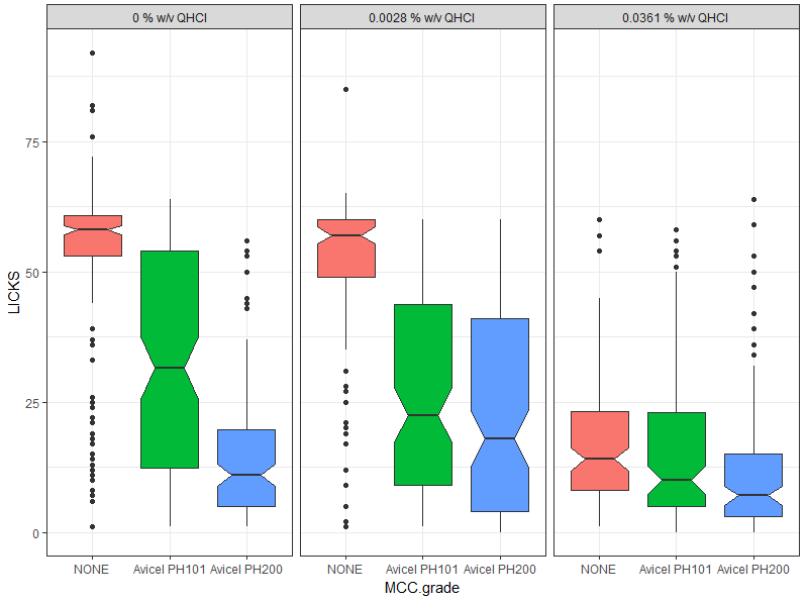


Figure 5-4 Lick number as a function of MCC grade at increasing concentrations of QHCl

5.5.1.2 *Assessing aversiveness as a function of increasing bitterness at increasing levels of viscosity*

Figure 5-5 demonstrates that as bitterness increases, lick number reduces as the formulation becomes more aversive. However, the taste masking effect of XG is also observed in Figure 5-5. The most significant differences between increasing concentrations of QHCl are observed at 0 % w/v XG. However, as the concentration of XG increases, the difference observed between increasing levels of QHCl reduces. Indeed, at 0.15 % w/v XG, no significant difference ($p > 0.05$) is observed between 0 and 0.0028 % w/v QHCl: see Figure 5-5. Moreover, comparison of mean lick number at the maximal QHCl concentrations at 0 and 0.15 % w/v XG, reveals significant differences ($p < 0.05$), further demonstrating the taste masking effect of XG.

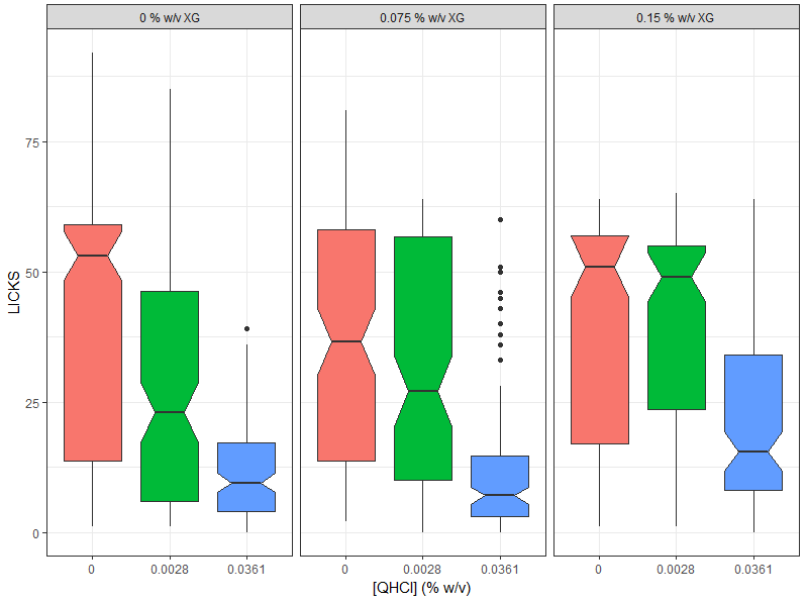


Figure 5-5 Lick number as a function of increasing QHCl concentration at increasing concentrations of XG

5.5.1.3 *Assessing aversiveness as a function of increasing bitterness at increasing levels of grittiness*

In direct contrast with viscosity, grittiness compounds the aversiveness of formulations of increasing bitterness. The highest mean lick numbers were observed in the absence of MCC for 0 and 0.0028 % w/v QHCL – see Figure 5-6. Significant reductions in mean lick number were observed when introducing a grittiness component to the formulations. No significant differences ($p > 0.05$) were observed between 0.0028 and 0.0361 % w/v QHCl at increasing grittiness levels from Avicel PH101 and Avicel PH200 (Figure 5-6), while significant differences ($p < 0.05$) were noted between formulations at 0 % w/v QHCl at said grittiness levels, indicating an inability of the rats to distinguish between bitterness and grittiness in combination.

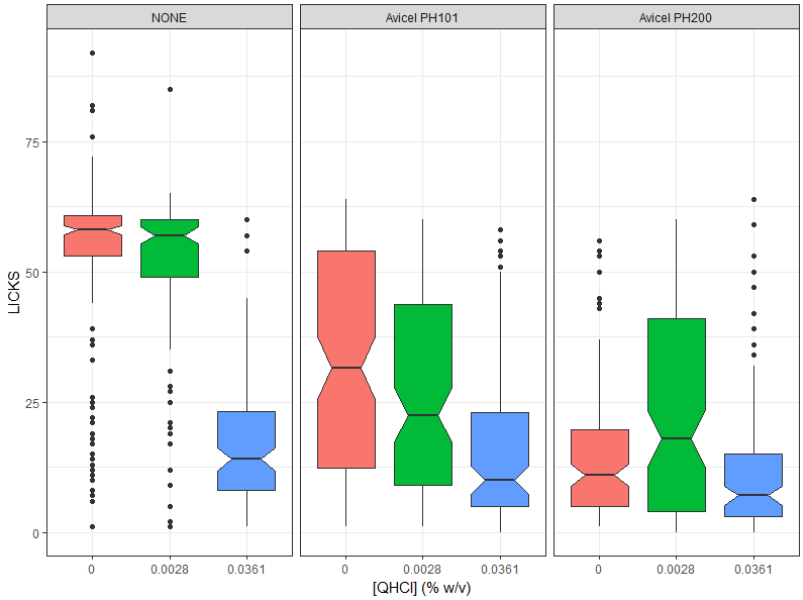


Figure 5-6 Lick number as a function of increasing QHCl concentrations at increasing particle sizes of MCC

5.5.1.4 *Assessing aversiveness as a function of increasing viscosity at increasing levels of grittiness*

No significant difference ($p > 0.05$) was observed between formulations of increasing viscosity in the absence of MCC: see overlapping notches in Figure 5-7. However, when a grittiness component is introduced in the form of increasing particle sizes of MCC, differences between increasing levels of viscosity are observed, identifying a grittiness masking effect of the XG. At the grittiness level imparted by Avicel PH101, significant differences ($p < 0.05$) were observed between all XG levels, with increases in mean lick number observed with increasing viscosity (Figure 5-7). The same is observed at Avicel PH200, however no significant difference ($p > 0.05$) is observed between 0 and 0.075 % w/v XG, but at 0.15 % w/v XG, a significant difference ($p < 0.05$) is observed as noted by the distance between notches in Figure 5-7. This observation demonstrates the need for a greater viscosity level in order to mask the grittiness of Avicel PH200, given the larger particle size.

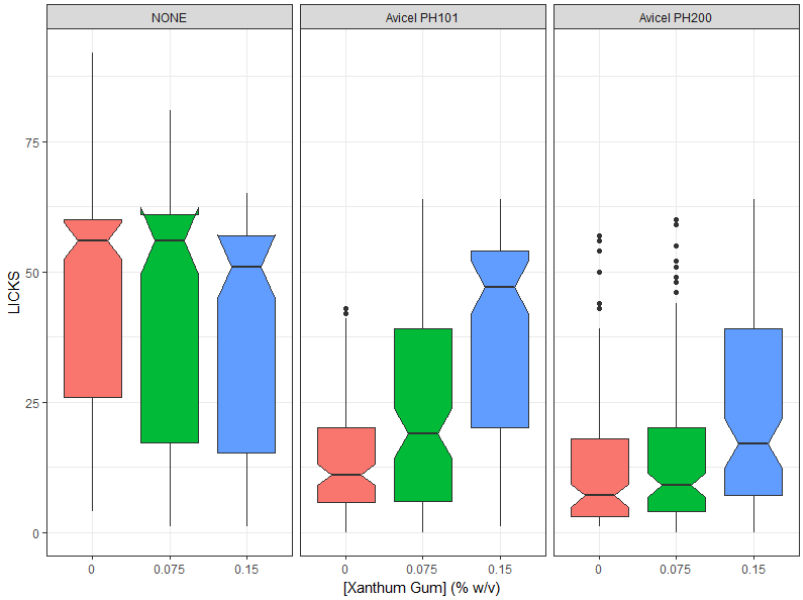


Figure 5-7 Lick number as a function of increasing xanthan gum concentration at increasing particles sizes of MCC

5.5.1.5 *Assessing aversiveness as a function of increasing grittiness at increasing levels of viscosity*

As grittiness increases, the aversiveness increases as indicated by a reduction in mean lick number in Figure 5-8. However, Figure 5-8 also provides further evidence of the ability of a viscosity-enhancing agent to mask the grittiness of a formulation. As the concentration of XG increases – thus viscosity increases – the difference observed between formulations of increasing grittiness (MCC particle size) reduces. Indeed, Figure 5-8 demonstrates that at 0.15 % w/v XG, the rats were unable to distinguish between an absence of MCC and Avicel PH101 ($p > 0.05$) – see overlapping notches – therefore the grittiness was masked. However, the grittiness achieved by Avicel PH200 was not masked by any concentrations of XG, as indicated by the significant differences ($p < 0.05$) in lick number shown in Figure 5-8: see distance between notches.

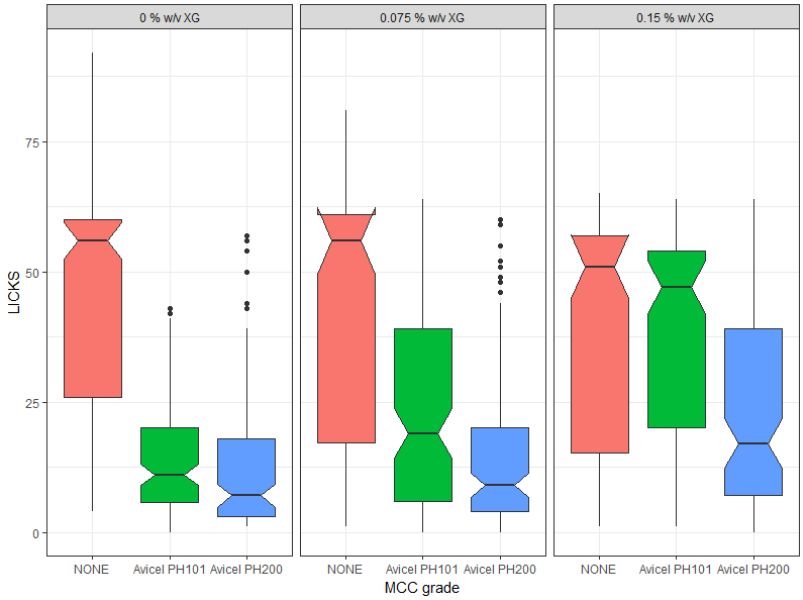


Figure 5-8 Lick number as a function of MCC particle size at increasing concentrations of xanthan gum

5.5.1.6 Ranking the effect of formulation characteristics on aversiveness

Utilising design of experiment principles, the impact of bitterness, grittiness and viscosity on reduction in lick number was assessed, with a view to ascertaining which formulation characteristic had the greatest impact on reducing lick number, thus which formulation characteristic had the greatest impact on aversiveness (Figure 5-9).

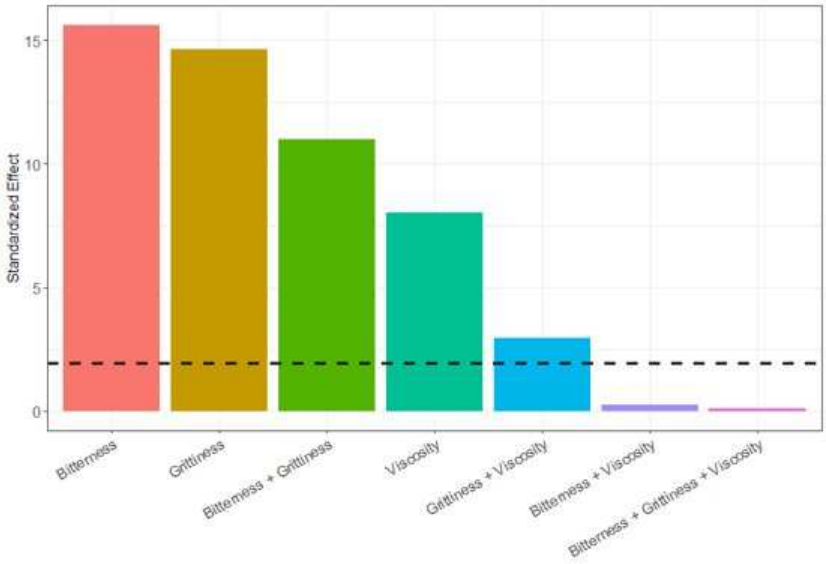


Figure 5-9 Pareto chart demonstrating the contribution of bitterness, grittiness and viscosity on reduction in lick number. The dashed line is indicative of the level above which a given variable has a significant effect

Figure 5-9 demonstrates that bitterness, grittiness and viscosity result in a reduction in lick number relative to water, given that all standardized effects observed were above the dashed line. As indicated by the increasing standardized effects in Figure 5-9, the ranking of contribution to reduction in lick number is bitterness > grittiness > viscosity.

5.5.2 Model development

5.5.2.1 Review of the dependent variable

In order to develop the model, it was first important to understand the data, its distribution and how the general relationship between the variables, which will aid in understanding the coefficients of the models to be developed. Here, the lick number is the dependent variable, and the quinine hydrochloride concentration (bitterness), microcrystalline cellulose particle size (grittiness) and the viscosity as dictated by xanthan gum concentration are the regressors.

Figure 5-10 demonstrates the distribution of the data, immediately demonstrating how difficult it will be to model, given that it is non-normally distributed, with the majority of the counts being at the extremes of the scale, and subject to extensive variation.

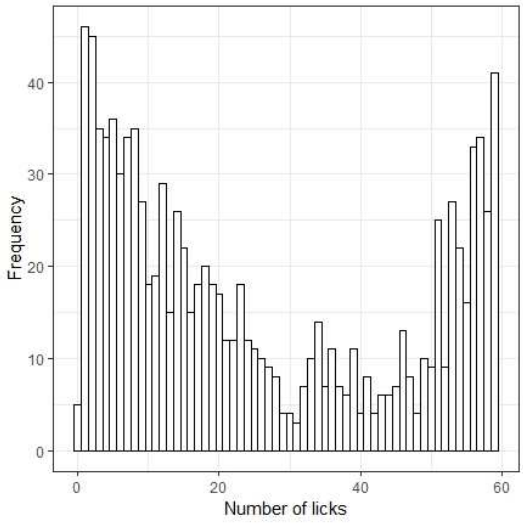


Figure 5-10 The distribution of the rat BATA data

5.5.2.2 Pairwise bivariate analysis

The partial relationships between the lick number dependent variable and its regressors was simply evaluated by assessing producing notched boxplots to demonstrate the bivariate relationship, thus informing the general trend that must be captured by the model.

Figure 5-11 reveals the basic relationship between bitterness and the dependent variable: lick number. It can be seen that as the bitterness increases, as governed by the quinine hydrochloride concentration, there is a significant reduction ($p < 0.05$) in lick number shown by the lack of overlap of the notches. Thus, in the models to be developed, the coefficients must be negative for the bitterness variable.

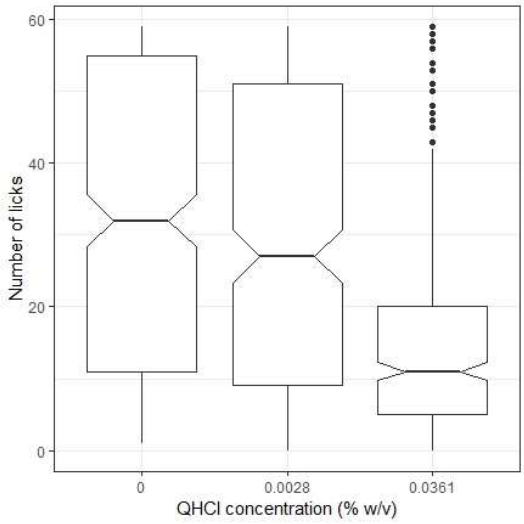


Figure 5-11 Bivariate relationship between lick number and bitterness

Viscosity demonstrates a generally positive relationship with lick number when all the data are considered as a whole. Figure 5-12 demonstrates that as the viscosity increases, the lick number positively correlates, although a significant difference ($p < 0.05$) is only noted at 0.36 PaS. The importance of this to model development being that the coefficients must be positive for the viscosity regressor.

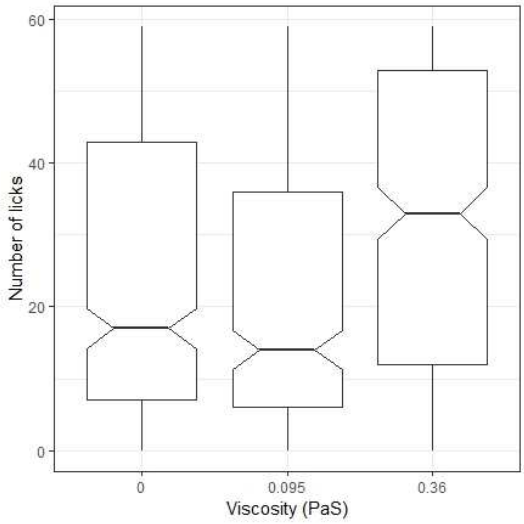


Figure 5-12 Bivariate relationship between lick number and viscosity

Lastly, Figure 5-13 shows the bivariate relationship between grittiness and lick number, in which a significant reduction ($p < 0.05$) in the number of licks is observed as the grittiness is increased as governed by the particle size of microcrystalline cellulose. Thus, the coefficients in the developed models must be negative for the grittiness regressor.

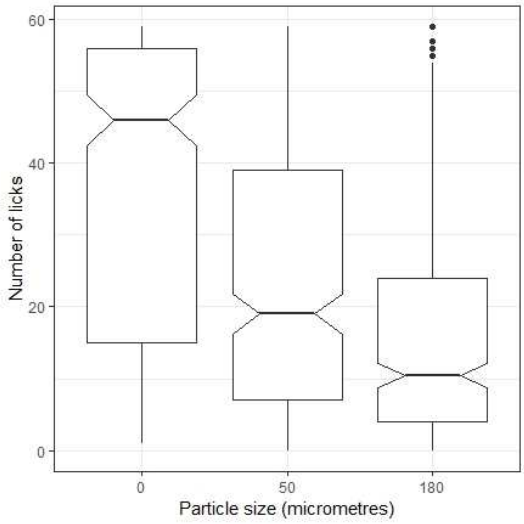


Figure 5-13 Bivariate relationship between lick number and grittiness

5.5.2.3 Fitting the models to the data

5.5.2.3.1 Poisson regression

A Poisson regression model was initially chosen to describe the relationships within the data. As per Table 5-2, it can be seen that this model does adhere to what was found in the exploratory analysis of the data, i.e. the coefficient estimates are of the correct *signum:* quinine hydrochloride and particle size are negative, while viscosity is positive. However, the estimated number of zeros predicted by the model was 0.04, which is not in concordance with the actual data, which consist five zeros. Furthermore, both the AIC and BIC were very large, 19008 and 19028.69, respectively. As described in the methods section, both the AIC and BIC are not absolute values, so their meaning can only be gained through comparison with other models, which will be explored as the remaining models are described.

5.5.2.3.2 Quasi-Poisson regression

Secondly, a quasi-Poisson model was fitted to the data. As per Table 5-2, the coefficient estimates are identical to the Poisson model, and thus the estimates are in line with the exploratory analysis of the data. However, the standard errors are smaller demonstrating the superiority of this model. Neither the AIC nor the BIC could be computed for this model, thus the aforementioned serves as the sole comparison. Of note, however, is that

the quasi-Poisson model incorporates an estimated dispersion parameter, which was found to be 11.36, compared to 1 within the Poisson model, thus demonstrating the presence of over-dispersion in this model and explaining the superiority and smaller error found within this model.

5.5.2.3.3 Negative-binomial regression

The third model to be explored was a negative-binomial regression model, which is a more formal way of accommodating over-dispersion within the data. Again, it can be seen from Table 5-2 that the coefficient estimates are similar to the former two models explored and with similar standard errors to the quasi-Poisson model. However, the AIC and BIC, 9859.915 and 9885.239 respectively, are far superior to the Poisson model, with values for the AIC and BIC as 19008 and 19028.69, respectively. The lower these information criteria, the better the model fits to the data, thus confirming superiority. Furthermore, the estimated number of zeros within the model was 14.98, which is beneficial given that BATA data does frequently consist a large number of zeros, but noting that the actual data had five zeros, it can be concluded that this is an excessive estimate.

5.5.2.3.4 Zero-augmented hurdle negative binomial regression

Finally, a zero-augmented hurdle negative-binomial was explored, which differs from the preceding models by incorporating an additional hurdle component modelling zero vs count, hence the two sets of coefficient estimates shown in Table 5-2. The coefficient estimates are similar to the other models and the AIC is comparable to that of the negative binomial regression, demonstrating a similar fitting of the respective models to the data. However, the estimated number of zeros in this model was five, which exactly matches that which was observed in the actual data, representing a possible superiority over the negative-binomial regression.

Table 5-2 Summary of model findings

Type	**Generalised linear model**			**Zero-augmented**
Distribution method	**Poisson**	**Quasi-Poisson**	**Negative binomial**	**Hurdle-negative binomial**
[Quinine hydrochloride] (% w/v)	-2.414e-1 (4.27e-1)	2.414e-1 (1.436e+0)	-2.267e-1 (1.428e+0)	-2.286e+1 (1.506e+0)
Particle size (µm)	-5.302e-3 (8.63e-5)	-5.302e-3 (2.903e-4)	-4.815e-3 (3.019e-4)	-4.871e-3 (3.104e-4)
Viscosity (Pa S)	9.016e-1 (3.452e-2)	9.016e-1 (1.161e-1)	1.257e+0 (1.494e-1)	1.281e+0 (1.567e-1)
[Quinine hydrochloride] (% w/v)	-	-	-	-64.185484 (32.825723)
Particle size (µm)	-	-	-	-0.009078 (0.006144)
Viscosity (Pa S)	-	-	-	0.833959 (3.085515)
Log L	-9500.213 (df=4)	-	-4924.957 (df=5)	-4921.206 (df=9)
AIC	19008	-	9859.915	9860.411
BIC	19028.69	-	9885.239	NA
Estimated No. zeros	0.04	-	14.98	5

5.5.2.4 Visualising the models with the data

Based on the aforementioned exploration of the four models, the negative-binomial and Zero-augmented hurdle negative binomial regression models were best able to describe the data, thus these were chosen to undergo further exploratory analysis through visualisation with experimental data.

The visualisation of the data is shown in Figure 5-14 and clearly demonstrates the importance of visualisation when modelling data. The models were used to predict the rat response to over 1000 proposed samples, differing by levels of bitterness, viscosity and grittiness, and subsequently plotted with the actual experimental data. Indeed, the exploratory analysis revealed that the negative binomial and zero-augmented hurdle negative binomial regression models were very similar, with the only key difference being the estimated number of zeros, with the zero-augmented hurdle negative binomial model better aligning with the actual data. However, Figure 5-14 reveals stark differences in the two models, with the negative binomial model incapable of tracking the experimental data. Thus, it can be concluded that the Zero-augmented hurdle negative binomial regression model is the superior model for predicting rat response to samples of varying viscosity, bitterness and grittiness, and will be further explored in Chapter 6 as a means to predict rat response to fully formulated antibiotic suspensions.

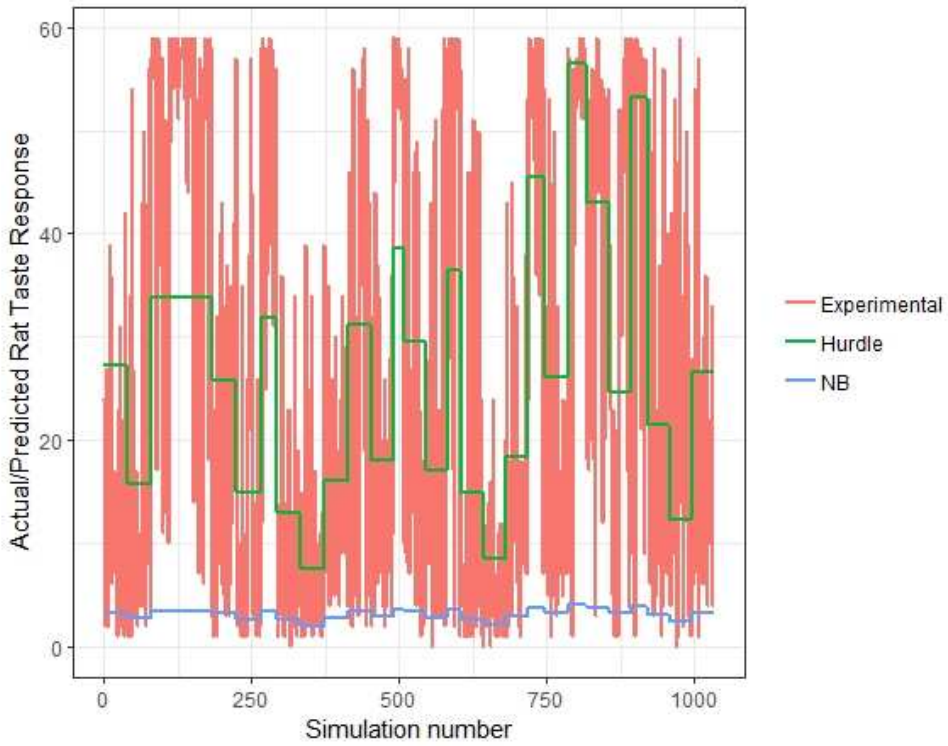


Figure 5-14 Visualising the developed models with experimental data

5.6 Discussion

In this study, mouthfeel was highlighted as a key component of medicine acceptability, but a dearth in the literature of ways to test mouthfeel without humans was identified, making assessment during early drug development impossible. Given that Maier et al. demonstrated that rats are capable of sensing mouthfeel, it was proposed that the rat BATA model may be used to provide information on not just the taste of an administered sample, but its mouthfeel too, as governed by the viscosity and grittiness [202]. In order to test this hypothesis, the rats were administered with samples consisting three levels of bitterness, viscosity and grittiness, such that a total of 27 samples were administered, with the interplay between said variables assessed.

In the first study of its kind, it was demonstrated that rats are capable of distinguishing significantly between samples of varying mouthfeel as well as bitterness using the rat BATA model. Indeed, the rats were able to distinguish between samples of increasing bitterness, grittiness and viscosity. Bitterness was identified as the most important factor in aversiveness, with grittiness the second-most important factor. Viscosity also had a significant impact on lick number, but this was a positive effect.

In addition, an interplay between said formulation variables was also identified, with both taste-masking and grittiness-masking demonstrated for the viscosity component: xanthan gum. This is a fascinating finding given that the same grittiness-masking and taste-masking effect of increased viscosity has been observed in humans. Indeed, in a study by Lopez et al. in which the effect of formulation variables on oral grittiness and preferences of multiparticulate formulations in adult volunteers was investigated, it was found that increasing the viscosity of the oral vehicle reduced the human grittiness score on a visual analogue scale (VAS). Indeed, samples dispersed in the least viscous vehicle (0.08 Pas) were rated at a mean VAS score of 32 +/- 13, while those of the intermediate (0.43 Pas) and highest viscosity (2.80 Pas) yielded VAS scores of 28 +/- 11 and 26 +/- 10, respectively [187]. Furthermore, in a second study by Lopez et al. looking at the effect of administration media on palatability and ease of swallowing of multiparticulate formulations, participants showed preference for samples dispersed in thickened vehicles over those dispersed in water [186]. In addition to mouthfeel, the effect of viscosity

on taste is very well documented in the literature. Indeed, viscosity has been shown to decrease both sensitivity to [205–207] and intensity of [208–214] bitter, sweet, sour and salty taste in humans. Exactly why a reduction in taste sensitivity/intensity with increasing viscosity is observed is outside of the scope of this chapter, but may be due to impaired diffusion of the tastant [215], accessibility to the taste receptors or otherwise [216,217]. The way in which the results observed in the rat BATA model correlate to humans is very exciting, but must be further investigated, by performing an identical study assessing the same levels of bitterness, viscosity and grittiness using the same design of experiment principles in humans to yield a direct correlation. This study does, however, point to the possibility of using rats to assess more than just taste during early drug development, thus enabling the assessment of new platform technologies which, as mentioned, may solve bitterness, but may suffer with grittiness acceptability issues.

An additional component to this study was the development of a model to describe the highly complex interplay between the studied formulation variables and the rat response. Such a model will leverage the data from the rats, thus minimising excessive animal use and enabling prediction of rat response *in silico*, in line with the principles of the 3Rs. The use of *in silico* models to describe this complex relationship has not been investigated in any previous study.

Four regression models were assessed for their ability to track the rat response to samples of varying bitterness, viscosity and grittiness. Model suitability was initially identified by assessing the coefficient estimates and standard errors, AIC/BIC and estimated number of zeros before visualising the selected superior models with experimental data.

The basic Poisson model was found to be the poorest performing model, with the highest AIC and standard error, and a small number of estimated zeros. The likely explanation for the failing of this model is the assumption regarding dispersion built into the model. Indeed, in this model, the variance is equal to the mean and thus the dispersion is fixed to 1. Given the excessive dispersion in the data, it is thus no surprise that this model failed. An improved model which accounts for dispersion and estimates a dispersion parameter from the data was thus assessed in the form of the quasi-Poissson model,

which demonstrated improved standard errors. A further two models were assessed, namely the negative-binomial regression, which is a more formal way of accounting for over-dispersion in the data and the zero-augmented hurdle negative-binomial regression model. Given that the negative-binomial model is better adept at accounting for over-dispersion, it performed better than the former two models, with a far improved AIC. However, it yielded an excessive estimated number of zeros, largely due to the zero counts observed in the data, which cannot adequately be dealt with by a negative – binomial regression. Thus, the zero-augmented hurdle negative-binomial model was investigated, which is a two-compartment model, which in this case consisted a negative-binomial component to account for the positive data points and a hurdle component to account for the zero and larger data points, therefore accounting for excessive zeros in the data, while also accounting for over-dispersion. Therefore, the zero-augmented hurdle negative-binomial model was best able to describe the data, and through visualisation, outperformed the negative-binomial model in tracking the complex rat response to samples of varying viscosity, grittiness and bitterness.

This is the first time *in silico models* have been used to predict rat response to samples of varying taste and mouthfeel. They provide a real opportunity to leverage the data from the BATA model if response can be adequately predicted. Szczesniak stated as part of her definition of mouthfeel that only certain properties of mouthfeel may be measured using physical techniques, thus mouthfeel assessment necessitates a living being to enable sensory interpretation [193]. Therefore, *in silico* models based on the response of living beings may provide the only opportunity to assess mouthfeel without the use of any living beings, per se. For example, by capturing the experimental space within which different medicines may lie in terms of bitterness and mouthfeel, the developed models may be used to predict the rat response and estimate aversiveness, while also reducing the use of animals in research. Indeed, the two highest performing models – the zero-augmented hurdle negative-binomial model and the negative-binomial model – will be further explored in chapter 6 in the assessment of antibiotic suspensions.

5.7 Conclusion

Given the identified importance of mouthfeel to the acceptability of medicines, and the resulting problems poor mouthfeel may have on patient adherence, methods for testing mouthfeel were explored, which identified a lack of methods without the use of humans, thus precluding their use during early drug development. Given that rats have been shown to be capable of sensing mouthfeel, the BATA model was proposed as a method to provide a more holistic evaluation of administered samples, thus going beyond taste, but also providing information on mouthfeel. To explore this hypothesis, samples of varying viscosity, grittiness and bitterness were administered to rats in the BATA model using design of experiment principles. It was found that rats are capable of distinguishing between samples of varying viscosity, grittiness and viscosity. Furthermore, grittiness- and taste-masking by increasing viscosity was observed, which has also been observed in humans, thus potentially demonstrating a human-rat correlation, but this must be further explored before real conclusions can be made. Nonetheless, this study has demonstrated the exciting potential of the rat BATA model to evaluate a wider range of samples, extending beyond tastants dissolved in water and moving towards formulated medicines, which will be explored in chapter 6, where antibiotic suspensions will be assessed using both the BATA model and the developed *in silico* models.

6 Elucidating the acceptability of antibiotic suspensions using the rat BATA model

6.1 Introduction

Anecdotally from the author's experience, in a discussion on the taste of medicines, most people will recall with horror a sickly yellow, foul-tasting antibiotic suspension they were force-fed by their parents as an infant. The fact that such medicines are able to elicit these emotions in people several decades later means a thesis on the taste of medicines would surely not be complete without addressing this particular set of fear-inducing medicines.

The described emotions above may be largely due to the age at which children are most likely to be prescribed antibiotics. Indeed, in a study assessing paediatric antibiotic use in six countries across five continents, it was found that the number of antibiotic prescriptions per child per year was highest below the age of 5 (0.5 – 3.4) and lowest in children aged 6 – 12 (0.2 – 0.8) [218]. Nonetheless, if a child of any age is receiving a medicine in Europe, it is most likely going to be an antibiotic, given that antibiotics are the most commonly prescribed medicines for children in Europe [219].

The poor acceptability of antibiotic medicines in children means that parents may have a very difficult time administering such medicines; surveying French parents on antibiotic use in their children found that in 22 % of cases, their child spat out at least one dose [220]. Therefore, parents have to resort to a range of methods in order to ensure their child receives the correct dose. A study by Bergene *et al.* categorised the strategies used by parents as open, hidden or force [221]. Open techniques include hiding the taste or smell by, for example, numbing the taste buds using ice cream before and after administration; giving the child an active role through play by, for example, 'giving teddy the medicine first'; or persuasion be it through reward or threat [221]. Hidden techniques include administering the medicine while the child is sleeping, in food or drink, or while the child is distracted [221]. However, Bergene *et al.* also reports the use of restraint by parents in which the child is held down and the medicine forced down; one report describes parents 'sitting on their child to get the medicine down' [221]. Such methods can induce significant

distress in both parents and children. Bergene *et al.* describes the emotions that some parents feel when they have to resort to such tactics: 'evil', 'abuse' and 'really bad' [221]. The issues parents face in the administration of medicines, particularly antibiotics, in some cases leads parents to request a different, usually second-line and broad spectrum, antibiotic. In a more recent study by Bergene *et al.,* it was found that parents requested a new prescription within 2 days in 3 % of all paediatric antibiotic prescriptions between 2004 to 2016 registered on the Norwegian Prescription Database (NorPD) [222]. After stratification by age, the most frequently proposed reason for the requested change was taste [222].

Therefore, as Bergene *et al.* alludes to, differences between the paediatric acceptability of antibiotics varies depending on the API. It is regarded that children will normally swallow co-amoxiclav, cefaclor, cephalexin and co-trimoxazole; children might swallow penicillin V, amoxicillin and clarithromycin; while children will often spit out or grimace when administered with trimethoprim or erythromycin [34].

Furthermore, in addition to differences in API, different brands of the same API have also been reported as having different acceptability in children [34,220]. In a study of 953 children prescribed either amoxicillin, co-amoxiclav or cefpodoxime proxetil and dispensed as either branded or generic form, parents were asked to complete a questionnaire which included a taste assessment using a facial hedonic scale [220]. While no significant differences in acceptability were found between branded and generic forms for amoxicillin, significant differences were identified for co-amoxiclav with the generic form more likely to be spat out [220]. Cefpodoxime proxetil was not available as a generic, thus it was not possible to assess brand-generic differences for this API. The data in the literature assessing differences between the acceptability of antibiotic brands are sparse and incomplete, but what data are there point to branded products being more acceptable than their generic equivalents [34].

The need to elucidate the acceptability of an antibiotic suspension, be it a newly developed or generic equivalent, is clear. While a pharmaceutical company developing a new antibiotic would have to demonstrate acceptability as part of the PIP or PSP, generic antibiotics are not subject to this, instead generic pharmaceutical companies are only

required to provide information on the quality of the medicine and demonstrate that the generic medicine produces the same levels of the active substance in the human body as the reference medicine. However, with the importance of acceptable paediatric medicines very much in the limelight of pharmaceutics, it may just be a matter of time before generic pharmaceutical companies are also required to demonstrate acceptability.

Methods for taste evaluation have been discussed extensively in this thesis, but those pertaining to antibiotics in children have yet to be addressed. The data on such testing in the literature are sparse; one study looked at the time taken for a medicine to be administered to a child [223], while another assessed the child's own spontaneous verbal judgments as an indicator of acceptability [224]. Both studies demonstrated promising results, but importantly these methodologies rely both on the sufficient child understanding and the medicine to have been proven as safe in humans, thus precluding their use during early drug development in the case of a newly developed antibiotic. The same problems are seen when using visual analogue scales. Collecting data on acceptability during the clinical trial phase is a promising answer, and several randomised control trials have been shown to effectively differentiate between antibiotics in terms of both efficacy and taste [225–227]. However, performing such studies at this stage risks product attrition due to acceptability issues, with significant financial implications for pharmaceutical companies.

Thus, acceptability of developing paediatric suspensions must be assessed as early on in the drug development process as possible, but how? A lack of safety data in humans precludes their use, and the complex nature of suspensions including multiple excipients and organoleptic properties such as grittiness and viscosity, which affect mouthfeel mean the use of *in vitro* tools, such as e-sensors would be in vain. The answer may lie with the BATA model. In chapter 5, it was demonstrated that rats are capable of distinguishing between varying levels of bitterness and mouthfeel as elicited by grittiness and viscosity. Therefore, it is hypothesised that the BATA model may provide data on the differing acceptability of antibiotic suspensions, both by API and brand. This chapter will hence assess three antibiotic APIs: co-amoxiclav, which children will normally swallow; clarithromycin, which children might swallow; and erythromycin, which children usually

spit out [34]. Multiple brands of the aforementioned APIs will also be assessed to ascertain whether the BATA model may also provide data on the different acceptability of brands.

6.2 Aims

Differences between human acceptability of antibiotic suspensions of both different API and brand have been observed in the literature. Given the identified issues with treatment adherence to poorly acceptable antibiotic suspensions, it is important to assess acceptability of antibiotic suspensions during drug development. It was demonstrated in chapter 5 that rats, in the BATA model, are able to distinguish between samples varying in both bitterness and mouthfeel, as governed by viscosity and grittiness. Thus, the aim of this chapter is to identify whether the acceptability of antibiotic suspensions can be elucidated using the BATA model.

6.3 Objectives

1. Assess antibiotic suspensions differing by API, brand and strength using the BATA model.
2. Test the developed in silico models from chapter 5 for their ability to predict rat response to antibiotic suspensions based on their measured properties of viscosity, grittiness (particle size) and bitterness.

6.4 Materials and methods

6.4.1 Materials

Mylan clarithromycin 125 mg/5 mL, Mylan clarithromycin 250 mg/5 mL, Sandoz clarithromycin 125 mg/5 mL, Sandoz clarithromycin 250 mg/5 mL, GSK co-amoxiclav 125 mg/31 mg/5 mL, GSK co-amoxiclav 250 mg/62 mg/5 mL, Mylan co-amoxiclav 250 mg/62 mg/5 mL, Sandoz co-amoxiclav 125 mg/31 mg/5 mL, Sandoz co-amoxiclav 250 mg/62 mg/5 mL, Sandoz co-amoxiclav 400 mg/57 mg/5 mL, Pinewood erythromycin 125 mg/5 mL, Pinewood erythromycin 125 mg/5 mL SF, Pinewood erythromycin 250 mg/5 mL, Pinewood erythromycin 250 mg/5 mL SF, Pinewood erythromycin 500 mg/5 mL, Teva erythromycin 125 mg/5 mL SF, Teva erythromycin 250 mg/5 mL SF were purchased from AAH Pharmaceuticals (Coventry, UK). Mylan co-amoxiclav 125 mg/31 mg/5 mL was purchased from John Bell & Croyden (London, UK).

6.4.2 Methods

6.4.2.1 Rat BATA model

6.4.2.1.1 Antibiotic suspensions

Each antibiotic suspension was assessed for its aversiveness using the rat BATA model. The methodology utilised was identical to that which has been discussed in previous chapters. However, in order to best replicate a human taking the medicine, it was identified that the suspension must be inverted prior to administration to the rats as one would do before pouring a 5 mL spoonful of antibiotic suspension for administration to a child. Thus, the lickometer apparatus was adjusted to incorporate a platform and handle which enabled the researcher to invert all suspensions through 180° every 8 s prior to presentation to the rats. Each suspension was placed in the lickometer rack in duplicate thus presented four times. A broad range of suspensions, consisting three different APIs, multiple strengths and multiple brands as per Table 6-1 were assessed. Each suspension was reconstituted on the morning of the experiment in which the volume of potable water as specified in the 'instructions to the pharmacist' section on the box was added before shaking. Table 6-1 also includes information on the excipient and flavour differences between the assessed suspensions, as per their summary of product characteristics (SmPC). Where an excipient is listed, it is indicative of it being present in the relevant suspension but absent from at least one other. Quantities of excipients are stated if they are provided in the SmPC.

Table 6-1 Antibiotic suspensions assessed in the BATA model. Details of the excipient composition and flavour are provided as per the SmPC. Where an excipient is deemed unique, it refers to it not being present in at least one of the other suspensions assessed.

API	Brand	Strength	Sugar(S)/sugar free (SF)	Unique excipients	Flavour
Clarithromycin	Mylan	125 mg/5 mL	550 mg/mL sucrose	Castor oil, Citric acid	Fruit punch
		250 mg/5 mL	455 mg.mL sucrose		
	Sandoz	125 mg/5 mL	480 mg/mL sucrose	Macrogol, methacrylic acid: ethylacrylate (1:1) co-polymer, triethyl citrate, glyceryl monostearate, polysorbate 80	Fruit punch
		250 mg/5 mL	480 mg/mL sucrose		
Co-amoxiclav	GSK	125 mg/31 mg/5 mL	-	Aspartame (2.5 mg/mL), hypromellose, succinic acid, xanthan gum,	Orange, raspberry & golden syrup
		250 mg/62 mg/5 mL	-		
	Mylan	125 mg/31 mg/5 mL	-	Aspartame (1.7 mg/mL), citric acid, sodium citrate, talc, guar galactomannan	Lemon-peach, apricot and orange containing essence of bergamot
		250 mg/62 mg/5 mL	-		
	Sandoz	125 mg/31 mg/5 mL	-	Aspartame (unspecified amount), citric acid, sodium citrate, talc, guar galactomannan	Lemon-peach, apricot and orange containing essence of bergamot
		250 mg/62 mg/5 mL	-		
		400 mg/57 mg/5 mL	-		
Erythromycin	Pinewood	125 mg/5 mL	S	Sucrose, sodium citrate, sodium carboxymethyl cellulose, sodium saccharin	Banana
		125 mg/5 mL	SF	Sorbitol, riboflavin-5-sodium phosphate, disodium hydrogen phosphate, carmellose sodium, glyceryl stearate	Orange
		250 mg/5 mL	S	As per 125 mg/5 mL S	Banana
		250 mg/5 mL	SF	As per 125 mg/5 mL SF	Orange
		500 mg/5 mL	S	As per 125 mg/5 mL S	Banana
	Teva	125 mg/5 mL	SF	Unavailable	Unavailable
		250 mg/5 mL	SF		

6.4.2.1.2 Antibiotic APIs

The rat BATA model was also used to assess the aversiveness of the antibiotic APIs dissolved solely in water. The methodology used was identical to that which has been discussed in previous chapters. The concentrations were chosen based on the maximum saturation solubility of the API balanced with the rat toxicity of the API, with serial dilutions from said maximums: see Table 6-2.

Table 6-2 Antibiotic APIs and their respective aqueous concentrations assessed in the BATA model

API	Concentrations assessed (mg/mL)
Clarithromycin	0.00000045, 0.00000136, 0.00001222, 0.00003667, 0.00011, 0.00033
Amoxicillin trihydrate	0.004, 0.013, 0.04, 0.12, 0.37, 1.1, 3.3
Potassium clavulanate	0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1
Erythromycin ethylsuccinate	0.00021, 0.00062, 0.0019, 0.0056, 0.0167, 0.05, 0.15

6.4.2.2 Particle size analysis

The Mastersizer 3000, fitted to a Hydro MV feeding system, enable analysis of the particle size distribution of the antibiotic suspensions using laser diffraction (Malvern Scientific, Worcestershire, UK). Deionised water was used as the dispersant. Each antibiotic suspension was reconstituted with potable water and allowed to stand for 10 minutes prior to commending the experiment. Immediately prior to the experiment, samples were shaken to enable re-suspension. Six replicates of each sample were taken.

The data were plotted using R, in which the volume density (%) was plotted as a function of particle size (μm). The mode particle size was utilised in comparing between suspensions given the bimodal distributions encountered for many of the samples, and was again computed using R.

6.4.2.3 In silico prediction of aversiveness

The statistical programming software R was used to predict the mean lick number using the 'predict()' function. The models used were those chosen as superior in chapter 5, namely the negative binomial generalised linear model (model 3) and the: zero-augmented hurdle negative binomial model (model 4). The predicted mean lick number for both models was compared to the actual experimental value. These values were also

visualised by plotting all rat responses and showing the model predictions as horizontal lines using R.

6.4.2.4 Rheology

The rheological properties of each antibiotic suspension were assessed using the Malvern Bohlin Gemini HR Nano Rheometer with associated Rotonetic 2 Drive. The CP 4 degrees/40 mm plate was used. The gap was set at 70 as this was found to achieve the best spread of sample without being affected by the suspended particles. The temperature was set to 25 °C. The system was set to run a log increase from 0.05 to 1750 shear rate (s^{-1}).

The data were presented as viscosity (Pa s) as a function of shear rate (s^{-1}) plotted using R. Statistical analysis was also completed using R, in which the viscosities at 1 s^{-1} shear rate were compared using a pairwise t-test utilising the Hochberg p adjustment method.

6.5 Results

6.5.1 Assessing antibiotic suspensions using the BATA model

Taste assessment of multiple brands and strengths of three antibiotic API suspensions was successfully carried out using the rat BATA model. The results are discussed by API below.

6.5.1.1 Clarithromycin

Assessing the rat response to different brand and concentrations of clarithromycin suspension reveals some differences. Indeed, it can be observed in Figure 6-1 that there was an inverse correlation between concentration and lick number for the Mylan suspensions, representing an increase in aversiveness with strength of API, while the reverse was seen for Sandoz suspensions with an increase in lick number with increasing concentration, thus revealing a reduction in aversiveness with strength of API. However, statistical analysis using the Kruskal-Wallis one-way analysis of variance reveals that neither observed difference was statistically significant ($p > 0.05$). However, when compared to water using Gao's posthoc analysis, it was revealed that all suspensions

assessed achieved a lick number significantly lower than that of water ($p < 0.05$), thus all were deemed aversive by the rats.

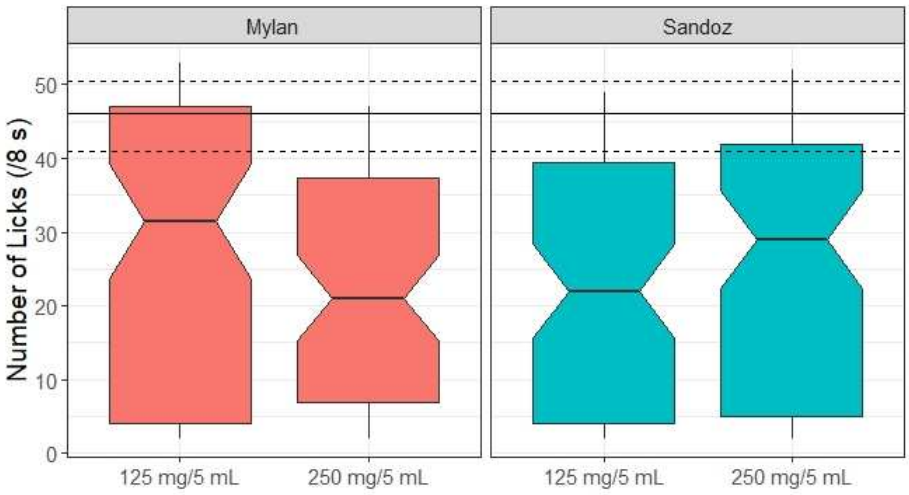


Figure 6-1 Rat BATA assessment of clarithromycin suspensions showing the differences between concentrations by brand. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

Figure 6-2 assesses the rat response to different brands of clarithromycin suspension, revealing that for the 125 mg/5 mL strength, the Mylan brand achieved a higher lick number relative to the Sandoz brand indicative of enhanced palatability, while conversely at the 250 mg/5 mL strength, the Sandoz brand achieved a higher lick number relative to the Mylan brand. Furthermore, the observed differences at both concentrations were found to be statistically significant with p values of 1.949e-07 and 1.065e-08 at concentrations of 125 mg/5 mL and 250 mg/5 mL, respectively.

Figure 6-2 Rat BATA assessment of clarithromycin suspensions showing the differences between brand per concentration. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

6.5.1.2 Co-amoxiclav

Different brands and concentrations of co-amoxiclav also underwent rat BATA assessment with some differences observed. By first assessing how concentration affects rat response for each brand of suspension, some stark differences can be seen. Firstly, Figure 6-3 demonstrates some minor differences between the GSK suspensions, with the 250 mg/62 mg/5 mL strength eliciting a more broad response than that observed for the 125 mg/31 mg/5 mL strength. However, no significant difference was observed between strenghs with Gao's posthoc analysis producing a p value exceeding 0.3, while both differed significantly from water ($p < 0.05$). The Mylan suspensions however demonstrated extremely different rat responses, with the 125 mg/31 mg/5 mL strength achieving an extremely large lick number comparable to that of water, while the 250 mg/62 mg/5 mL strength achieved a lick number below 20. Indeed, Gao's posthoc analysis revealed that the observed differences between the Mylan suspensions were statistically significant ($p < 0.05$). The 125 mg/31 mg/5mL strength did not differ statistically to water ($p = 0.21$), while the 250 mg/62 mg/5 mL strength did differ significantly from water ($p < 0.05$). Finally, some differences were also observed for the Sandoz suspensions assessed, however converse to the differences observed for the Mylan suspensions, the highest concentration assessed – 400 mg/57 mg/5 mL – achieved the highest lick number, while visually there was no discernible difference between 125

mg/31 mg/5 mL and 250 mg/62 mg/5 mL strengths. Gao's post hoc analysis confirmed the observed differences, with no statistical difference observed between 125 mg/31 mg/5 mL and 250 mg/62 mg/5 mL strengths ($p = 0.961$), but both 125 mg/31 mg/5 mL and 250 mg/62 mg/5 mL differed significantly from 400 mg/57 mg/5 mL ($p < 0.05$).It was also found that the 400 mg/57 mg/5 mL strength did not differ significantly from water ($p = 0.961$), while all other strength did differ significantly from water ($p < 0.05$).

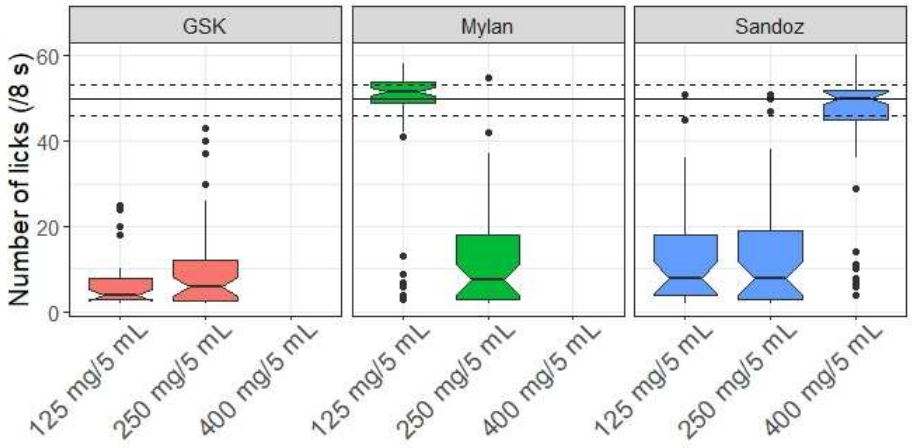


Figure 6-3 Rat BATA assessment of co-amoxiclav suspensions showing the differences between concentrations by brand. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

The inter-brand differences were also explored as shown in Figure 6-4. At a strength of 125 mg/31 mg/5 mL, stark differences can be observed between brands, with the Mylan suspension demonstrating reduced aversiveness relative to both the GSK and Sandoz brands as indicated by the increased lick number. Indeed, statistical analysis revealed that the Mylan brand achieved a significantly higher lick number than both the GSK and Sandoz brands ($p < 0.05$), while no significant differences were observed between GSK and Sandoz ($p = 0.19$). At a strength of 250 mg/62 mg/5 mL, it can be seen from Figure 6-4 that the breadth of lick numbers observed for each brand appear to differ, however it was found that, at this strength, no significant difference between brands was observed ($p > 0.05$).

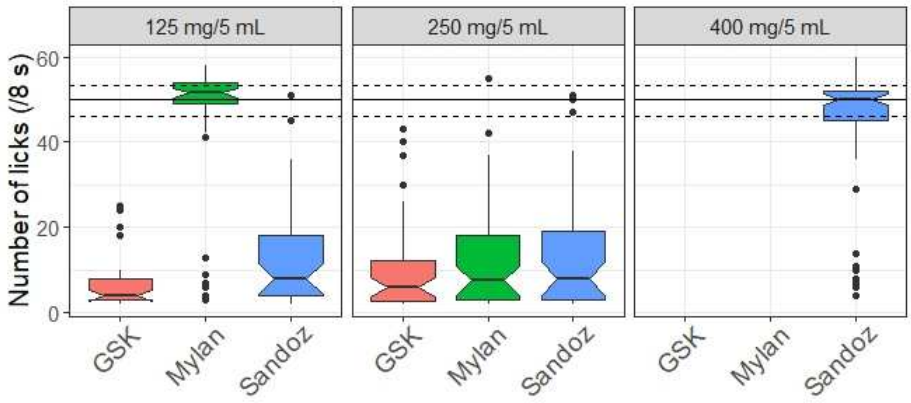


Figure 6-4 Rat BATA assessment of co-amoxiclav suspensions showing the differences between brand per concentration. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

6.5.1.3 Erythromycin

Erythromycin suspensions were also assessed for aversiveness using the rat BATA model. There appears to be no effect of suspension strength on the aversiveness of Pinewood sugared as indicated by the lick number which remains approximately equal across all strengths: see Figure 6-5. This observation was confirmed by Gao's post hoc analysis ($p > 0.05$). The same observation is seen with both Pinewood sugar free and Teva sugar free suspensions. Kruskal-Wallis one way analysis of variance confirmed that no statistically significant differences were seen between concentrations of these suspensions ($p > 0.05$).

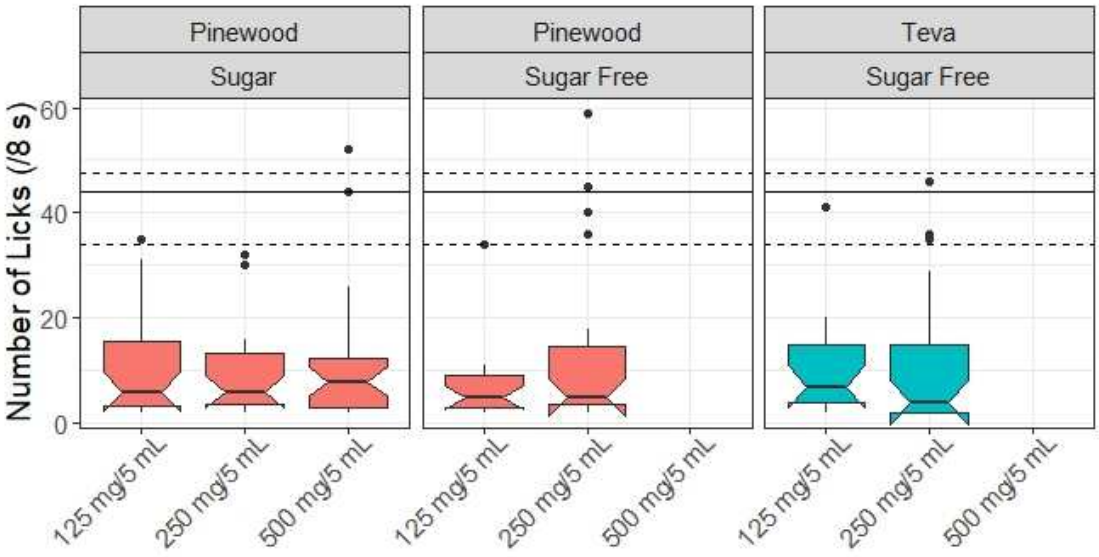


Figure 6-5 Rat BATA assessment of erythromycin suspensions showing the differences between concentrations by brand and sugar content. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

Figure 6-6 shows the effect of brand, and thus formulation, on the dependent variable: lick number. The only comparisons that were possible however, were between the sugar free suspensions as the Teva brand was only formulated without sugar. Furthermore, the maximum strength of Teva erythromycin available is 250 mg/5 mL, thus no comparison was possible for the 500 mg/5 mL Pinewood sugar suspension. Looking first at the 125 mg/5 mL sugar-free suspensions, Figure 6-6 demonstrates that the Teva formulation appears to achieve a slightly higher lick number indicative of reduced aversiveness. However, statistical analysis reveals a p value of 0.32, thus indicating no statistically significant differences between the assessed brands. Teva and Pinewood sugar free brands of erythromycin 250 mg/5mL appear to achieve very similar lick numbers: Figure 6-6. Indeed, this visual observation was confirmed by statistical analysis, which revealed no statistically significant difference ($p = 0.43$).

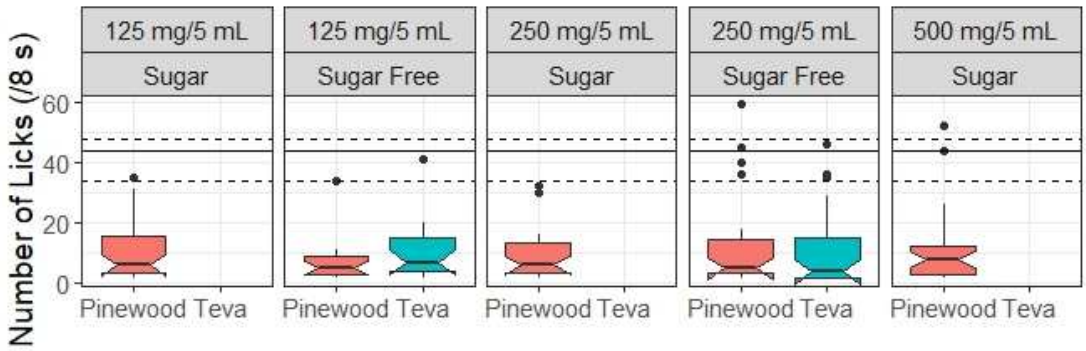


Figure 6-6 Rat BATA assessment of erythromycin suspensions showing the differences between brand per concentration and sugar content. The median water lick number and interquartile range are shown as solid and dashed lines, respectively.

Finally, the effect of sugar was explored as per Figure 6-7. Here, all brand data are pooled and the effect of sugar was assessed by plotting sugar and sugar free suspensions of the same erythromycin strength together. At 125 mg/5 mL, the sugared suspensions elicit a much broader rat response as indicated by the broader interquartile range, relative to that of the sugar free suspensions: Figure 6-7. However, the observed differences were not statistically signficant achieving with a p-value exceeding 0.4. At a strength of 250 mg/5 mL, it is the sugar free suspensions that elicited a broader rat response relative to the sugared suspensions, as demonstrated by the broaderinterquartile range: Figure 6-7. However, similarly to the 125 mg/5 mL suspensions, the observed differences were found to be insignificant (p=0.70).

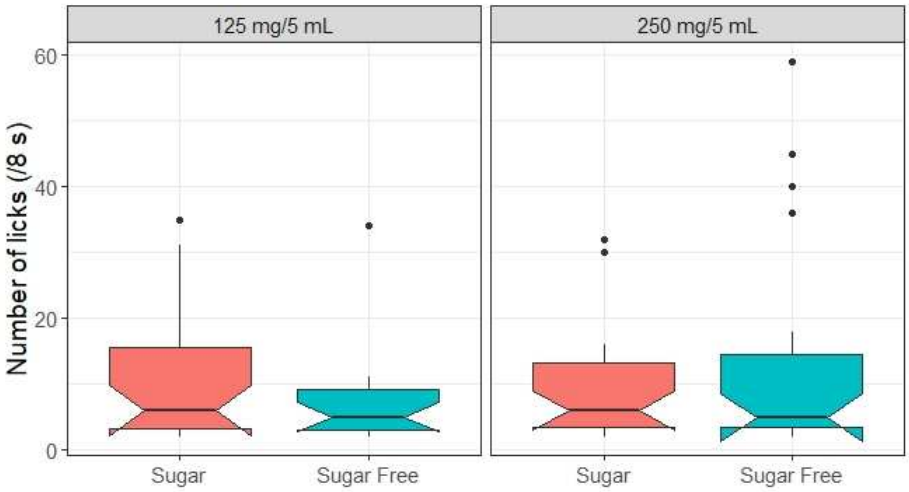


Figure 6-7 Rat BATA assessment of erythromycin suspensions, in which all data have been pooled to assess the effect of sugar content for each concentration.

6.5.1.4 Inter-API comparison

As a means to assess the ranking of each API suspension as provided by the BATA model. The data from all suspensions at strengths of 125 mg/5 mL (or 125 mg/31 mg/5 mL in the case of co-amoxiclav) and 250 mg/5 mL (or 250 mg/62 mg/5 mL in the case of co-amoxiclav) were pooled and compared.

6.5.1.4.1 125 mg/5 mL or 125 mg/31 mg/5 mL

As demonstrated in Figure 6-8, difference rat responses to suspensions of different APIs were found. Extensive variabiility in rat response was observed for both clarithromycin and co-amoxiclav, while only a small range in rat response was observed for erythromycin, as demonstrated by the differences in interquartile range shown in Figure 6-8. Indeed, statisitical analysis revealed that all brands of erythromycin suspensions were signifcantly more aversive than both clarithromycin and co-amoxiclav suspensions ($p < 0.05$). However, no signficiant difference was observed between clarithromycin and co-amoxiclav suspensions at this concentration ($p = 0.08$).

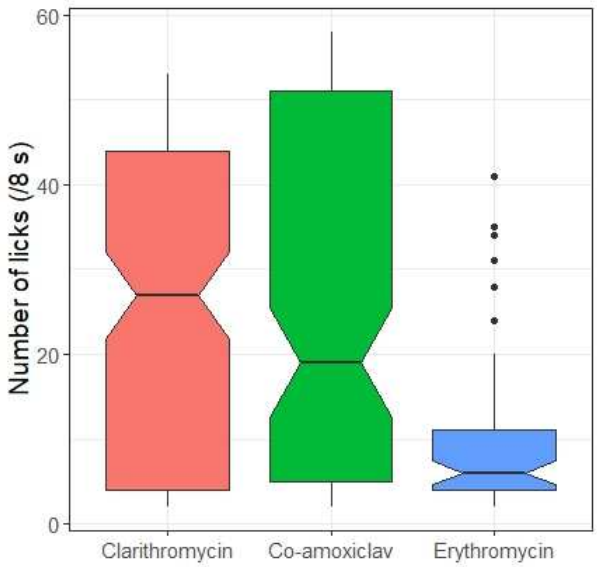


Figure 6-8 Rat BATA assessment of antibiotic suspensions by API. All data at concentrations of 125 mg/5 mL or 125 mg/31 mg/5 mL have been pooled to assess differences in rat response to APIs

6.5.1.4.2 250 mg/5 mL or 250 mg/62 mg/5 mL

At concentrations of 250 mg/5 mL, or 250 mg/62 mg/5 mL in the case of co-amoxiclav, differences in rat response to different APIs, regardless of brand were observed: see Figure 6-9. Clarithromycin elicits a more broad lick response than co-amoxiclav and

erythromycin, as indicated by the differences in interquartile range among these APIs. This finding suggests clarithromycin is less aversive than erythromycin and co-amoxiclav. Indeed, statistical analysis revealed that clarithromycin elicited a singificantly higher lick number than both co-amoxiclav and erythromycin ($p < 0.05$). However, no statistically significant difference was found between the rat response to co-amoxiclav and erythromycin, regardless of brand, at this concentration ($p = 0.50$), demonstrative of an equal aversiveness of these suspensions.

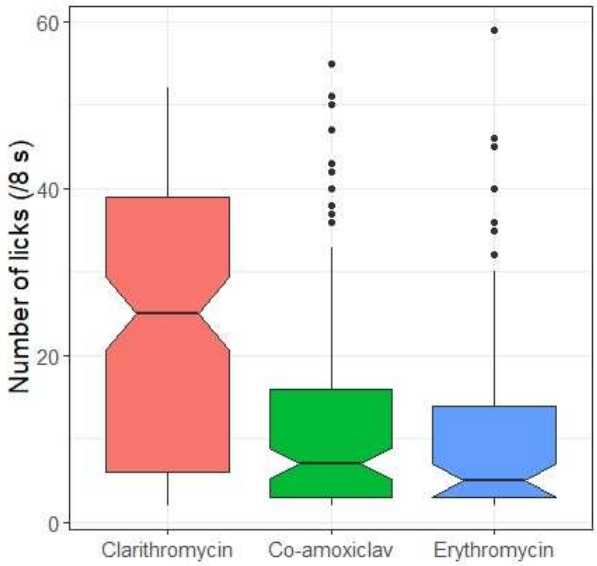


Figure 6-9 Rat BATA assessment of antibiotic suspensions by API. All data at concentrations of 250 mg/5 mL or 250 mg/62 mg/5 mL have been pooled to assess differences in rat response to APIs

6.5.2 Characterising the antibiotic suspensions

In order to gain a greater insight into the way in which the assessed antibiotic suspensions elicit their aversiveness, and ascertain why the identified differences in lick number might be, all assessed suspensions were characterised for their innnate bitterness – that which is elicited by the API alone, and their mouthfeel as governed by the viscosity and particle size.

6.5.2.1 Aversiveness of the API

6.5.2.1.1 Clarithromycin

A full concentration range of clarithromycin dissolved in water was assessed using the rat BATA model. The results - Figure 6-11 – indicate a lack of aversiveness of the API alone. The concentration-response curve is rather flat with only one concentration eliciting a

lick response significantly different to the other concentrations assessed: 0.0033 mg/mL elicited a significantly lower lick response than 0.00000136 mg/mL ($p = 0.0014$), 0.00001222 mg/mL ($p = 0.0049$) and 0.00003667 mg/mL ($p = 0.0030$); all concentrations were not significantly different to each other ($p > 0.05$). Indeed, all but one concentrations of clarithromycin assessed, including the saturation solubility concentration were found to elicit a lick number not significantly different from water ($p > 0.05$). Indeed, only 0.00000136 mg/mL was found to be significantly different to water ($p = 0.045$). As such, it was not possible to compute an IC_{50} for this API due to the flat lick response provided by the rats.

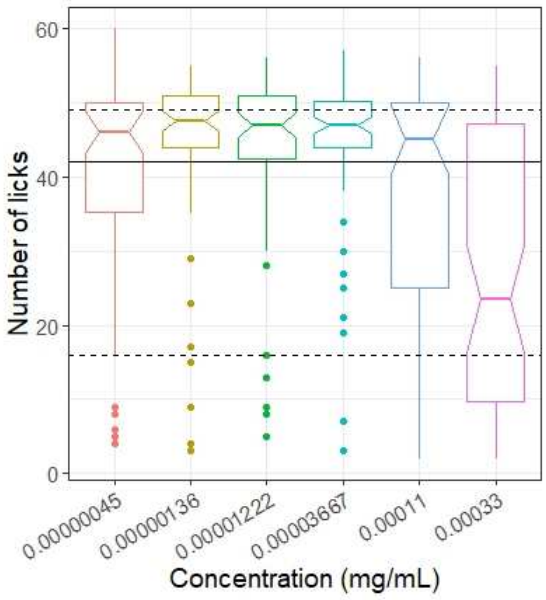


Figure 6-10 Rat response to increasing concentrations of clarithromycin dissolved in water. The median lick number and interquartile range are shown as solid and dashed lines, respectively

An unusually broad interquartile range for water was identified in this experiment - Figure 6-10 – which can be explained by the lack of aversiveness of the clarithromycin solutions. The rats therefore demonstrated maximum lick counts at the start of the experiment, but reduced lick counts for all solutions, including water, towards the end of the experiment. The rats were essentially 'full up'. Indeed, Figure 6-11 demonstrates that the majority of rats on days 1 and 2 licked water at a far lower rate at the end of the experiment relative to the beginning.

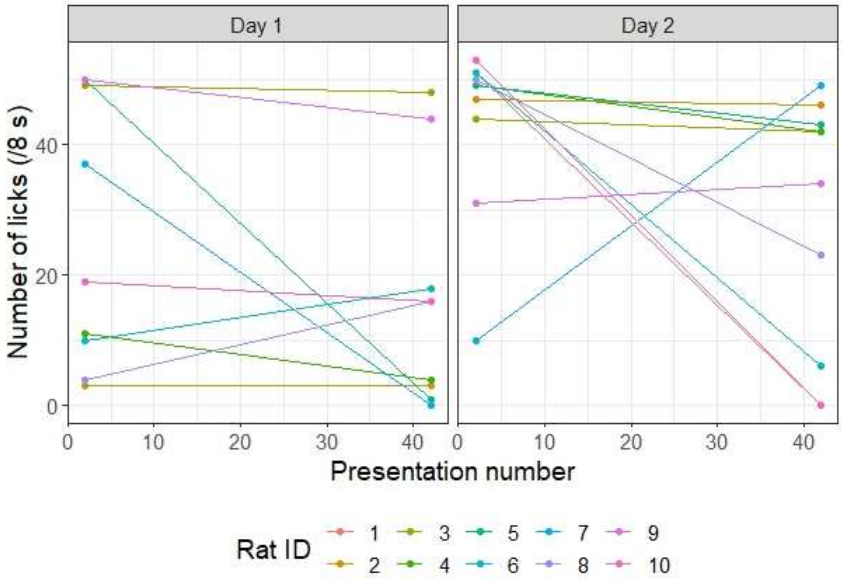


Figure 6-11 Rat response to water as a function of session time, showing each individual lick number value for each rat for each water presentation.

6.5.2.1.2 Amoxicillin trihydrate

A full concentration range of amoxicillin trihydrate was assessed using the rat BATA model: Figure 6-12. A flat concentration-response was attained demonstrating a lack of innate aversiveness of amoxicillin trihydrate. Indeed, all concentrations were found to elicit lick responses that were not significantly different to each other with the exception of 0.013~1.1 mg/mL ($p = 0.023$), 0.12 ~1.1 mg/mL ($p = 0.0032$) and 1.1~3.3 mg/mL ($p = 0.0081$ mg/mL). Furthermore, when compared to the water response, as shown by the solid and dashed black horizontal lines in Figure 6-12, the majority of concentrations including the saturation solubility concentration did not elicit a lick response significantly different from water ($p > 0.05$), with the exception of 0.004 mg/mL ($p = 0.016$), 0.37 mg/mL ($p = 0.0041$) and 1.1 mg/mL ($p = 0.000034$). Thus, this demonstrates a general inability of rats to distinguish water from that adulterated with amoxicillin trihydrate, and as such a lack of aversiveness of this compound. For this reason, it was therefore not possible to compute an IC_{50} for amoxicillin trihydrate.

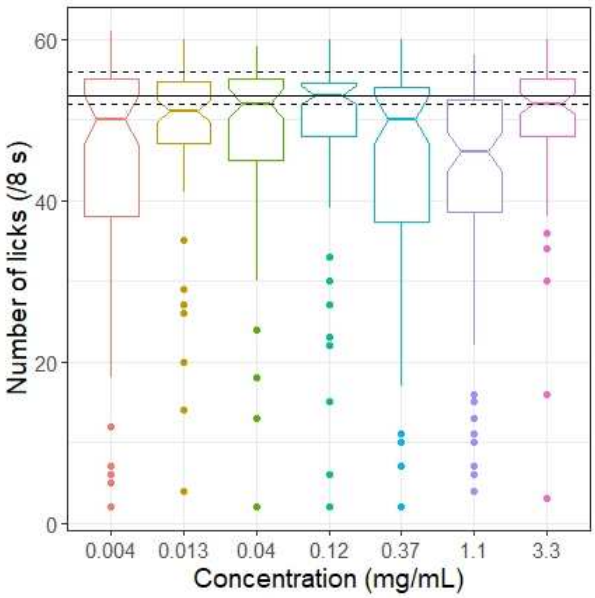


Figure 6-12 Rat response to increasing concentrations of amoxicillin trihydrate dissolved in water. The median lick number and interquartile range are shown as solid and dashed lines, respectively

6.5.2.1.3 Potassium clavulanate

A full concentration range of potassium clavulanate dissolved in water was assessed using the rat BATA model, as shown in Figure 6-13. Among all concentrations, there were no significant differences identified ($p > 0.05$), as reflected by the flat lick response with increasing concentration up to its saturation water solubility. Furthermore, among all concentrations assessed, none were found to elicit a lick response significantly different to water ($p > 0.05$). An IC_{50} could therefore not be calculated given that all concentrations, including the saturation solubility, did not achieve a significantly different lick number to water. Potassium clavulanate is therefore not an aversive compound, and cannot be differentiated from water at any concentration in the rat BATA model.

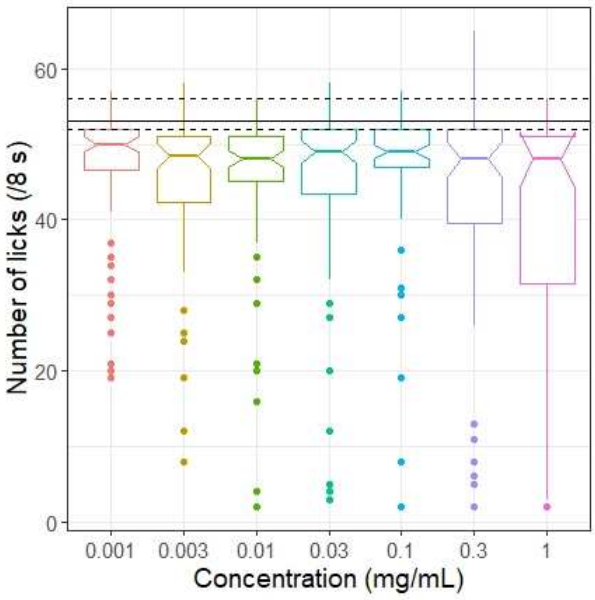


Figure 6-13 Rat response to increasing concentrations of potassium clavulanate dissolved in water. The median lick number and interquartile range are shown as solid and dashed lines, respectively

6.5.2.1.4 Erythromycin ethylsuccinate

Erythromycin ethylsuccinate was assessed in the rat BATA model at a full concentration range up to its saturation water solubility. As demonstrated in Figure 6-14, some significant differences in lick number were identified among the concentrations assessed. Indeed, 0.0056 mg/mL elicited a significantly different lick number to 0.00021 mg/mL ($p = 0.0011$), 0.00062 mg/mL ($p = 0.03$) and 0.0019 mg/mL ($p = 0.0041$). Furthermore, 0.05 mg/mL elicited a significantly different lick number to 0.0056 mg/mL ($p = 0.049$). Finally, the maximum concentration assessed – 0.15 mg/mL – elicited significantly different rat responses to 0.00021, 0.00062, 0.0019, 0.0167 and 0.05 mg/mL, with all p values being inferior to 0.0001. However, of all concentrations assessed, only 0.15 mg/mL – the saturation solubility concentration – differed significantly to water ($p = 0.012$), thus only at its peak concentration in water is erythromycin ethylsuccinate distinguishable from water. Therefore, with only a single concentration eliciting a significantly different response to water, it was not possible to compute an IC_{50} value. Erythromycin ethylsuccinate can be deemed non-aversive from this analysis.

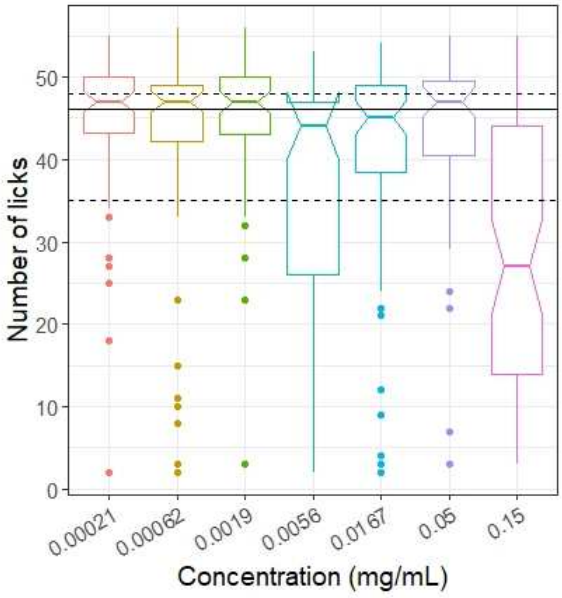


Figure 6-14 Rat response to increasing concentrations of erythromycin ethylsuccinate dissolved in water. The median lick number and interquartile range are shown as solid and dashed lines, respectively

6.5.2.2 Assessing the grittiness: particle size analysis

In order to assess the grittiness of the suspensions under test as a means to ascertain the source of the aversiveness elicited, the antibiotic suspensions underwent particle size analysis. A very broad range of particle sizes were encountered, with several orders of magnitude between some measurements: Figure 6-15. The data will now be split based on the API, and discussed in turn.

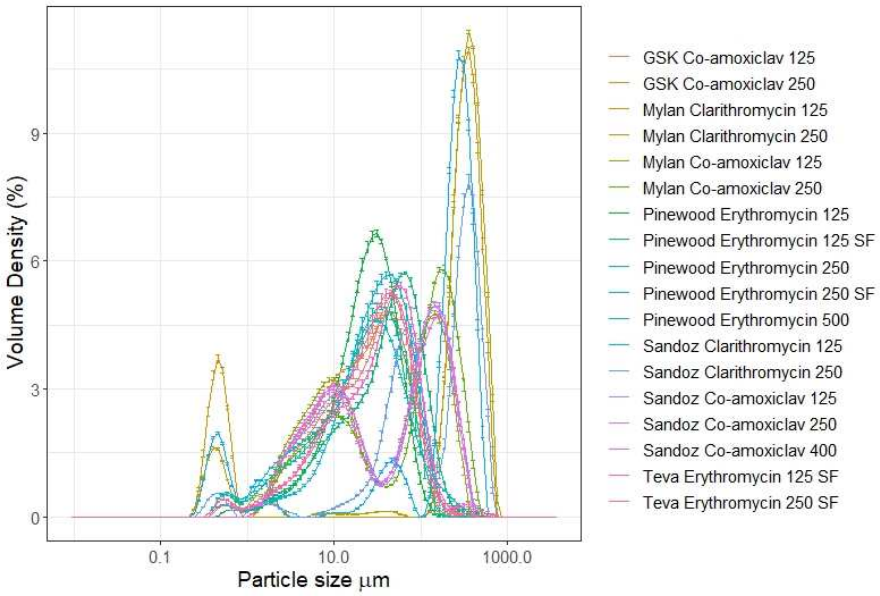


Figure 6-15 Particle size distributions of all antibiotic suspensions under scrutiny

6.5.2.2.1 Clarithromycin

All clarithromycin suspensions under scrutiny were assessed for their particle size distribution. The mode particle size among the assessed suspensions were found to be dramatically different, particularly among brands: Figure 6-16. Indeed, the Sandoz suspensions were found to have mode particle sizes of 4.14 and 30.06 µm for the 125 and 250 mg/5 mL strengths, respectively. While, the Mylan suspensions were found to have mode particle sizes of 517.48 and 359.52 µm for the 125 and 250 mg/5 mL strengths, respectively.

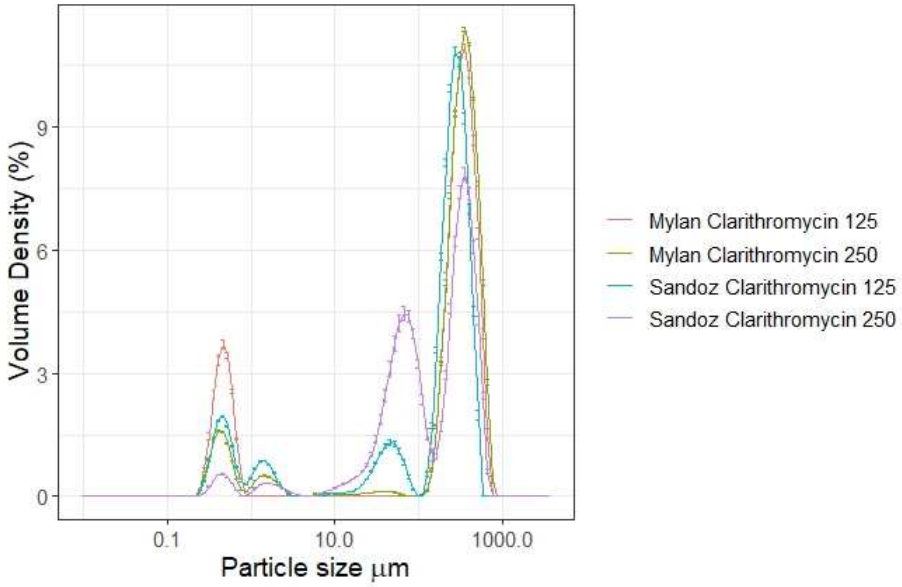


Figure 6-16 Particle size distributions of clarithromycin suspensions

6.5.2.2.2 Co-amoxiclav

The range of particle sizes observed for the co-amoxiclav suspensions was not as broad as those seen for clarithromycin: Figure 6-17. It was found that the GSK suspensions had the lowest particle size of all brands assessed, and had no difference between strengths, with both the 125/31 and 250 mg/62 mg/5 mL strengths have mode particle sizes of 45.6 µm. The Sandoz 125/31 and 250 mg/62 mg/5 mL strengths were also found to have an identical mode particle size of 144 µm. However, the highest strength assessed – Sandoz 400 mg/57 mg/5 mL – was found to have a higher mode particle size of 163 µm. Lastly, the Mylan co-amoxiclav suspensions assessed measured mode particle sizes that differed

by strength, with values of 144 and 186 µm for the 125 mg/31 mg/5 mL and 250 mg/62 mg/5 mL strengths, respectively.

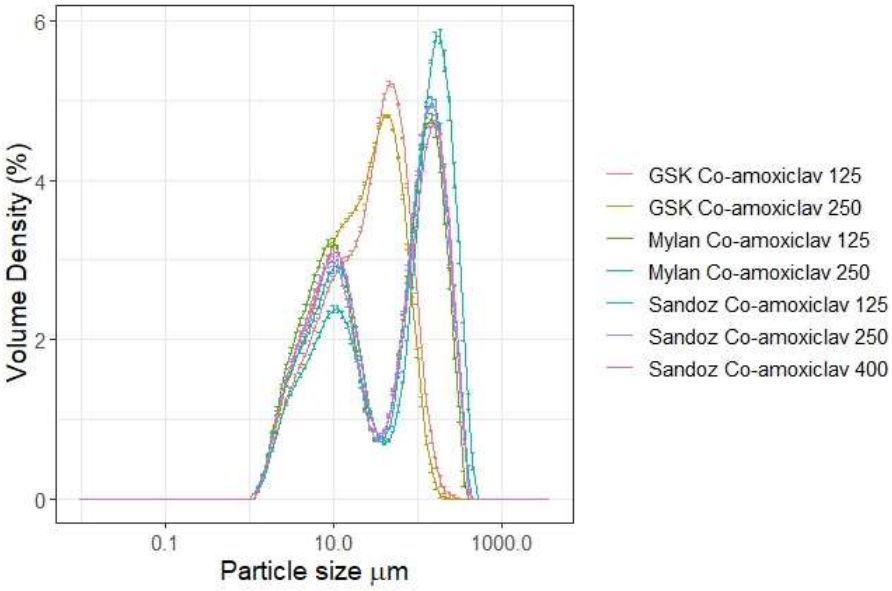


Figure 6-17 Particle size distributions of co-amoxiclav suspensions

6.5.2.2.3 Erythromycin

The measured particle sizes for erythromycin were found to be broadly similar: Figure 6-18. Some minor differences in mode particle sizes by strength and sugar content were noted with both the Pinewood and Teva suspensions. The Pinewood 125 mg/5 ml sugar free suspension was found to have a mode particle size of 66.9 µm, while the sugared form had that of 31.1 µm. However, at a strength of 250 mg/5 mL, no real difference was identified between the sugar/sugar free Pinewood suspensions, with respective mode particle sizes of 35.3 and 31.1 µm. Finally, small strength differences were noted between the Teva suspensions assessed, with respective mode particles sizes of 58.9 and 45.6 µm for the 125 mg/5 mL and 250 mg/5 mL strengths.

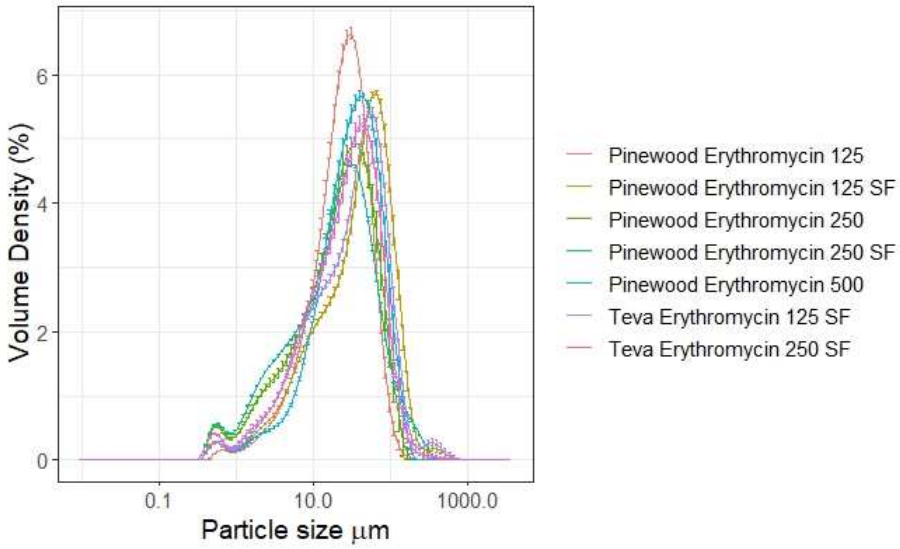


Figure 6-18 Particle size distributions of erythromycin suspensions

6.5.2.3 Assessing the viscosity

The rheological properties of all antibiotic suspensions under scrutiny were assessed, with some significant differences identified. However, all suspensions were found to be non-Newtonian, exhibiting shear-thinning behaviour with a reduction in viscosity with increased shear rate: Figure 6-19. All findings will now be discussed in turn by API.

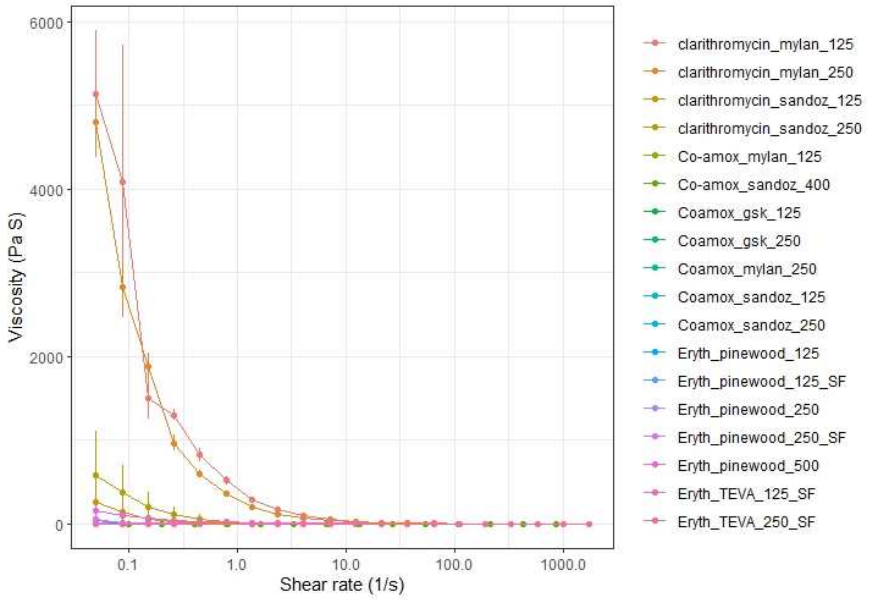


Figure 6-19 The rheological properties of the assessed antibiotic suspensions. The data are plotted as the mean, with error bars indicative of the standard error of the mean (SEM).

6.5.2.3.1 Clarithromycin

The clarithromycin suspensions assessed demonstrated very different rheological properties, both between brands and strengths: Figure 6-20. Analysis at a shear rate of 1/s, reveals that statistically significant differences were observed among all suspensions assessed ($p < 0.05$), with the exception of the intra-Sandoz suspension differences, which were found to be not significant ($p = 0.553$).

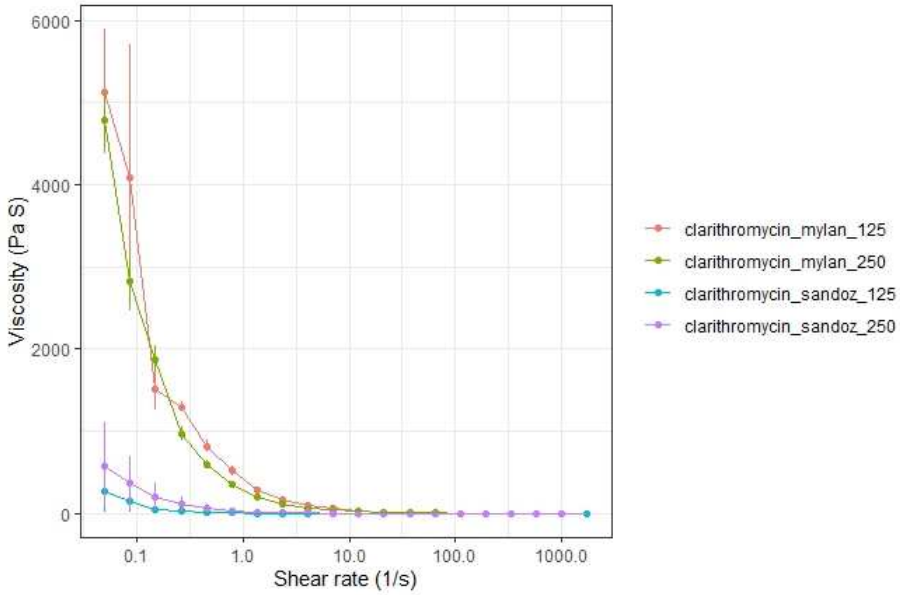


Figure 6-20 The rheological properties of the assessed clarithromycin suspensions. The data are plotted as the mean, with error bars indicative of the SEM.

6.5.2.3.2 Co-amoxiclav

As demonstrated in Figure 6-21, differences in rheological behaviour were observed between different brands and concentrations of co-amoxiclav. However, Figure 6-21 also shows the significant error associated with the mean values of viscosity at given shear rates, as indicated by the wide error bars. This might be due to the suspended particles affecting the rheometer. As such, no significant differences were observed between any of the co-amoxiclav suspensions assessed ($p > 0.05$).

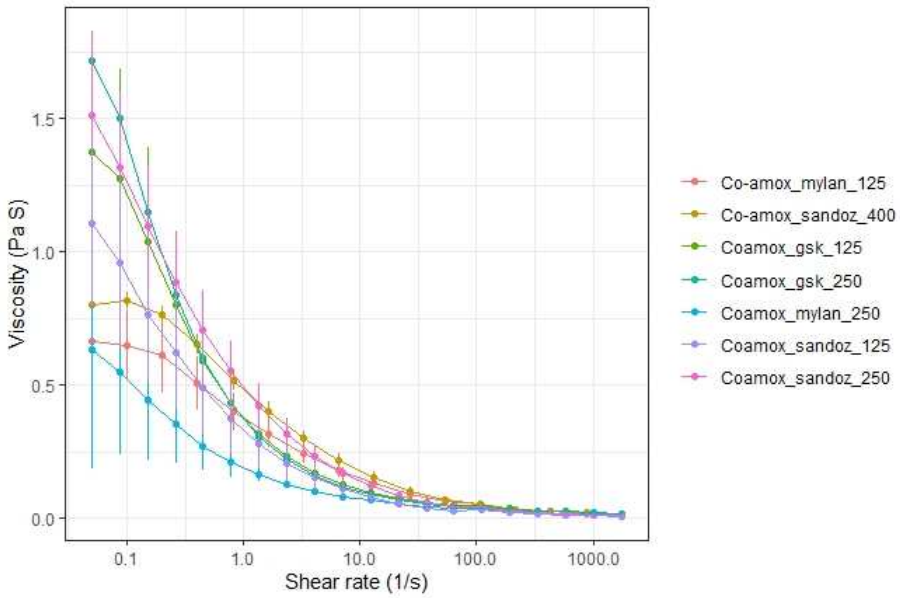


Figure 6-21 The rheological properties of the assessed co-amoxiclav suspensions. The data are plotted as the mean, with error bars indicative of the SEM.

6.5.2.3.3 Erythromycin

Stark differences in rheological behaviour were observed among some of the antibiotic suspensions assessed: Figure 6-22. At 1/s shear rate, Pinewood 500 mg/5 mL was found to have a significantly higher viscosity than all other suspensions assessed ($p < 0.05$). However, pairwise statistical analysis of all other suspensions combinations revealed no statistically significant differences in viscosity at 1/s shear rate ($p > 0.05$).

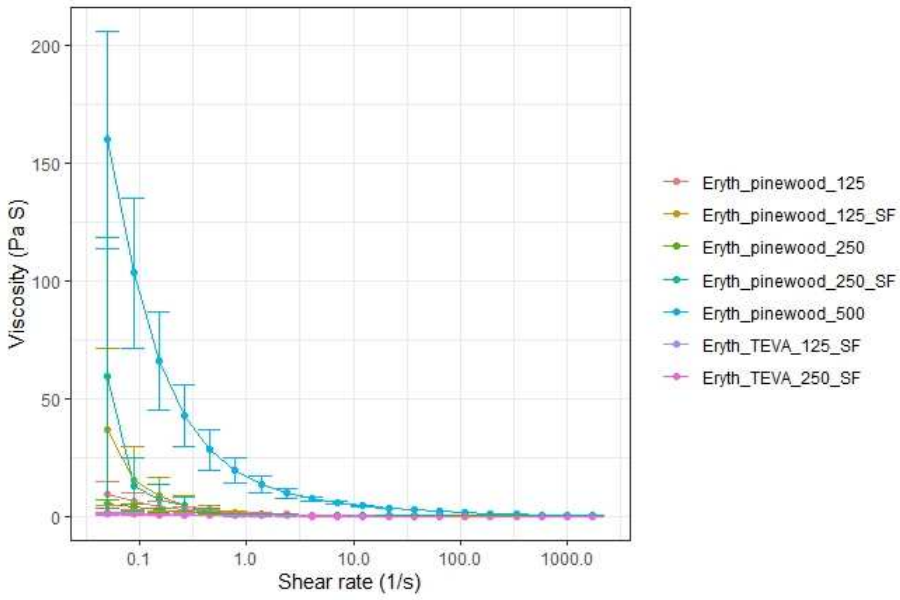


Figure 6-22 The rheological properties of the assessed erythromycin suspensions. The data are plotted as the mean, with error bars indicative of the SEM.

6.5.3 Predicting aversiveness in silico

In order to ascertain whether or not it is possible to predict *in silico* the rat response to a given suspension by knowing its bitterness level relative to quinine hydrochloride (QHCl) and its mouthfeel as governed by viscosity and particle size, the favoured models developed in chapter 5 were put to the test, namely model 3: negative binomial generalised linear model and model 4: zero-augmented hurdle negative binomial model. The relative model performances will be discussed in turn by API.

6.5.3.1 Clarithromycin

Neither the negative binomial model (model 3) nor the zero-augmented hurdle negative binomial model (model 4) were able to provide realistic predictions of lick number of the assessed Mylan clarithromycin suspensions based on their respective bitterness, grittiness and viscosity: Table 6-3. This is likely to be due to the excessive viscosities measured for these suspensions, which is a favourable parameter in the developed model, in combination with the very large mode particle sizes. Thus both models provided unrealistic predictions exceeding 60 licks/8 s.

Table 6-3 Summary of suspension parameters and model predictions for clarithromycin

Suspension	Viscosity at 1/s shear rate (PaS)	Mode Particle size (µm)	Bitterness (QHCl equivalence)	Actual mean lick number	Predicted model 3	Predicted model 4
Mylan clarithromycin 125 mg/5 mL	517.48	325	0	17.01	>60	>60
Mylan clarithromycin 250 mg/5 mL	359.52	325	0	12.72	>60	>60
Sandoz Clarithromycin 125 mg/5 mL	4.14	272	0	14.68	8.17	>60
Sandoz Clarithromycin 250 mg/5 mL	30.06	352	0	16.46	44.14	>60

More realistic predictions were provided by model 3 for the Sandoz suspensions with the predicted lick number for 125 mg/5 mL being 8.17 licks/8 s relative to the experimental value of 14.68 licks/8 s: Figure 6-23. While, model 3 predicted the lick number of the 250 mg/5 mL suspension to be 44.14 licks/8 s relative to the experimental value of 16.46 licks/8 s: Table 6-3. The observed over prediction for the 250 mg/5 mL suspension is again likely to be due to the large particle size skewing the model. It is however important to visualise the findings in Figure 6-23, which shows the range of individual rat responses to each suspension and where the model prediction lies in relation to the experimental values. Thus, the complexity of rat response, and what is trying to be predicted, is evident.

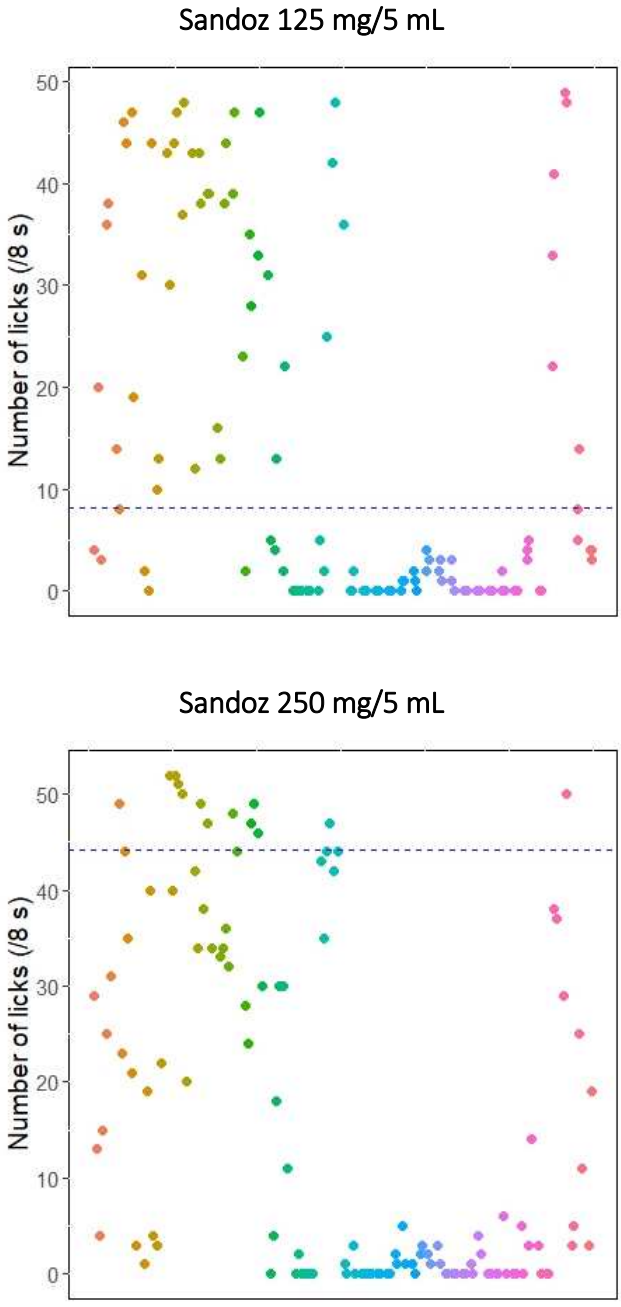


Figure 6-23 Assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

6.5.3.2 Co-amoxiclav

Both models 3 and 4 provided realistic predictions of lick number for all co-amoxiclav suspensions assessed: Table 6-4.

Table 6-4 Summary of suspension parameters and model predictions for co-amoxiclav

Suspension	Viscosity at 1/s shear rate (PaS)	Mode Particle size (µm)	Bitterness (QHCl equivalence)	Actual mean lick number	Predicted model 3	Predicted model 4
GSK coamoxiclav 125 mg/31 mg/5 mL	0.43	45.6	0	3.19	3.93	51.29
GSK coamox 250/62 mg/5 mL	0.43	45.6	0	6.18	3.93	51.29
Mylan co-amoxiclav 125/31 mg/5 mL	0.40	144	0	37.82	3.47	32.25
Mylan co-amoxiclav 250/62 mg/5 mL	0.21	186	0	8.10	3.03	20.63
Sandoz co-amoxiclav 125/31 mg/5 mL	0.37	144	0	6.58	3.43	30.91
Sandoz co-amoxiclav 250/62 mg/5 mL	0.55	144	0	7.82	3.68	39.92
Sandoz co-amoxiclav 400/57 mg/5 mL	0.52	163	0	37.43	3.56	35.26

However, as shown in Table 6-4, model 3 generally performed better at predicting the lick number of the co-amoxiclav suspensions assessed. Indeed, with the exception of Mylan co-amoxiclav 125 mg/31 mg/5 mL, which was very well received by the rats with a mean lick number of 37.82 licks/8 s, the range between model 3 predicted mean lick number and experimental lick number did not exceed 5.07 licks/8 s, thus demonstrating excellent predictability of rat response. Model 4 largely over predicted rat response to the co-amoxiclav suspensions, with the exception of Mylan 125 mg/31 mg/5 mL, which model predicted to elicit a rat response of 32.25 licks/8 s, relative to the actual response of 37.82

licks/8 s: Table 6-4. The performance of each model relative to the individual rat data can be seen in Figure 6-24 through Figure 6-30.

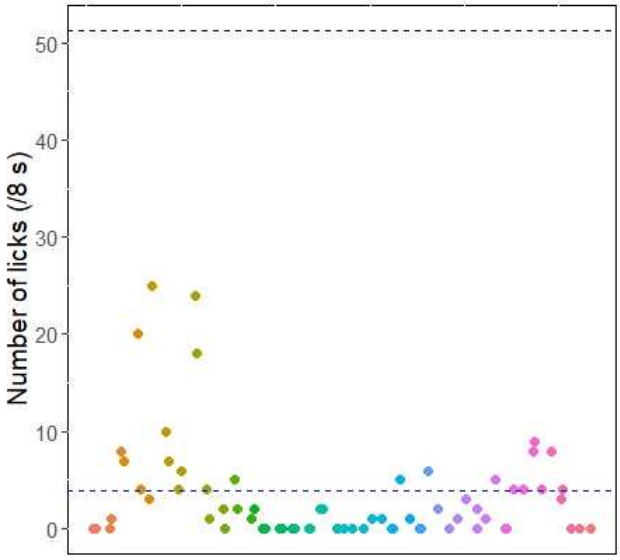


Figure 6-24 GSK Co-amoxiclav 125 mg/31 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

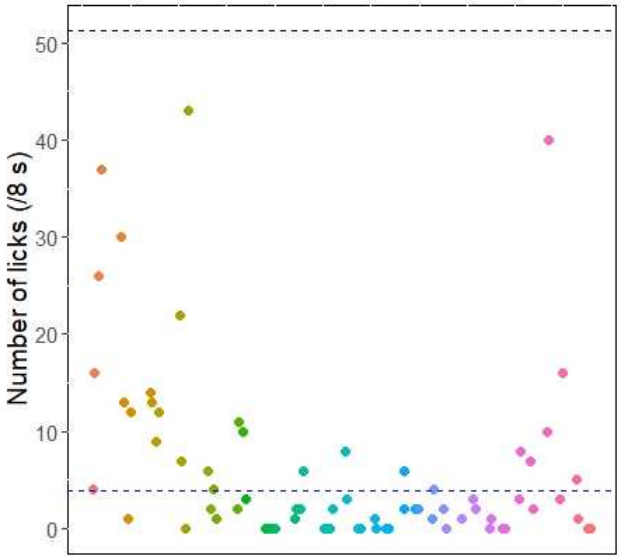


Figure 6-25 GSK Co-amoxiclav 250 mg/62 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

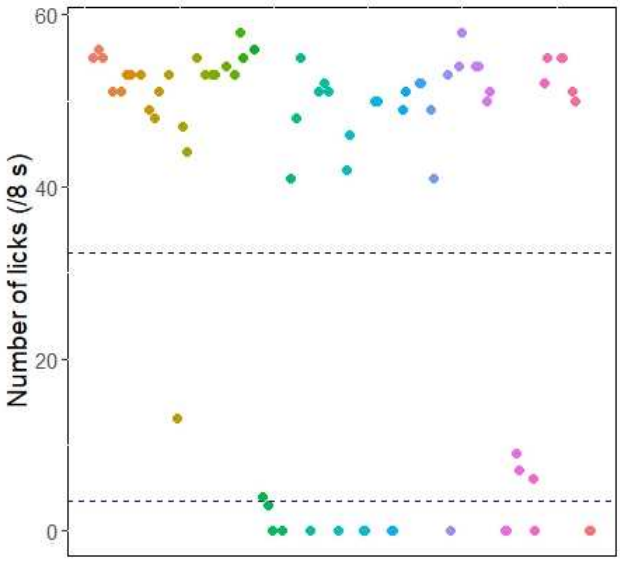


Figure 6-26 Mylan Co-amoxiclav 125 mg/31 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

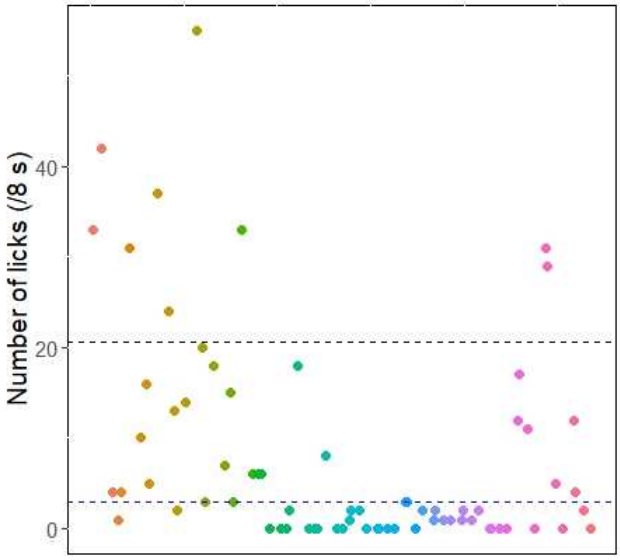


Figure 6-27 Mylan Co-amoxiclav 250 mg/62 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

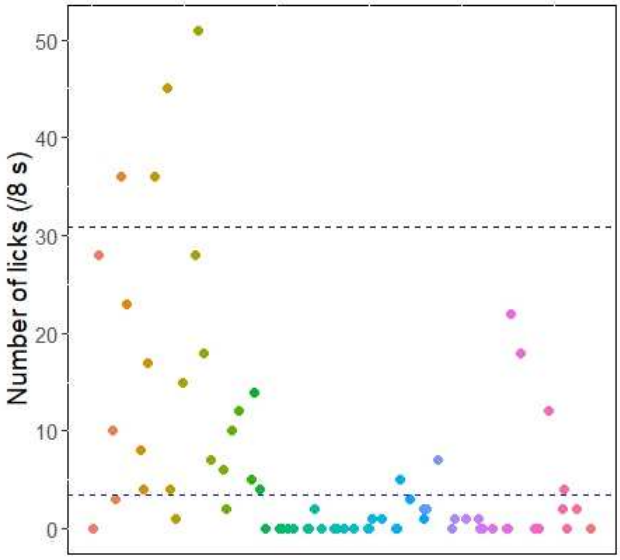


Figure 6-28 Sandoz Co-amoxiclav 125 mg/31 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

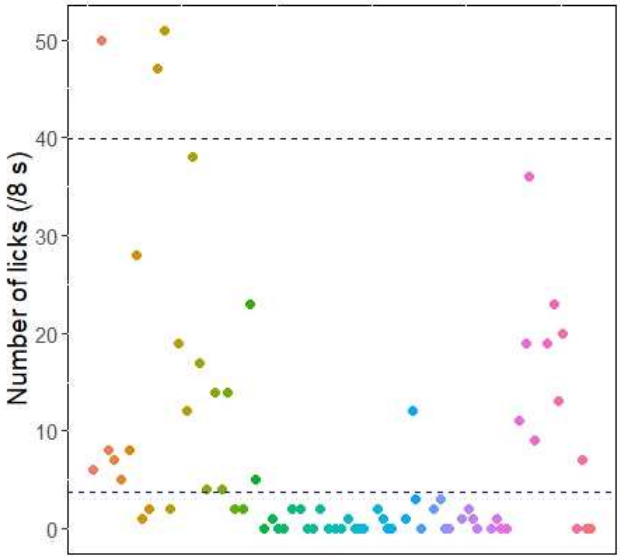


Figure 6-29 Sandoz Co-amoxiclav 250 mg/62 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

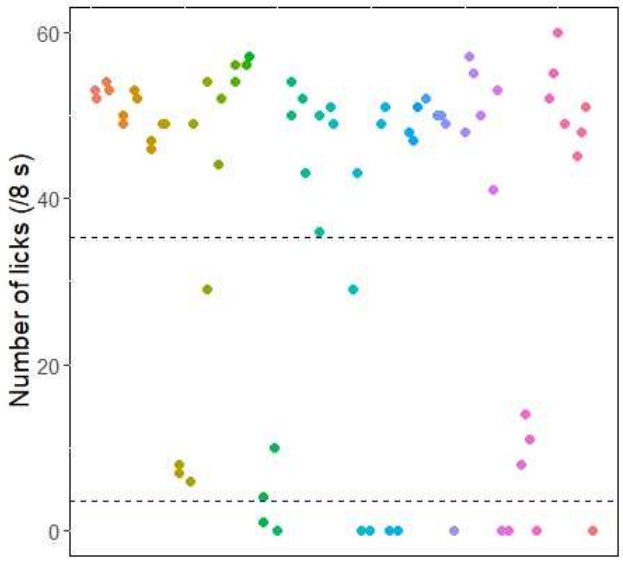


Figure 6-30 Sandoz Co-amoxiclav 400 mg/57 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

6.5.3.3 Erythromycin

For the assessed erythromycin suspensions, model 3 was also found to outperform the predictability of model 4, with some astonishingly close predictions of lick number. For example, in the case of Teva erythromycin 250 mg/5 mL SF, model 3 predicted the mean lick number to within 0.11 licks/8 s, with predicted and experimental values of 3.65 and 3.54, respectively: Table 6-5.

Table 6-5 Summary of suspension parameters and model predictions for erythromycin

Suspension	Viscosity at 1/s shear rate (PaS)	Mode Particle size (µm)	Bitterness (QHCl equivalence)	Actual mean lick number	Predicted model 3	Predicted model 4
Pinewood erythromycin 125 mg/5 mL	1.43	31.1	0	3.60	5.39	>60
Pinewood erythromycin 125 mg/5 mL SF	1.36	66.9	0	1.93	5.14	>60
Pinewood erythromycin 250 mg/5 mL	1.04	35.3	0	3.11	4.83	>60
Pinewood erythromycin 250 mg/5 mL SF	0.71	31.1	0	3.75	4.39	>60
Pinewood erythromycin 500 mg/5 mL	19.3	45.6	0	4.23	30.37	>60
Teva Erythromycin 125 mg/5 mL SF	0.27	58.9	0	2.80	3.65	38.57
Teva Erythromycin 250 mg/5 mL SF	0.23	45.6	0	3.54	3.65	38.57

The only suspension for which model 3 provided a poor prediction was Pinewood 500 mg/5 mL: Figure 6-35. For this suspension, model 3 predicted a rat response of 30.37 licks/8 s, relative to the experimentally observed value of 4.23 licks/8 s. The relatively large viscosity of this suspension – 19.3 Pa s – which is a favourable parameter within model 3, is the likely explanation for this over prediction.

Model 4 provided unrealistic predictions for all Pinewood suspensions, with values exceeding 60 licks/8 s. The small particle size, combined with the higher viscosity values of these suspensions is the likely cause of these over predictions. Model 4 was, however, capable of predicting realistic values for the Teva suspensions, but the values were incorrect by a factor of ten.

A visualisation of how each model performed, and the individual experimental values are shown in Figure 6-31 through Figure 6-37.

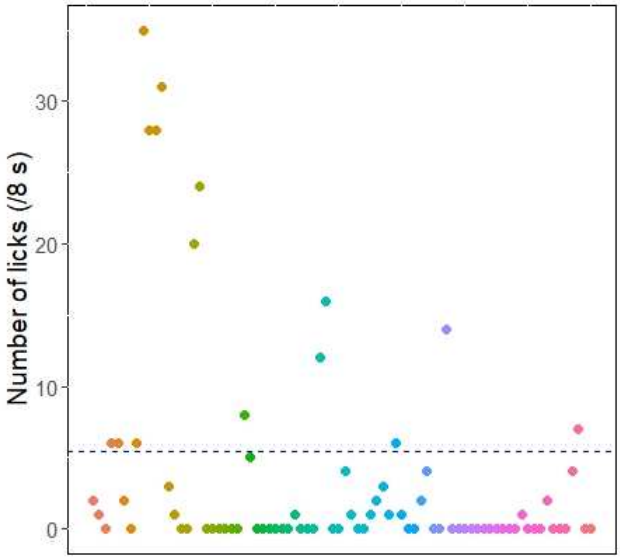


Figure 6-31 Pinewood erythromycin 125 mg/5 mL: assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

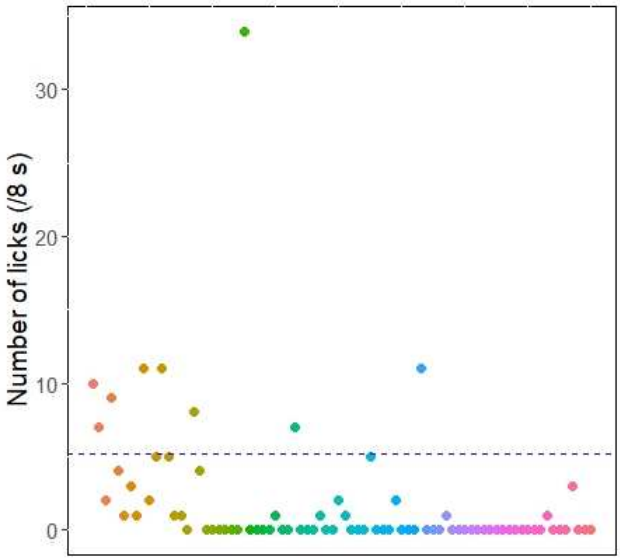


Figure 6-32 Pinewood erythromycin 125 mg/5 mL SF: assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

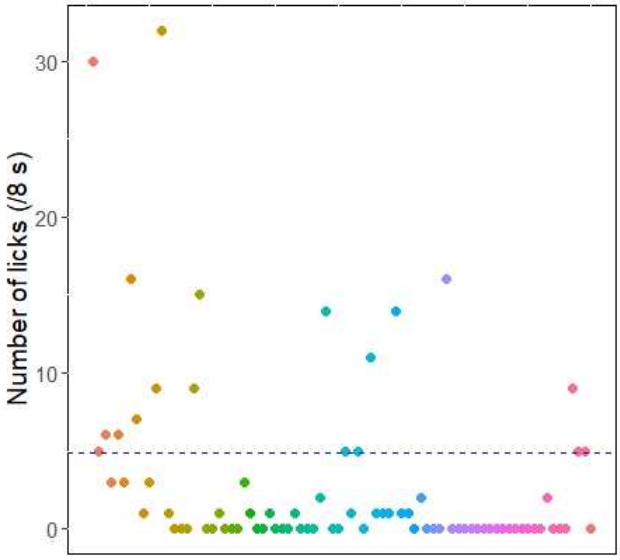


Figure 6-33 Pinewood erythromycin 250 mg/5 mL: assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

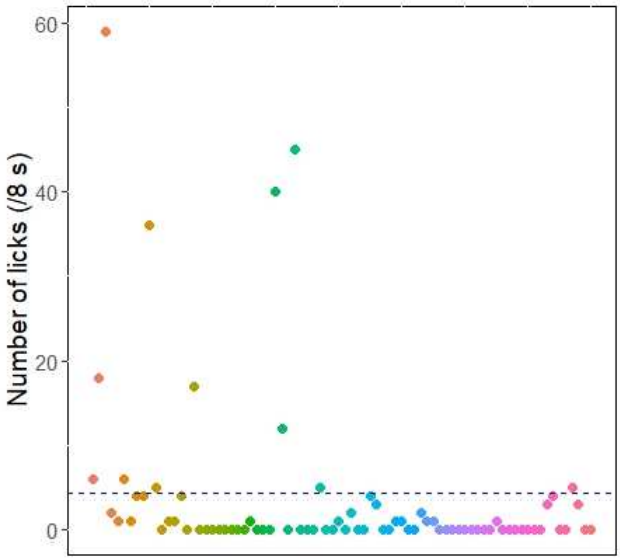


Figure 6-34 Pinewood erythromycin 250 mg/5 mL SF: assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

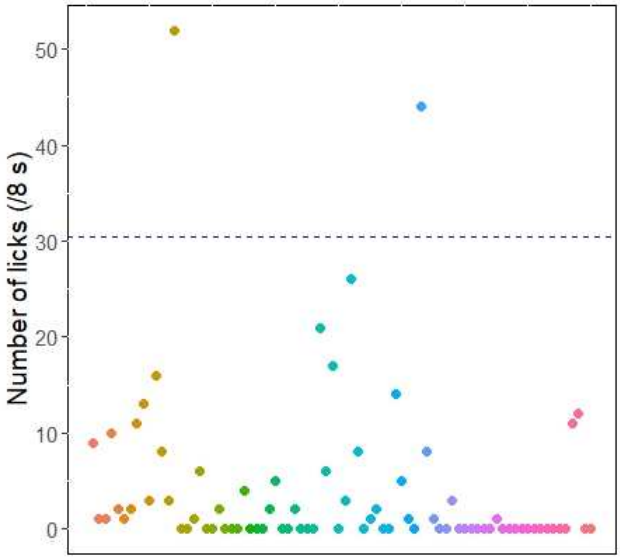


Figure 6-35 Pinewood erythromycin 500 mg/5 mL: assessing how the models fit the data. Model 3 is represented as a blue dashed horizontal line. Each coloured point is representative of a single rat response.

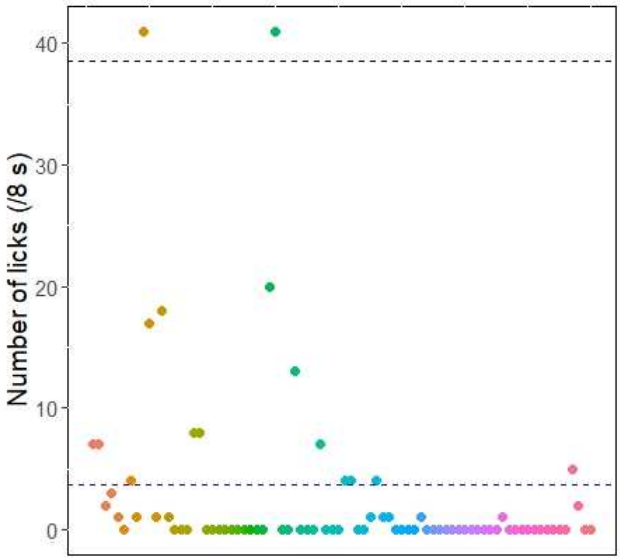


Figure 6-36 Teva erythromycin 125 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

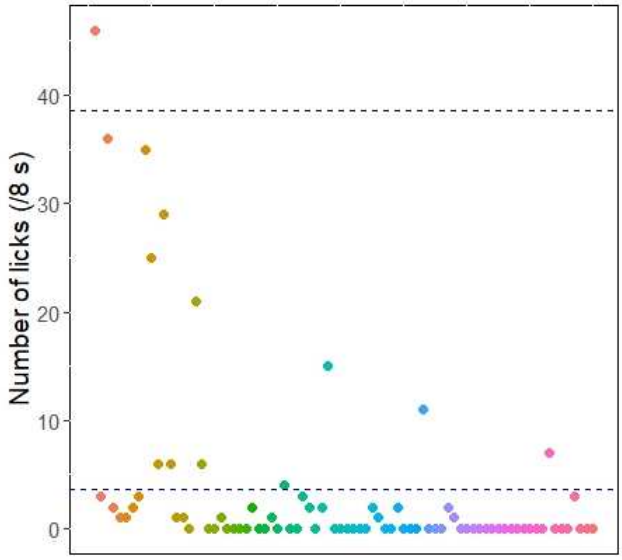


Figure 6-37 Teva erythromycin 250 mg/5 mL: assessing how the models fit the data. Models 3 and 4 are represented as blue and black dashed horizontal lines, respectively. Each coloured point is representative of a single rat response.

6.6 Discussion

The problem of taste in antibiotic suspensions and the subsequent issues with acceptability and thus treatment compliance were highlighted in section 6.1 including a demonstration of the lengths to which parents must go in order to successfully administer antibiotics to their children. Thus, the need for acceptability testing of antibiotic suspensions was identified, and the earlier this can occur during the drug development process the better so as to enhance efficiency in the development process and mitigate late-stage acceptability-related product attrition. Given the promising data presented in chapter 5 in which the ability of rats in the BATA model to distinguish between suspensions of differing mouthfeel as well as bitterness, the BATA model was proposed as a method by which paediatric suspensions might be screened for acceptability during early-phase drug development.

This is the first study of its kind to assess the aversiveness of suspensions using the rat BATA model. Indeed, a search of the literature returns no results when all combinations of the search terms: 'rat', 'taste', 'suspensions', 'antibiotic', 'clarithromycin', 'co-amoxiclav' or 'erythromycin'. Thus, it was not possible to correlate the findings of the presented data to those already published.

An ability of the rat BATA model to differentiate between different brands of antibiotic suspensions was identified in this study, although this was API-dependent. Indeed, no significant differences between brand were identified for erythromycin, however significant differences were identified for both clarithromycin and co-amoxiclav.

The data in the literature assessing differences between the acceptability of antibiotic brands are sparse and incomplete, but what data are there point to branded products being more acceptable than their generic equivalents [34]. However, this study found that branded co-amoxiclav at a strength of 125 mg/31 mg/5 mL (Augmentin®) was more aversive than the Mylan generic brand. This may be due to the differences between aspartame levels in the branded suspension relative to that of the generic, being 2.5 mg/mL and 1.7 mg/mL, respectively: see Table 6-1. However, looking at the BATA response to sweeteners, Soto, J found that concentrations of aspartame between 0.05 mg/mL and 2.6 mg/mL, do not elicit a lick number significantly different to that of water; only at 7.9 mg/mL was a significantly different lick number observed (unpublished data). Other excipient differences exist between the different brands: the branded suspension uniquely contains hypromellose, succinic acid and xanthan gum, while the Mylan and Sandoz brands contain citric acid, sodium citrate, talc and guar galactomannan (Table 6-1). Flavour differences were also found with the branded suspension containing orange, raspberry and golden syrup flavours, while the generic Sandoz and Mylan suspensions both contained lemon-peach, apricot and orange with an essence of bergamot (Table 6-1). Thus the BATA output is describing a highly complex mixture, and the reason for the differences observed cannot be fully elucidated. In humans, Cohen et al. identified that the generic co-amoxiclav suspension is more likely to be spat out that the branded version, however the exact generic against which the branded product was compared was not stated, thus making correlation to this study difficult [220]. However, the exciting finding is that the rats are capable of distinguishing between highly complex mixtures.
Differences between brands were also identified among the clarithromycin suspensions assessed, with the superior brand being dependent on the strength. At a strength of 125 mg/5 mL, the Mylan brand was found to be less aversive, while at 250 mg/5 mL, the Sandoz strength was found to be less aversive. The differences cannot be due to differences in flavour given that all clarithromycin suspensions contained fruit punch

flavour. However the identified differences may be a result of differences in sucrose levels between both brands and strengths: at 125 mg/5 mL, the Mylan brand contains 550 mg/mL sucrose and achieves a higher lick number than the Sandoz brand, which contains 480 mg/mL sucrose; whereas at 250 mg/5 mL, the Sandoz brand is more accepted than the Mylan brand, containing 480 mg/mL and 455 mg/mL sucrose, respectively. Further excipient differences can be seen in Table 6-1, which shows that the Mylan suspension contains two unique excipients to the Sandoz suspensions, namely castor oil and citric acid. While, the Sandoz suspension contains five excipients unique to the Mylan brand, namely macrogol, methacrylic acid:ethylacrylate (1:1) copolymer, triethyl citrate, glyceryl monostearate and polysorbate 80. Therefore, as for the differences between the co-amoxiclav suspensions, the BATA output is a description of a highly complex mixture, thus it is difficult to determine exactly the reason for the identified differences. However, the aim of the study was not to assess why differences between antibiotic suspensions occur, it was to identify whether the BATA model was capable of picking up such differences, which excitingly it has done In humans, two studies in the literature have assessed the differences between branded clarithromycin suspensions, both identifying the Mylan brand to be superior [228,229]. The first study compared the taste of the Mylan brand at a strength of 250 mg/5 mL to other commercially-available clarithromycin suspensions in Palestine using a facial hedonic scale in paediatric patients and community pharmacists [229]. The second study sought to evaluate the ability of patient-reported outcome measurements as a reliable tool to evaluate acceptability in a paediatric inpatient population, thus assessment of brand differences in acceptability was a secondary outcome of this study and thus the sample sizes were very small to enable statistical analysis [228]. Nonetheless, the study did find that the Mylan brand was superior to the Sandoz brand, but the strength of the assessed suspensions were not reported [228]. Thus, comparison with the literature is difficult, given the sparsity of the data available. However, the identified studies do point to brand differences in acceptability, which the BATA model has been able to replicate for both clarithromycin and co-amoxiclav, thus suggesting an ability of the BATA model to differentiate among complex formulation differences.

This study also sought to investigate the ability of the BATA model to differentiate between APIs, regardless of brand. The identified ranking of aversiveness was dependent on the strength of API. At a strength of 125 mg/5 mL (including 125 mg/31 mg/5 mL co-amoxiclav), clarithromycin and co-amoxiclav were found to not be significantly different to each other in terms of aversiveness, while both were significantly less aversive than erythromycin. At a strength of 250 mg/5 mL (including 250 mg/62 mg/5 mL co-amoxiclav), erythromycin was also found to be the most aversive API, but was not significantly different co-amoxiclav, while clarithromycin was significantly less aversive than the aforementioned. To summarise, see Figure 6-38:

	Increasing aversiveness →
125 mg/5 mL	Clarithromycin = Co-amoxiclav > Erythromycin
250 mg/5 mL	Clarithromycin > Co-amoxiclav = Erythromycin

Figure 6-38 Summary of aversiveness of antibiotic suspensions by API

Baguley *et al.*[34] characterised the assessed antibiotics as follows:

- Co-amoxiclav: children will normally swallow
- Clarithromycin: children might swallow
- Erythromycin: children will normally spit out and/or grimace

Thus, the aversiveness as per Baguley *et al.*[34] is co-amoxiclav > clarithromycin > erythromycin, thus some agreement can be seen with the BATA model findings. However, other studies refute the aforementioned ranking by Baguley *et al.*[34]. Indeed, Mistry *et al.* found clarithromycin suspensions to be worse than co-amoxiclav in terms of acceptability [228]. Other studies have also reported co-amoxiclav to be an acceptable suspension, and clarithromycin poorly accepted [230–236]. Thus, it is not clear which ranking is correct, thus necessitating a future human taste panel assessing the acceptability of the assessed suspensions. However the findings do show an ability of the BATA model to distinguish between suspensions of different antibiotic APIs, a promising finding in itself given the complexity of this formulation consisting multiple excipients and flavours.

The suspensions were further characterised by assessing the innate aversiveness of the API by assessing the taste of API alone dissolved in water at its saturation water solubility and the mouthfeel, by assessing both the viscosity and particle sizes. In the first study of its kind, the data on bitterness, viscosity and grittiness were used to predict *in silico* the

rat response to the assessed antibiotics using two models developed in chapter 5, designed by using design of experiment principles to assess the interplay of viscosity, grittiness and bitterness. It was found that generally, the negative binomial generalised linear model (model 3) outperformed the: zero-augmented hurdle negative binomial model (model 4), however this was API specific with excellent predictability observed for erythromycin, intermediate predictability with clarithromycin and very poor predictability with co-amoxiclav. The exact reasons for the different performances of the models for different APIs are unknown, and thus require further investigation, specifically looking into the different excipient compositions of the antibiotic suspensions, and incorporation of the effect of excipients into the *in silico* models. Importantly however, this study has demonstrated the power that *in silico* models may hold in the future, and provide a further example of how animal data may be leveraged using *in silico* models to reduce animal research to a minimum.

6.7 Conclusion

The poor acceptability of antibiotic suspensions was identified in the literature, with differences observed both between antibiotic APIs and among different brands of the same antibiotic. The lack of acceptability testing of antibiotics was also noted, particularly during early phase drug development during which a lack of demonstrated human safety preclude human taste panels. Previous data using the rat BATA model showing an ability of rats to distinguish between suspensions of varying bitterness and mouthfeel (chapter 5) pointed towards the use of the BATA model in the assessment of antibiotic suspension acceptability. Indeed, the rat BATA model demonstrated an ability to distinguish both between antibiotic APIs and brands of the same API, with some agreement with the literature. However the lack of agreement in the literature as to the acceptability of different antibiotic suspensions means such conclusions must be taken carefully, and require a human taste panel to be conducted on the assessed antibiotic suspensions in order to best assess the human-rat correlation. Furthermore, *in silico* models developed in chapter 5 were put to the test in predicting rat response using the bitterness, particle sizes and viscosity of the assessed suspensions, with varying API-dependent success. Indeed, the predictive power of the models reduced from erythromycin to clarithromycin to co-amoxiclav. Further model development is necessary to elucidate why such different

predictability was observed, perhaps incorporating different excipient profiles. Nonetheless, this work has demonstrated an exciting way in which the rat BATA data may be leveraged, and point to a future in which computer models may reduce the use of animals in sensory research in line with the principles of the 3Rs.

7 Expanding the BATA model to solid dosage forms

7.1 Introduction

There are multiple ways in which the identified problem of bad-tasting medicines may be mitigated. These methods can be classified as either masking the taste of the bitter 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 [9]. However, the use of excipients in paediatric preparations comes with additional considerations, encompassing technical, safety and regulatory challenges. Therefore any additional excipients can yield issues given the associated regulatory constraints, particularly when considering use in younger children [9] while the use of more complicated techniques introduces challenges in manufacture and product development, which may affect the commercial viability of a product [237].

There is growing interest in the use of multiparticulates as a means to overcome the identified challenges. The multiparticulate dosage form is a platform technology that can 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 [45]. These are drug delivery systems (DDSs) comprised of multiple solid units, such as pellets or minitablets [187].

Alkindi® provides an example of a newly developed formulation utilising multiparticulate technology to overcome the lack of paediatric hydrocortisone formulations for paediatric adrenal insufficiency [238]. Prior to the development of Alkindi, clinical practice governed pharmacists to extemporaneously prepare paediatric hydrocortisone formulations by tablet crushing or using a hydrocortisone base to produce a powder or solution, while in some countries such as the UK, parents were expected to crush tablets, with the inherent risk of dosing inaccuracy [239–241]. Indeed, a study found that pharmacist-compounded hydrocortisone batches failed to meet European pharmacopoeial guidelines in 21.4 % of assessed samples, while 3.6 % of batches contained no hydrocortisone at all [240]. The problem is far worse when tablet crushing by parents is assessed, with a UK study

revealing more than 50 % of doses were at least 10 % out of specification [241]. Alkindi overcomes this issue by presenting the hydrocortisone as a multiparticulate granule formulation in a transparent capsule as 0.5, 1, 2 and 5 mg strengths, which can be opened for dosing. The granules consist an inert cellulose core, a spray coat of hydrocortisone and a taste-masking layer to prevent the bitter taste of hydrocortisone being tasted by the patient [238].

Thus, Alkindi provides an example that 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 such as Opadry EC. Further, the pH dependent water insoluble polymers can be further classified based on their release profile within the stomach, and include reverse enteric such as Smartseal 100P, enteric such as Eudragit L 100 and Acryl EZE, and their combinations [242,243].

Although, taste masking using coating technologies seemingly provides a simple solution to this complex problem, it is important that the formulator is capable of achieving sufficient taste-masking without hindering bioavailability through excessive coating. Thus, how can we test the taste of coated solid oral dosage forms such as multiparticulates, particularly during early drug development when insufficient toxicological data prevents the use of human taste panels? Given that only API dissolved in the human oral cavity is capable of interacting with the taste receptors and thus eliciting a taste, assessment of drug release from a coated solid oral dosage form within a system replicative of the human oral cavity may provide some insight [93]. Thus, dissolution testing may provide some of the answers. Further, given that different APIs elicit aversiveness at different concentrations, a threshold concentration above which aversiveness may be deemed likely must be linked to the proposed dissolution test. However, in a review paper by Gittings et al. it was identified that there is currently no such dissolution test that replicates closely enough the human oral cavity and linking taste thresholds that enables the prediction of *in vivo* taste masking efficacy [100].

When a solid oral dosage form is placed in the mouth, it will reside in the oral cavity for a small amount of time before the patient swallows it, thus drug release within the first 30 seconds is critical to taste-masking assessment. The dosage form will contact a small volume of saliva, and swallowing may occur before the dosage form is actually swallowed, thus non-cumulative drug release is of the greatest interest as this will govern the concentration present within the sink conditions of the mouth at any given time and thus the taste that will be elicited. Therefore, the concentration at which the API elicits an aversive taste must be known and linked to drug release when assessing taste-masking. Finally the dissolution test must be 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 [100,101]. Furthermore, such a test would have to be able to discriminate between different coating technologies, predictive of taste and inform formulation design.

This chapter questions if it is possible to replicate/simulate conditions within the human oral cavity *in vitro* thus enabling a biorelevant dissolution test capable of assessing taste-masking efficacy [244].

7.2 Aims

Assess the feasibility of assessing taste-masking efficacy *in vitro* using drug release in a biorelevant dissolution test.

7.3 Objectives

- Design and manufacture a novel dissolution column replicating the volume of saliva within the human oral cavity and enabling a flow through set-up utilising a biorelevant dissolution medium.
- Generate taste thresholds for assessed APIs and link to dissolution such that drug release can be assessed in the context of taste.
- Validate method using a range of taste-masking technologies and APIs.

7.4 Materials and Methods

7.4.1 Materials

The chlorphenamine maleate (CPM) and sildenafil citrate (SDC) used in the BATA was purchased from Sigma Aldrich (St Louis, Missouri, USA), while those for the human taste panel, in addition to sodium chloride were purchased from Fagron (Rotterdam, The Netherlands). The sildenafil beads, including uncoated, Smartseal 30D and Eudragit EPO coated, were provide by Pfizer (Kent, UK). The CPM loaded multiparticulates were prepared by Colorcon 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); 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). Eudragit EPO coated sildenafil bead (BN: 709287).

7.4.2 Methods

7.4.2.1 Buccal dissolution test development: sildenafil citrate(SDC) multiparticulates

In order to develop the dissolution methodology, SDC multiparticulates were used as an initial test formulation as part of the SPeaDD consortium (www.paediatricscienceuk.com). They were coated (15 % weight gain) with pH sensitive reverse enteric coatings (Eudragit EPO and Kollicoat Smartseal 30 D) and had a drug loading of 20 mg SDC/500 mg. An uncoated control was also tested (drug loading: 20 mg SDC/425 mg). This technology was selected by the SPeaDD consortium to be investigated for their effectiveness in taste masking. Eudragit EPO has been the most widely used reverse enteric coating. More recently Kollicoat Smartseal 30D has become available which uses a copolymer comprising of methyl methacrylate (MMA) and diethyl-aminoethyl methacrylate copolymer (DEAEMA) which has a lower solubility at the pH of the oral cavity and therefore potentially has superior taste masking capabilities A methodology capable of both distinguishing between different coating technologies and providing an absolute prediction of taste-masking was sought.

The dissolution method was performed using an Icalis peristaltic pump PCP490 and Tygon R-3603 tubing, as a flow system (Figure 7-1).

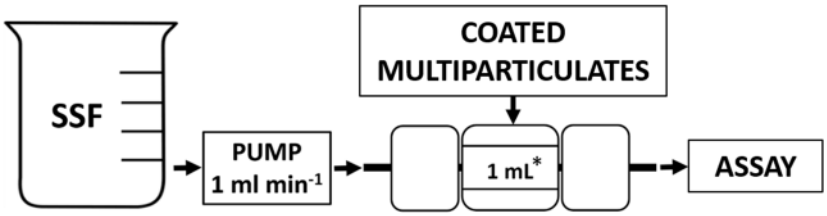


*Figure 7-1 Flow diagram representing the biorelevant buccal dissolution test (*calculated internal volume of the column)*

The speed of the rotators was set to 4.5 rotations per minute in order to achieve a flow rate of 1mL min^{-1}, simulating unstimulated salivary flow rate. The media supplied to the pump was simulated salivary fluid (SSF) of pH 7.4 (Table 7-1), used by Guhmann *et al.* [115].

Table 7-1 Composition of SSF (in full) as per Guhmann et al.[115]

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

A novel column was designed and built in-house to mimic the oral cavity (Figure 7-2). The column – machined using solid acrylic - has an inner diameter of 5 mm and length of 5 cm, giving a calculated internal volume of 0.98 mL. The coated beads had a drug loading as stated above (20 mg SDC/500 mg) and since the lowest dose of SDC given is 25 mg, the quantity of beads chosen to fill the column prior to dissolution assessment was 625 mg.

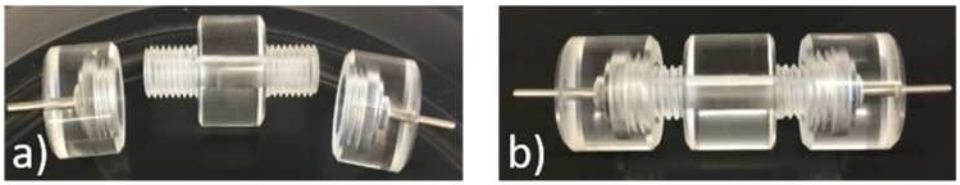


Figure 7-2 Buccal dissolution test column manufactured for the dissolution test showing a) three parts of the column that can be assembled together after sample placed within it and b).the column when completely assembled.

Meshes of size 50 µm and 100 µm were placed at each end of the column to keep the sample in place along with rubber washers to prevent leaking. After assembly, one side of the column was then attached to the Tygon tubing, which was connected to the peristaltic pump.

Sampling was carried out at the following time points: 60, 80, 100, 120, 180, 240 and 300 seconds. Sample collection began 10 seconds prior to the stated time points in order to allow collection of a sufficient volume to pipette 10µL.

7.4.2.1.1 SDC Assay

Samples were analysed using high-performance liquid chromatography (HPLC), equipped with an Agilent Technologies 1200 series degasser, quaternary pump, auto-sampler, thermostatted column compartment and a variable wavelength detector. A Synergi 4u Polar-RP 80A column (4µm, 250×4.60 mm; Phenomenex Inc.) was used and the column temperature was set at 40°C. 10µL of the contents from each Eppendorf tube was pipetted into HPLC vials, and diluted 15-fold with 140µL 20%v/v methanol in water. Two mobile phases were used in this method:

- mobile phase A consisting potassium phosphate buffer – containing potassium dihydrogen phosphate (2.6 ± 0.2g/L), dipotassium hydrogen phosphate trihydrate (1.4 ± 0.2g/L), acetonitrile (50mL/L), triethylamine (1.5mL/L) and adjusted to pH 6.5 with orthophosphoric acid
- mobile phase B was acetonitrile.

The volumes were set at 40% and 60% for mobile phases A and B respectively. The flow rate was 1.0mL/min and the injection volume was 10µL. A needle wash containing 100%

methanol was prepared. The ultra-violet (UV) detector was set at a wavelength of 290nm. A calibration curve was prepared with an R^2 value of 0.99923.

7.4.2.2 Testing the methodology with chlorphenamine maleate (CPM) multiparticulates

In order to further assess the ability of the developed dissolution methodology to provide information on taste-masking of multiparticulates, it was further tested using multiparticulates loaded chlorphenamine maleate, and coated with a wider range of coating technologies. Chlorphenamine maleate was chosen as it is known to suffer with problems of bitterness [245], and had not been explored by the research group previously, thus it was an API to which we were naïve. Thus, threshold concentrations in humans and rats had to be first identified, prior to *in vitro* evaluation of taste masking. In this instance, the developed dissolution methodology was compared to conventional USP I (basket) dissolution methodology to ascertain whether the developed methodology was able to yield more information.

7.4.2.2.1 Taste thresholds

7.4.2.2.1.1 BATA procedure

The BATA procedure and data analysis was identical to that which is described in other parts of this thesis, however the chlorphenamine maleate samples were presented at concentrations ranging from 0.005 to 18 mg/mL in triplicate and at random.

7.4.2.2.1.2 Human taste panel

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).

The human taste panel procedure and data analysis was identical to that which is described in other parts of this thesis. Participants were presented with 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).

7.4.2.2.2 Taste masking of CPM

Chlorphenamine maleate (CPM), a BCS class 1 API, was used as the model bitter drug [245]. 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) and a pH dependent water insoluble reverse enteric coating (developmental fully formulated system based on Kollicoat Smartseal 100P).

Drug layering and barrier membrane taste mask coating of the sugar spheres were performed at Colorcon. The Surelease:Opadry, an aqueous 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 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 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 7-2). The research team received them labelled randomly A to G to perform the dissolution experiments blindly.

Table 7-2 CPM multiparticulate 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

7.4.2.2.3 Drug release assessment

7.4.2.2.3.1 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.

7.4.2.2.3.1.1 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.

7.4.2.2.3.1.2 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.

7.4.2.2.3.2 Buccal dissolution test with CPM multiparticulates

The developed novel dissolution apparatus was used to assess drug release from the CPM multiparticulates (600 mg) (drug loading: ~30 mg/g) under investigation. All parameters regarding experimental methodology were identical to those detailed in 7.4.2.1. 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.

7.4.2.2.3.2.1 CPM HPLC-UV assay

Samples 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.

7.4.2.2.3.2.2 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 above.

7.4.2.2.3.3 USP I (Basket) dissolution test in simulated gastric fluid (SGF)

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.

7.4.2.2.3.3.1 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 CPM concentration within each sample.

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.

7.5 Results

7.5.1 Column development: SDC

A novel flow through dissolution column was developed satisfying the requirements as outlined in 7.1 to replicate the cavity, namely an internal volume of 1 mL, a flow rate of 1 mL min $^{-1}$ and a dissolution medium replicative of human saliva, was manufactured successfully and used to simulate the human oral cavity in the assessment of the taste-masking efficacy of SDC multiparticulates coated using Eudragit EPO and Smartseal 30 D, in addition to an uncoated control.

The values shown in Table 7-3 were used to inform the thresholds above which, the formulation would be deemed aversive and thus not adequately taste-masked. Thus, release data from the multiparticulates assessed was linked to said thresholds, thus allowing comparison of taste-masking efficacy. Indeed, this test was able to distinguish between formulations coated using different coating technologies.

Table 7-3 Taste thresholds – EC_{50} and IC_{50} – for SDC taken from rat BATA experiments and human taste panels respectively [157]*,*

Human EC_{50}	1.05 mg/mL
Rat IC_{50}	1.32 mg/mL

Figure 7-3 demonstrates the ability of this novel dissolution method to distinguish between multiparticulates of varying coatings: it shows the cumulative release as % drug release. As expected, the uncoated particles (coating = NONE) show the greatest amount of release. Comparison of Eudragit EPO (coating = EPO) and Smartseal 30D (coating = SSD) reveal interesting differences. EPO prevents release of any detectable level of SDC up to 240 seconds, far exceeding the time that an oral dosage form would be retained in the mouth. By contrast SSD shows immediate release of sildenafil citrate, up to approximately 3 % non-cumulative release at 100 seconds. The significance of this in terms of aversiveness is dependent on the link between the measured release values and the bitter taste threshold values: see Figure 7-4.

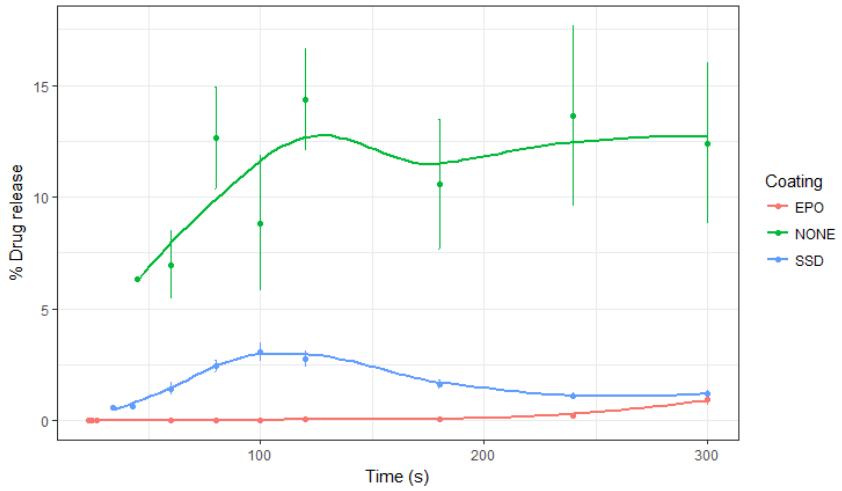


Figure 7-3 Cumulative release of SDC from multiparticulates consisting different coatings: None (n = 6), Eudragit EPO (EPO) (n = 7) and Smartseal 30D (SSD) (n = 8).

Figure 7-4 shows the non-cumulative concentrations achieved in the column, as this is most reflective of condition of that which would be expected in the mouth given the constant production of saliva and subsequent swallowing, thus non-cumulative concentration-time plot is the most biorelevant. It demonstrates that a lack of coating on sildenafil citrate multiparticulates – shown in green – allows release of sildenafil citrate to an extent significantly greater than both the IC_{50} and EC_{50}, indicative of an inadequately taste-masked formulation. By contrast, Eudragit EPO sufficiently prevents release of sildenafil citrate with minimal release even after 300 seconds. Indeed, the release values are significantly lower than both the IC_{50} and EC_{50}, indicative of an adequately taste-masked formulation. Smartseal 30D, however was unable to prevent the release of sildenafil citrate to a point significantly lower than the human EC_{50}, as indicated by the overlap of error bar with the EC_{50} horizontal line in Figure 7-4, thus indicating a poorly acceptable formulation. It must be noted, however that the non-cumulative release only just exceeds the EC_{50}, which may question whether this would actually be significant in terms of taste. To address this question, it is important to identify what the EC_{50} tells us; importantly it is not a detection threshold, thus the taste can be detected at much lower concentrations. It is the concentration eliciting 50 % of the maximum aversiveness response by humans, thus it does represent an aversive taste and therefore any concentration that equals or exceeds this value will certainly be aversive to the patient, demonstrating a marked failure of the Smartseal 30D coat. Smartseal 30D did not however enable release of sufficient drug to exceed the rat IC_{50}.

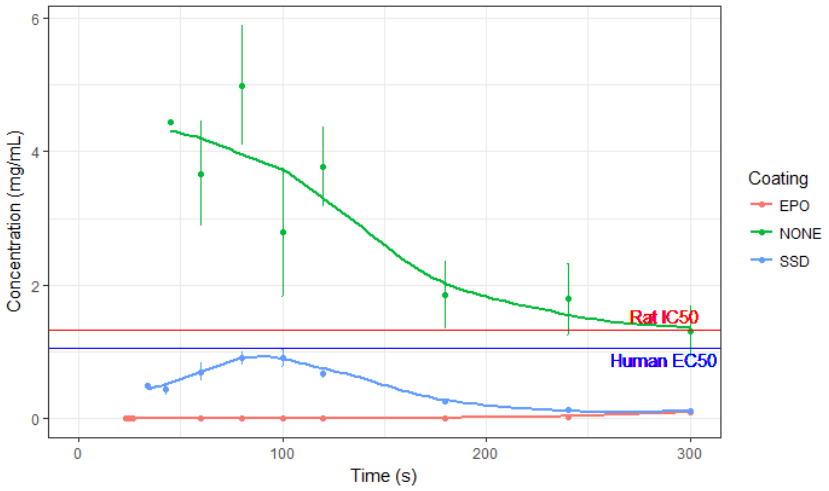


Figure 7-4 Linking drug release data to bitter taste thresholds. A non-cumulative concentration-time plot showing the human EC50 and rat IC50 values as blue and red horizontal lines respectively. Coatings: None (n = 6), Eudragit EPO (EPO) (n = 7) and Smartseal 30D (SSD) (n = 8).

7.5.2 Testing the methodology: CPM

7.5.2.1 Taste Thresholds

7.5.2.1.1 Rat BATA

Rat BATA analysis of increasing concentrations of CPM in water was successfully carried out with the results shown in Figure 7-5. 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 ($p>0.05$).

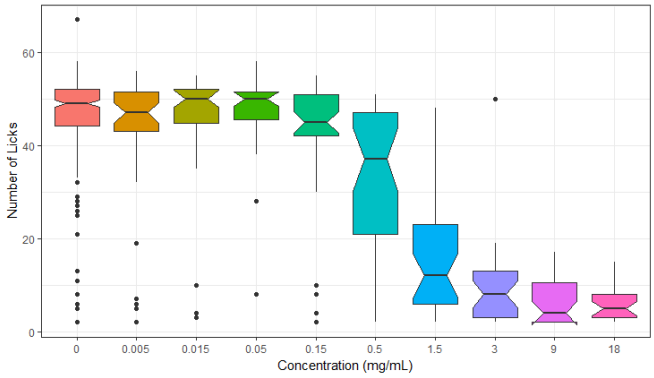


Figure 7-5 Rat response (number of licks) to increasing concentrations of CPM in water.

Figure 7-6 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[152]. 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.

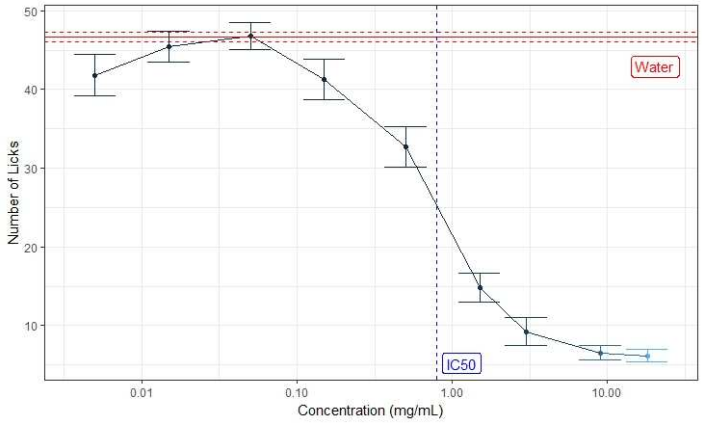


Figure 7-6 Mean number of licks [+/- standard error of the mean (SEM)] as a function of increasing CPM concentration (mg/ml). The water control is shown as a solid red line (mean number of licks), with the SEM as dashed red lines. The IC_{50} is shown as a blue line

7.5.2.1.2 Human Taste Panel

The human taste panel assessing increasing concentrations of CPM was successfully carried out, with results shown in Figure 7-7. 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 ($p<0.05$).

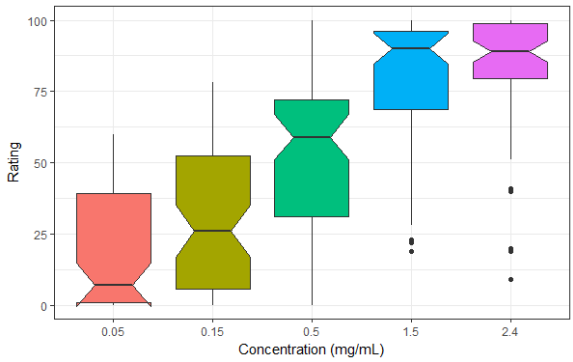


Figure 7-7 Participant aversiveness (VAS) response to increasing concentrations of CPM in water.

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.

7.5.2.2 Taste masking assessment of CPM

7.5.2.2.1 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 7-8 and Figure 7-9 summarise the findings and differ based on the inclusion of the taste thresholds, IC_{50} and EC_{50}, indicated as grey and black dashed lines, respectively. As shown in Figure 7-8, 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.

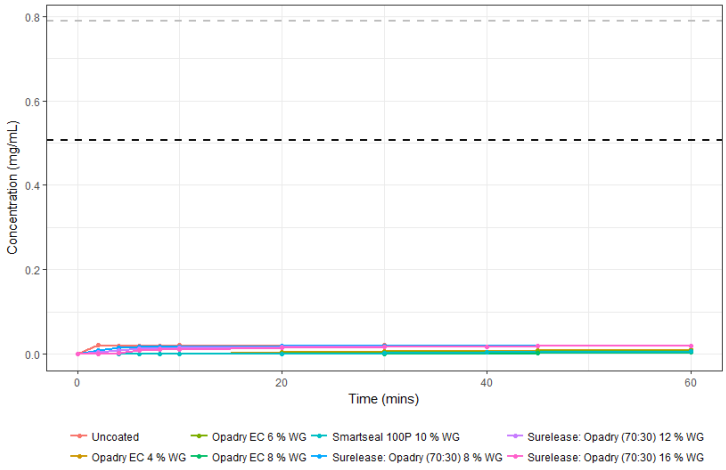


Figure 7-8 Drug release [mean +/-SEM] from CPM loaded sugar spheres with varying coatings in PBS using USP I dissolution apparatus. Taste thresholds are shown as grey and black dashed lines; the IC_{50} and EC_{50} respectively

However, when the taste thresholds are disregarded, distinction between both the type and extent (% WG) of coating is possible (Figure 7-9). 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 7-9). 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).

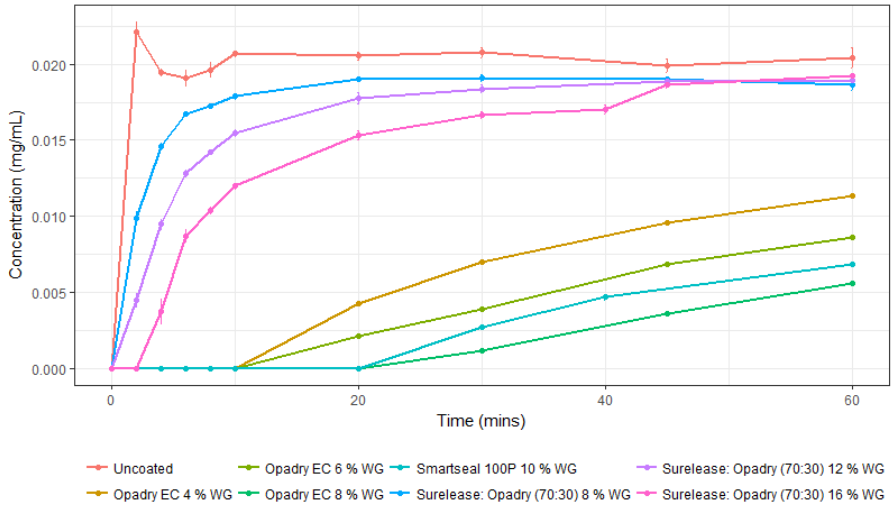


Figure 7-9 Cumulative Drug release (mean +/-SEM) from CPM loaded sugar spheres with varying coatings in PBS using USP I dissolution apparatus

7.5.2.2.2 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 7-10 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.

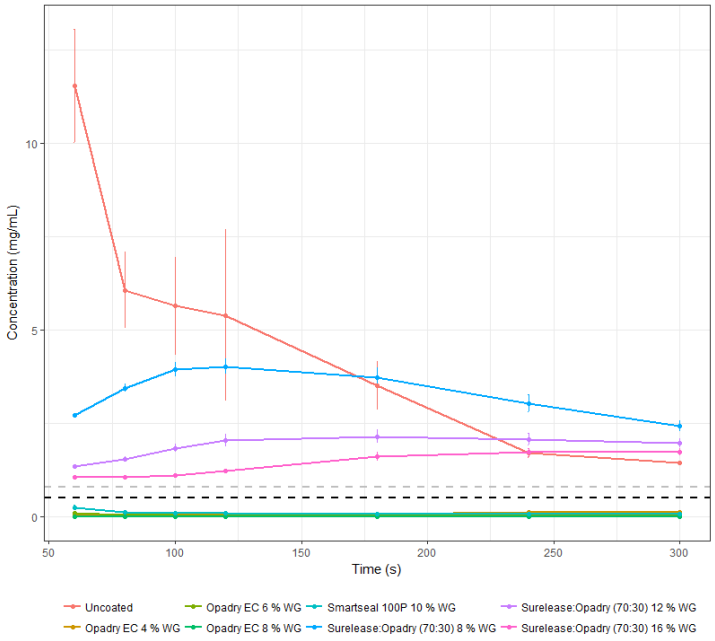


Figure 7-10 CPM release (mean +/- SEM) as a function of time showing different types and levels of coating. The taste thresholds are represented as grey and black dashed lines; the IC_{50} and EC_{50} respectively.

7.5.2.2.2.1 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 7-11).

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

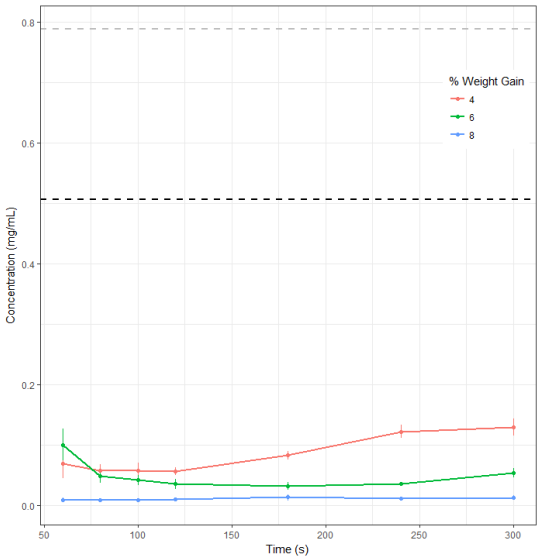


Figure 7-11 CPM release (mean +/- SEM) as a function of time showing different levels of Opadry EC coating. The taste thresholds are represented as grey and black dashed lines; the IC50 and EC50 respectively.

7.5.2.2.2.2 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 7-12). 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.

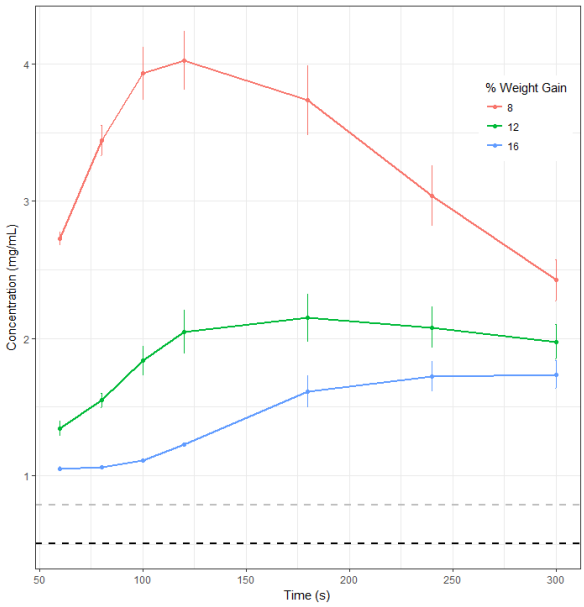


Figure 7-12 CPM release (mean +/- SEM) as a function of time showing different levels of Surelease:Opadry coating. The taste thresholds are represented as grey and black dashed lines; the IC50 and EC50 respectively.

7.5.2.2.2.3 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 7-13). 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.

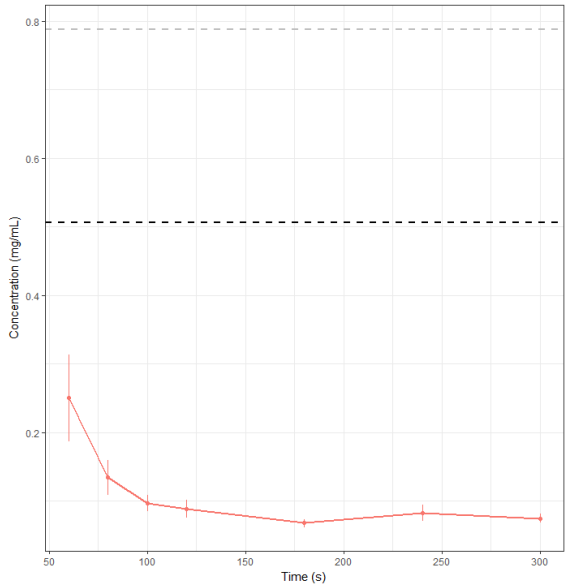


Figure 7-13 CPM release (mean +/- SEM) from a developmental formula based on Smartseal 100P coated sugar spheres, showing the taste thresholds as grey and black dashed lines; the IC50 and EC50 respectively.

7.5.2.2.3 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 7-14).

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.

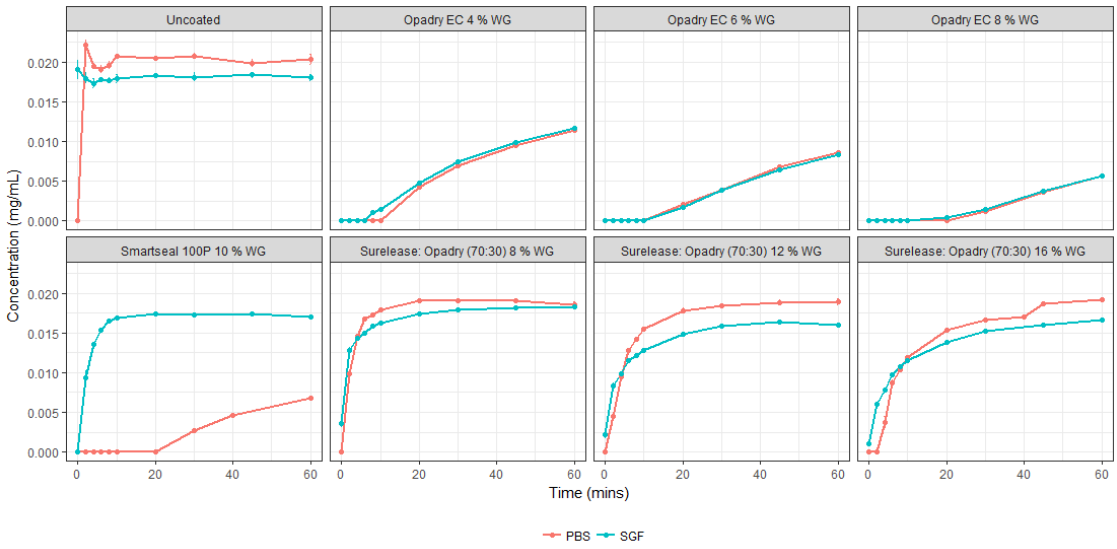


Figure 7-14 Drug release [mean +/- SEM] of CPM from sugar spheres in SGF following pre-soaking in SSF (blue) and in PBS (red).

7.6 Discussion

In her review paper, Gittings *et al.* highlighted an absence of adequate dissolution methodology for taste masked oral dosage forms [100]. 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. The methodology was developed using SDC multiparticulates and further tested using CPM multiparticulates.

However, a literature search reveals several attempts at the development of such a dissolution methodology, but all suffer from a lack of biorelevance and/or lack of correlation to real taste data. For example, in a study assessing a novel paediatric formulation of midazolam, the authors utilised dissolution as one of their means to assess taste [246]. The dissolution test used by the authors consisted simulated salivary fluid as dissolution medium, and a total media volume of 300 mL, with sampling at 0 and 5 minutes and up to 60 minutes. Such an excessive volume 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, the sampling time points do not allow for assessment of drug release within the first 5 minutes, 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, while the BATA model was utilised in the study, the authors did not adequately link the BATA data to the *in vitro* dissolution data, thus a the opportunity to gain a real insight into the absolute taste of the developed formulation *in vitro* was missed [246]. In another example, taste-masking by means of film coating of granulated core particles was investigated [247]. 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 the developed novel 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 [247].

The development of the novel dissolution methodology was achieved using SDC multiparticulates, and provided promising results by linking previously determined taste thresholds from humans and rats to drug release data *in vitro*. The developed methodology demonstrated an ability to discriminate between different coating technologies and provide an absolute assessment of taste. However, in order to confirm the utility of the developed test and ensure such data were not achievable using established methodologies, taste-masked CPM multiparticulates were investigated. This provided the opportunity to replicate a formulation development scenario in which the team were naïve to the API. Thus, both human and rat taste thresholds needed to be generated prior to assessment of taste masking *in vitro.*

It was possible to distinguish between both different types of coating and extents to which the coat had been applied using the USP I (basket) dissolution test. 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 [246,247]. 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 [248], 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 excipients, 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 [9]. 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.

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 given the demonstrated excellent ability of the BATA model to predict human taste [122]. Furthermore, minimal animal experimentation is necessary to achieve adequate taste assessment using the developed dissolution methodology; a single API dose-aversiveness response curve using the BATA model is required to yield the IC50, which can then be used for all further *in vitro* dissolution taste assessment. Thus, the principles of the 3Rs are satisfied by leveraging the rat data.

Some key limitations were however identified in this study. Firstly, the buccal dissolution test was performed at room temperature (25 °C), however the temperature of the human mouth ranges for men and women between 35.7-37.7 and 33.2-38.1 °C, respectively. Thus future testing should incorporate heated SSF or fully-submerged buccal dissolution test column in a ~37 °C water bath. Secondly, due to the flow rate of 1 mL min-1 to replicate the salivary flow rate, it frequently took up to 60 s for the dissolution media to pass through the column and allow for sampling. Earlier sampling is preferred in taste assessment as feasibly the patient will not hold an administered formulation in their mouth for an extensive period of time. This may be achieved by incorporating a mid-sampling in the dissolution column into which a syringe may be placed to sample at an earlier time point. Indeed, a mid-point sampling column is currently under development. Finally, the assessed APIs – sildenafil citrate and chlorphenamine maleate – are BCS class

I compounds being both highly soluble and highly permeable. Thus, the capability of the developed buccal dissolution test to assess the taste-masking efficacy of solid oral dosage forms consisting less amenable APIs must be questioned. A range of BCS class APIs must be assessed in the model in order to provide further validation.

Furthermore, in order to better understand the benefits and challenges of this novel dissolution test, it must be further tested using 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.

7.7 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.

8 General discussion, conclusions and future work

The research described in this thesis serves to demonstrate the importance of palatability assessment during pharmaceutical development of paediatric medicines and strives to enhance our understanding of this area. This chapter provides a general discussion of the research; it begins with a justification for research into pharmaceutical taste assessment, before discussing the key findings, limitations and future work.

8.1 The importance of pharmaceutical palatability assessment

Medicines taste bad. Children do not like bad tasting things and so do not take their medicines. If a medicine is not taken, no matter what type of new formulation technology it is utilising or new drug target it is targeting, it will have no therapeutic effect. These are incredibly simple principles, yet we are only just waking up to the importance of palatable medicines for the effective treatment of children.

Historically, children have been neglected in pharmaceutical development. In the USA, most drugs (75 %) do not have approved paediatric formulations [4]. Instead, children receive medicines that have not been evaluated as suitable for this patient population, receiving medicines 'off label' or without license or marketing authorisation [2]. The medicines are frequently unpalatable, providing a significant barrier to treatment adherence in children. Indeed in a survey, 90 % of paediatricians identified taste and palatability as the greatest barrier to treatment completion among their patients [7]. Furthermore, a more recent study which asked children directly their views on taking medicines identified taste as the most commonly reported reason for problems in taking medicines with 416/653 respondents between 10 and 18 years old stating 'don't like the taste' when asked 'why do you find some of the medicines difficult to take?' [8].

As highlighted in chapter 6, parents have to resort to a wide range of measures in order to administer a bad tasting medicine to their children. The techniques may range from giving the child an active role through play by, for example, 'giving teddy the medicine first' to the use of restraint in which the child is held down and the medicine forced down; one report describes parents 'sitting on their child to get the medicine down' [221]. It is absurd that in 2019, a medicine can be so poorly designed that parents must resort to such measures.

Efforts have however been under way to improve the picture of paediatric pharmaceutical development, largely thanks to the advent of the Paediatric Regulation (EC) 1901/2006, which was largely inspired by developments in the USA addressing paediatric drug development [37]. This legislation provided a legal obligation for pharmaceutical companies to develop paediatric medicines and established incentives for doing so [38].

A key component of the Paediatric Regulation is patient acceptability, defined by the EMA as the overall ability and willingness of the patient to use and its care giver to administer the medicine as intended [40]. Palatability is regarded as the most important aspect of acceptability for paediatric medicines, and is defined as the overall appreciation of a medicinal product in relation to its smell, taste, aftertaste and texture [40,41]. The regulation requires palatability to be demonstrated but little further guidance is given. Given that poor palatability is such a key issue in paediatric medicine, it is critical that any such problems are highlighted as early on in the drug development process as possible, thus enabling mitigation and preventing product attrition due to palatability issues. The only way in which such problems may be highlighted is to test the palatability. Several taste assessment methodologies are available, each with varied success and validity. Of the available methods, it was highlighted in the introduction that while human taste panels form the gold standard of palatability assessment, the BATA model has shown the most promising results of the non-human tools. Indeed, the research presented in this thesis has focused on human taste panels and the BATA model; it strives to enhance the understanding of these palatability assessment methodologies and, in the case of the BATA model, push the limits of what can be done.

To reiterate the aims of this PhD, they were to: 1) review current knowledge on the importance of palatability in paediatric medicine, how palatability may be assessed and identify where developments are required; 2) explore the methodological limitations of palatability assessment methodologies; 3) expand the formulation repertoire and push the limits of the BATA model; 4) reduce the use of animals in pharmaceutical taste research, where possible, by leveraging the data from the BATA model.

8.2 Overview of original contributions, limitations and future work towards better palatability assessment for the development of better paediatric medicines

The main findings of the research documented in this thesis are summarised below in relation to the original aims and objectives:

- Review of the scientific literature in chapter 1 and, more briefly, in the introductions to the experimental chapters identified the importance of palatability testing and the methods by which this may be achieved. However, by reviewing the literature multiple key knowledge gaps were identified where developments are required. This thesis strived to bridge the identified knowledge gaps as detailed henceforth.

- Assessment of the literature pertaining specifically to taste panels conducted in humans revealed inconsistencies in sample size among the identified studies. Thus, the most basic initial consideration when conducting any human experiment – how many participants do I need – was not known. Thus, this most important of questions was the first to be addressed in this research. It was identified that different sample sizes are required to meaningfully differentiate between different levels of bitterness as elicited by quinine hydrochloride. Indeed, the more dichotomous the bitterness, the fewer participants necessary to achieve statistical significance between sample rating. Two key limitations were however identified as part of this study. Firstly, quinine hydrochloride was used as a model bitter drug. To ensure the validity and generalisability of the results, the same methodology must be applied to additional APIs that elicit different levels of bitterness. Furthermore, a greater initial sample size must be used as it was found that distinction between medium and high level bitterness required 49 participants to achieve the power of 54 participants. The closeness of these values warrants a greater initial sample size. However, this study served to answer this most important of questions, and will serve to provide justification for sample size selection in future human taste panels.

- Participant selection was also identified as a key unknown during review of the literature pertaining to human taste panels. Indeed, no studies were identified which documented how one might select participants, and which is superior. Thus a range of selection methodologies were explored from phenotyping to sensitivity to the API under assessment, for identifying participants sensitive to APIs with a range of bitterness levels. It was found that sensitivity to the tested drug identified the most sensitive participants, but where this is not possible, participants should be stratified by precision in rating of quinine hydrochloride as a model bitter drug. The results were validated using an anonymised drug of unknown bitterness.

- Finally, with regards aim 2 of this thesis but also incorporating aim 4, review of the BATA methodology identified a key question, that of gender. A lack of consensus was identified in the literature surrounding the gender effect on taste, however it points to there being a possible difference between male and female taste in rats as well as humans. Rats are used in the BATA model as analytical tools, and as with any experiment, one should use the most sensitive analytical tools available. Furthermore, a limited use of female rats in research was identified, thus the realisation that females are bred for research but ultimately culled due to a limited market. Thus, in line with the 3Rs and a desire for the most taste-sensitive rats, females were pitted against males for PROP phenotype status and taste sensitivity to a range of bitter APIs. It was found that males had a predominance of supertasters while females a predominance of medium tasters, and thus sensitivity to all APIs was generally greater in males relative to females. Importantly however, this study merely assessed the phenotypic differences in taste among females and males. There was no assessment of the genetic differences in taste, which is required to provide a complete picture of taste differences between the genders. However, this study satisfied the research questions posed to it by identifying that the use of males should be conserved in the BATA model, and there is no justification for use of females in terms of 3Rs.

- The remaining chapters within this thesis sought to address aims 3 and 4 of this PhD: expansion of the formulation repertoire of the BATA model and enhanced

leveraging of the BATA data, respectively. Starting with aim 3, the question of poorly soluble APIs was addressed. It was identified that solubility and taste are not so inextricably linked as one might reason as highlighted by several poorly soluble compounds that elicit an aversive taste. Thus, it is critical that the BATA model is capable of providing data on such compounds. Pharmaceutical co-solvents were suggested as a means to bridge this gap, but their use may be limited by their own taste, thus various co-solvents at a range of concentrations were investigated in the BATA model in order to identify concentrations at which they are not distinguishable from water and thus informing a toolkit of co-solvents for the sensory scientist when presented with a poorly soluble tastant. Various co-solvents and specified concentrations were identified, however the question of umami preference among the rats was identified. Thus it was questioned whether a lick number not statistically different to water was indicative of umami preference rather than neutrality as initially proposed. It is therefore critical that umami preference in the BATA model be explored by perhaps using a monosodium glutamate control in addition to a water control, and exploring the interplay between umami and bitterness in rats. However, this work has laid the foundations for a toolkit of co-solvents to expand the repertoire of the BATA model to those APIs limited by water solubility.

- In line with aim 3, the limits of what the BATA model can tell us were explored by questioning whether or not mouthfeel can be assessed by rats. This is pertinent to palatability testing as there is more to palatability than just taste; mouthfeel or texture is also a key element. It was identified that the BATA model may provide the only non-human means of assessing mouthfeel, as according to Szczesniak's definition of mouthfeel, physical techniques can only measure certain properties of mouthfeel, and require *sensory interpretation.* By assessing the interplay between bitterness and mouthfeel – as governed by viscosity and grittiness – it was found that rats are capable of providing information on acceptability in terms of mouthfeel as well as taste aversion. Moreover, exciting correlations to humans were found with rats identifying a grittiness-masking and reduction in taste sensitivity with increasing viscosity. It is however critical that the human-rat

correlation be further explored by performing an identical experiment in humans by using design of experiment principles to explore the interplay between bitterness, viscosity and grittiness in a human taste panel. Nonetheless, this study has provided very exciting data demonstrating that the rat BATA model can distinguish between samples of varying mouthfeel as well as bitterness which has never before been investigated and which may correlate to humans.

- Based on the promising results linking taste and mouthfeel, the BATA model was pushed even further to its limits by assessing fully formulated antibiotic suspensions: highly complex systems in terms of sensory assessment incorporating multiple tastes as governed by various excipients and flavours and mouthfeel as governed by a range of particle sizes and viscosities. Despite such complexities, in the first study of its kind, the rat BATA model demonstrated an ability to distinguish both between suspensions of different antibiotic APIs and different brands of the same API. Human-rat correlation was difficult due to some disagreement in the literature, thus warranting a human taste panel assessing the acceptability of the antibiotics suspensions under scrutiny in order to truly assess the correlation. Furthermore, the contribution of the vast number of excipients to the acceptability of the assessed suspensions to rats and humans must be elucidated through experiments involving individual excipients and gradually increasing the complexity of the systems by combining excipients and flavours. However, the key finding here is that the rat BATA model is capable of assessing more than just API in aqueous solution; it can provide a more holistic assessment of the palatability of a sample, providing information on not just the taste but also the mouthfeel; something other non-human palatability assessment methodologies have yet to demonstrate.

- Assessment of the interplay between taste and mouthfeel in the rat BATA model also provided an opportunity to leverage the data in the development of *in silico* prediction models, thus satisfying aim 4 of this PhD. By defining the experimental design space using design of experiment principles encompassing bitterness, viscosity and grittiness on three levels, it was realised that the data generated

could be used to build a model to predict the rat response. Indeed, multiple models were developed and tested by predicting the rat response to the antibiotic suspensions and comparing the predicted and actual response. Some success was found, although this was API dependent. However, it must be highlighted that for seemingly simple models incorporating three parameters – viscosity, grittiness and bitterness – to show some accuracy in the prediction of rat response to highly complex systems is hugely impressive and demonstrates the real promise of *in silico* models to aid in palatability assessment in the future and thus minimise animal use. Future work must serve to expand the experimental space within which the models function. Thus, they must incorporate excipients, flavours and a wider range of viscosities, particle sizes and tastes.

- The final part of this PhD expanded the BATA model towards the assessment of solid dosage forms by leveraging the data from the BATA model, thus satisfying aims 3 and 4. It was identified that the only way in which solid dosage forms may be assessed would be to take palatability assessment *in vitro* by linking drug release to taste thresholds from the BATA model. Of course, drug release had to be assessed in a system replicative of the human oral cavity. Review of the literature revealed no such adequate systems, thus a novel buccal dissolution test was developed. The developed test linked drug release in a biorelevant system to taste thresholds in both humans and rats, allowing an absolute assessment of taste-masking efficacy. It must be noted that only BCS class I compounds were used in the development of the dissolution methodology, thus it must be further challenged with APIs of different BCS class, notably those that are poorly soluble (classes II and IV). Furthermore, the human correlation must be explored by performing a human taste panel assessing the taste-masked multiparticulates used to develop the model.

- Overall, this PhD has served to enhance the understanding of pharmaceutical palatability assessment methodologies. It has pushed the boundaries of established conventions and laid the foundations for further development of

palatability assessment methodologies, towards more patient-centric paediatric medicines.

8.3 Conclusions

The need for better, more palatable medicines for children has been realised. It is no longer acceptable for parents to have to resort to holding their children down to administer often life-saving medicines. However, the only way in which this scenario can be prevented is to improve the palatability assessment of pharmaceuticals. Only if we know the palatability of a medicine can we work to improve it and thus mitigate any potential adherence issues at the point of medicine administration. The evaluation of palatability must therefore occur as early on in the drug development process as possible to stand the best chance of ensuring poor palatability can be addressed before the medicine meets patient and caregiver.

The research detailed in this thesis has served to enhance the understanding of pharmaceutical palatability assessment, answering key questions such as sample sizing. It has pushed the boundaries of the BATA model, providing optimism for further development of this already promising methodology. Palatability has been assessed *in vivo, in vitro* and *in silico* demonstrating how far the limitations of pharmaceutical palatability assessment have been pushed.

Of course, more questions have been realised, and extensive work is still required to improve pharmaceutical palatability assessment and thus paediatric medicines. Further collaboration between industry, academia and the pharmaceutical regulators is needed to achieve our goal of better medicines for children, but a future where children do not spit out their medicines and are thus treated effectively regardless of their disease, age or geography is looking promising.

9 Scientific communications

9.1 Journal articles

Soto, J.; Keeley, A.; Keating, A. V.; Mohamed-Ahmed, A. H. A.; Sheng, Y.; Winzenburg, G.; Turner, R.; Desset-Brèthes, S.; Orlu, M.; Tuleu, C. Rats Can Predict Aversiveness of Active Pharmaceutical Ingredients. *Eur. J. Pharm. Biopharm.* 2018, *133* (August), 77–84. https://doi.org/10.1016/J.EJPB.2018.09.027.

Keeley, A.; Teo, M.; Ali, Z.; Frost, J.; Ghimire, M.; Rajabi-Siahboomi, A.; Orlu, M.; Tuleu, C. In Vitro Dissolution Model Can Predict the in Vivo Taste Masking Performance of Coated Multiparticulates. *Mol. Pharm.* 2019, acs.molpharmaceut.9b00060. https://doi.org/10.1021/acs.molpharmaceut.9b00060.

Ruiz, F.; Keeley, A.; Patrick, L.; Tuleu, C.; Lachuer, C.; Rwabihama, J.; Bachalat, N.; Boulaich, I.; Abdallah, F.; Rabus, M. Sex Differences in Medicine Acceptability: A New Factor to Be Considered in Medicine Formulation. *Pharmaceutics* 2019, *11* (368), 1–12. https://doi.org/https://doi.org/10.3390/pharmaceutics11080368.

9.2 Journal articles (in draft)

Keeley A, Kloprogge F, Orlu M, Ernest T, Tuleu C. Assessing the sample size necessary for a human taste panel for a bitter API.

Keeley A, Orlu M, Ernest T, Tuleu C. Rats can mouthfeel too: assessing mouthfeel and taste using the BATA model.

Keeley A, Mohamed-Ahmed, A. H. A., Orlu M, Ernest T, Tuleu C. Assessing the taste of poorly soluble APIs: exploring the taste of pharmaceutical co-solvents.

9.3 Oral presentations

Keeley A, Orlu M, Ernest T, Tuleu C (2018). Seminar: "Sensory Pharmaceutics at UCL School of Pharmacy", *Monell Chemical Senses Center, Philadelphia (USA).*

9.4 Poster presentations

Keeley A, Georgopoulos D, Tuleu C (2019). Assessing the feasibility of using "taste-strips" for bitterness taste panels. 11th *EuPFI Conference: Formulating Better Medicines for Children, Malmo (Sweden)*

Keeley A, Sheng Y, Soto J, Ernest T, Orlu M, Tuleu C (2018). Are Rats Good Sensory Assessors According to ISO Norms 10th *EuPFI Conference: Formulating Better Medicines for Children, London (UK)*

Keeley A, Orlu M, Ernest T, Tuleu C (2018). Rat Brief-Access Taste Aversion Model Through a Gender Lens 10th *EuPFI Conference: Formulating Better Medicines for Children, London (UK)*

Keeley A, Orlu M, Ernest T, Tuleu C (2017). Assessing the sample size necessary for a human taste panel *9th EuPFI Conference: Formulating Better Medicines for Children, Warsaw (Poland)*

Keeley A, Orlu M, Ernest T, Tuleu C (2017). Are Rats Supertasters? *9th EuPFI Conference: Formulating Better Medicines for Children, Warsaw (Poland)*

Keeley A, Orlu M, Ernest T, Tuleu C (2017). Assessing the Interplay Between Bitter Taste and Mouth Feel Using the Rodent Brief-Access Taste Aversion (BATA) Model *AAPS Annual Meeting and Exposition, San Diego (USA)*

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