Is rat a good model for assessment of particulate-based taste-masked formulations?

Joseph Ali, Manting Chiang, Jong Bong Lee, Gregory O. Voronin, Joanne Bennett, Anne Cram, Leonid Kagan, Martin C. Garnett, Clive J. Roberts, Pavel Gershkovich

A R T I C L E   I N F O

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A B S T R A C T

Recently there has been an increased interest to develop specialised dosage forms that are better suited to specific patient populations, such as paediatrics and geriatrics. In these patient populations the acceptability of the oral dosage form can be paramount to the products success. However, many Active Pharmaceutical Ingredients (APIs) are known to cause an aversive taste response. One way to increase the acceptability and to enhance the palatability of the formulation is to design coated taste-masked particulate-based dosage forms. The masking of poorly tasting drugs with physical barriers such as polymer coatings can be utilised to prevent the release of drug within the oral cavity, thus preventing a taste response. However, currently, there are few assessment tools and models available to test the efficiency of these particulate-based taste-masked formulations. The rat brief access taste aversion model has been shown to be useful in assessment of taste for liquid dosage forms. However, the applicability of the rat model for particulate-based taste masked formulations is yet to be assessed. It is not understood whether dissolution, solubility and thus exposure of the drug to taste receptors would be the same in rat and human. Therefore, rat saliva must be compared to human saliva to determine the likelihood that drug release would be similar within the oral cavity for both species. In this study rat saliva was characterised for parameters known to be important for drug dissolution, such as pH, buffer capacity, surface tension, and viscosity. Subsequently dissolution of model bitter tasting compounds, sildenafil citrate and efavirenz, in rat saliva was compared to dissolution in human saliva. For all parameters characterised and for the dissolution of both drugs in rat saliva, a substantial difference was observed when compared to human saliva. This discrepancy in saliva parameters and dissolution of model drugs suggests that preclinical taste evaluation of particulate-based taste-masked formulations suggests rat is not a good model for predicting taste of solid dosage forms or undissolved drug where dissolution is required. Alternative preclinical in vivo models in other species, or improved biorelevant in vitro models should be considered instead.

1. Introduction

The oral route is the most popular and convenient route for drug administration [1]. For the prescription of medicinal products to patient populations such as paediatrics and geriatrics the acceptability of a solid oral dosage form can be paramount to the products success [2]. There are several challenges to overcome when designing solid oral dosage forms for children and older adults. For example, children can have issues with conventional solid dosage forms due to poor palatability, limited dose flexibility, difficulty swallowing, recalcitrance, and differences in anatomy and physiology [2–4]. Therefore, there can be an impact on therapeutic performance of the product. For paediatrics, there have also been recent developments in European Union (EU) legislation (Reg 1901/2006/EU and Reg 1902/2006/EU) stating that new medicines coming to commercial markets must demonstrate paediatric suitability [5]. Geriatrics are also receiving similar attention with calls for an update in legislation for geriatric drug development [6,7]. Consequently, there have been increased discussions on the development of patient-centric particulate-based oral dosage forms for these specific populations. Many Active Pharmaceutical Ingredients (APIs) and excipients are known to cause an aversive taste response [8]. An unpleasant taste has been shown to greatly affect acceptability of a
dosage form [9]. Taste-masking strategies are commonly used to overcome these challenges. Particulate-based oral dosage forms can have a polymer coating applied to provide a physical barrier between the aversive tasting API and taste receptors within the oral cavity. The addition of coatings to particulates to prevent a taste response reports have shown a significant increase in palatability of dosage forms when compared to API alone [10]. This demonstrates the effectiveness of coatings in particulate-based formulations for taste-masking and increasing acceptability in paediatrics. Particulate-based oral dosage forms may be used for better acceptability in paediatrics due to enhanced palatability [11], flexible dosing, and improved swallowability [12]. Particulate-based oral formulations such as minitablets, sprinkle capsules, suspensions, and granules are intended to be swallowed from the oral cavity intact. An appropriate preclinical assessment method is hence required to investigate the efficiency of taste-masking in particulate-based formulations.

Currently the most common method for taste evaluation for solid oral dosage forms is using human taste panels [13]. However, target populations that require taste-masked formulations, such as paediatrics, may not be suitable for this assessment method due to ethical concerns [13]. In addition, these human trials are expensive, resource intensive, and may cause significant delays to drug development timelines. Therefore, there are limited opportunities to conduct paediatric taste panel studies as part of a clinical trial. In vitro drug release and dissolution studies can be used for the determination of drug released from particulate-based taste-masked formulations, to determine if the concentration is above the taste threshold and has unacceptable taste. However, there is no general consensus and limited studies on what parameters and models should be used in an oral cavity dissolution test [14]. What is agreed on is that biorelevant dissolution testing is of great value and allows for a robust assessment method with better predictions of in vivo behaviour for the formulations of interest [15–19].

Currently the most common preclinical in vivo taste evaluation method for liquid dosage forms is the Brief-Access Taste Aversion (BATA) method. Often mice and rats are deprived of water for 16–24 h and given a solution of the drug present in several sipper tubes. A (BATA) method.Oftenmiceandratsaredeprivedofwaterfor16–24h method for liquid dosage forms is the Brief-Access Taste Aversion (BATA) method. Often mice and rats are deprived of water for 16–24 h and given a solution of the drug present in several sipper tubes. An appropriate preclinical assessment method is hence required to investigate the applicability of the rat taste aversion model to particulate-based formulations, to determine if the concentration is above the taste threshold and has unacceptable taste. Therefore, the aim of the present work was to determine if rat saliva has similar physicochemical properties to human saliva, and drug dissolution properties for potential adaptation of a rat taste preclinical taste model to the assessment of particulate-based taste-masked formulation

### 2. Materials and methods

Sildenafil citrate API powder was donated by Pfizer Ltd. (Sandwich, UK). Efavirenz API powder was purchased from ChemShuttle (Wuxi City, China). Pilocarpine hydrochloride was purchased from Sigma-Aldrich (UK). Methyl-tert-butyl ether (MTBE) was purchased from Fisher Scientific (UK). All solvents used in the study were HPLC grade or higher.

#### 2.1. Collection of human saliva from healthy adult volunteers

Human saliva was collected in accordance with Ethics Reference Number: R12122013 from Faculty of Medicine and Health Sciences, Nottingham University Hospitals, as previously described [28,29]. Informed written consent was obtained from all volunteers. All data was held in agreement with the Data Protection Act. Exclusion criteria for the volunteers included chronic or acute illness in the past 3 months, cold or flu symptoms, oral health concerns, and taking medication (except contraceptives). Before collection of saliva (at least 2 h prior) volunteers were asked to not eat, smoke, drink or use oral hygiene. To avoid differences in saliva composition due to circadian rhythms, all saliva was collected between 14:00 and 16:00 h.

## Table 1

| Drug             | Molecular weight (g/mol) | Log P | pKa            | pH solubility (mg/mL, 37°C) |
|------------------|--------------------------|-------|----------------|----------------------------|
| Sildenafil citrate | 666.7                    | 2.3   | Sildenafil free base: 6.5, 9.2 | Adapted from [25] |
|                  |                          |       |                | pH 3.0: 6.97               |
|                  |                          |       |                | pH 4.0: 7.08               |
|                  |                          |       |                | pH 5.0: 2.07               |
|                  |                          |       |                | pH 6.0: 0.11               |
|                  |                          |       |                | pH 7.0–pH 10.0: < 0.04     |
|                  |                          |       |                | pH 11.0: 0.02              |
| Efavirenz        | 315.7                    | 4.8   | 10.2           | Adapted from [26] |
|                  |                          |       |                | pH 1.2: 0.020              |
|                  |                          |       |                | pH 4.5: 0.019              |
|                  |                          |       |                | pH 6.8: 0.011              |
|                  |                          |       |                | pH 7.4: 0.082              |
|                  |                          |       |                | pH 9.0: 0.16               |

and gives burning mouth syndrome [24]. Both these model drugs have clinical use for paediatric populations, and both required formulation strategies to overcome issues with taste. A brief summary of physicochemical properties of sildenafil citrate and efavirenz is shown in Table 1.

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2.2. Collection of stimulated rat saliva

All procedures for rat saliva collection were reviewed and approved by the Rutgers University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Envigo, USA) 8 – 12 weeks old were used for saliva collection. Animals were housed under controlled temperature, twelve-hour light/dark cycle and free access to food and water.

For the collection of rat saliva there are two main approaches described in literature. Firstly, intra-oral cannulation of salivary duct orifices to gather saliva from their respective glands [34–38]. Secondly, an elevation collection method, that collects saliva passively from the mouth from anaesthetised animals [39,40].

The saliva collection method used in this work was an optimisation of the previously reported non-invasive, elevation sialometric method [39,40]. This sialometric method involves anaesthetising animals, then administering a sialogogue agent, and orientating animals head in a downwards slope to which then saliva can passively flow into collection tubes. General anaesthesia was induced with inhalation of 3% isoflurane, and anaesthesia was maintained throughout collection with isoflurane via a nose cone. Specific 3D printed platforms were necessary for efficient saliva collection as the platform must hold a number of components at once: a nose cone for anaesthesia, the collection tube, orientation of the animals’ head downwards without impacting cardio-vascular parameters, and engagement of the lower incisors to keep the mouth open during collection, as shown in Fig. 1 [41]. These platforms were tapered in height from 50 mm to 10 mm, width 100 mm, depth 125 mm.

Immediately before saliva collection 2 mL saline was injected for fluid replacement by subcutaneous bolus. Saliva was then stimulated by intraperitoneal administration of 2 mg/kg pilocarpine hydrochloride. Immediately following the injection, the animals were positioned on top of the platforms for saliva collection. After collection, the animals were left to recover for 48 h, and were given an additional 2 mL saline replacement 24 h post collection. Immediately following collection, pH of individual saliva samples was measured with a Mettler Toledo S220 Seven Compact pH/ion meter, connected to an InLab Micro pH electrode. Saliva samples were titrated with 0.01 M HCl until pH had decreased by 1 unit. Buffer capacity was then calculated in mmol H⁺/L, by the amount of acid added. A Modular Compact Rheometer MCR 302 (Anton Paar GmbH, Germany) was used with a cone-plate set up to measure viscosity of saliva. The cone used was a CP50-2-SN30270, diameter 49.972 mm, angle 2.016°, truncation 211 µm. Viscosity measurements were taken at 37 °C over three logarithmic decades for shear rate from 1 to 1000 s⁻¹ with measurements taken at 8 points per decade. Data was recorded on Rheopuls software (Anton Paar GmbH, Germany) for analysis. A DSA 100 Drop Shape Analyser with DSA 4 software (Kruss GmbH, Germany) was used to measure surface tension of saliva by using the pendant drop method with Laplace-Young computational method. Temperature was set to 37 °C using an MB-5 heat circulator and water bath (Julabo GmbH, Germany).

2.4. Oral cavity dissolution studies of sildenafil citrate and efavirenz in pooled rat and human stimulated saliva

The volume of saliva used in the oral cavity dissolution method was based on average saliva volumes reported for adult humans [42,43]. Multiple small-scale dissolution vessels were kept at 37 °C each with a magnetic stirrer in a setup as was previously described for human saliva dissolution studies [29]. Separate dissolution vessels were used in parallel to measure the concentration of drug over different time points. At each time point pH of saliva was recorded. Pooled stimulated saliva (200 µL) was added to 10 mg of sildenafil citrate. Pooled stimulated saliva (250 µL) was added to 50 mg of efavirenz. Volume of saliva and amount of drug were selected to give relevant clinical dose in humans. A constant stirring speed of 200 rpm was used to allow adequate mixing of solid material in relatively small volumes of media. Both rat and human stimulated saliva were used as dissolution media for the two drugs of interest. After each time point was reached, the entire contents of the dissolution vials were transferred to Costar Spin-X centrifuge tubes with 0.22 µm pore CA filters (Corning B.V. Life Sciences, UK), and centrifuged for 10 min at 17,000 × g.

2.5. Analytical procedures

Filtered dissolution sample (10 µL) was transferred to a glass test tube, to which the following was added: 10 µL of internal standard (IS), 90 µL of blank saliva (for further dilution of the drug to the range suitable for HPLC analysis), and 400 µL of 50:50 acetonitrile (ACN), methanol mixture (stored at −20 °C). After filter centrifugation of efavirenz samples, 180 µL of filtered dissolution sample was transferred to a glass test tube, to which 20 µL of internal standard (IS), and 400 µL of bath. Initial pH was determined using Mettler Toledo S220 Seven Compact pH/ion meter, connected to an InLab Micro pH electrode.

Pooled stimulated human saliva was characterised for: pH, buffer capacity, viscosity, and surface tension as previously described [28,29]. Individual rat saliva samples were tested for pH immediately after collection. Pooled stimulated rat saliva was characterised for: buffer capacity, viscosity, and surface tension. Two hundred µL of rat saliva, and 4 mL of human saliva samples were brought to 37 °C in a water bath.
acetonitrile/methanol mixture (50:50, stored at −20 °C) was added. Then, the test tubes were vortex mixed for 2 min. Next, methyl-tert-butyl ether (MTBE) was added to each test tube (3 mL for sildenafil, 4 mL for efavirenz), and vortex mixed at 1200 min⁻¹ for 10 min in a multi-tube vortexer (VWR VX-2500). Then, samples were centrifuged at 1690 × g for 10 min. Following centrifugation, the organic layer was transferred and evaporated to dryness under nitrogen. The dry residue was then reconstituted with 100 μL of mobile phase (44% ACN, 56% water). Reconstituted samples were then vortex mixed and centrifuged, before transferring the contents to HPLC vials. All calibration and quality control samples underwent the same sample preparation procedures as stated for the dissolution samples.

Waters (Milford, USA) 2695 separations module HPLC system equipped with Waters 996 PDA UV detector was used. Samples in the autosampler were maintained at 4 °C and the column oven was set to 40 °C. Empower 2 software was used for data processing. Separation of the extracted sildenafil citrate and efavirenz samples were achieved with Waters Xterra C18 2.1 × 100 mm, 3.5 μm particle size column, with Xterra MS C18 2.1 × 10 mm 3.5 μm guard column and pre-column filter including a 0.5 μm stainless steel frit. Mobile phase was 56% 0.2 M ammonium acetate buffer (pH 7.0) and 44% ACN, eluted at isocratic conditions at 0.3 mL/min for sildenafil citrate, and 0.2 mL/min for efavirenz. Bifonazole was used as the internal standard (IS) for sildenafil. Sildenafil citrate was used as the internal standard (IS) for efavirenz. Sildenafil citrate and bifonazole were detected at 224 nm at 3.1 and 7.9 min respectively. Efavirenz and sildenafil were detected at 290 nm and 224 nm at 11.2 and 4.0 min respectively. Validation of both assays for these compounds was performed following FDA guidelines [44]. A summary of validation parameters is shown in Tables 2 and 3 for sildenafil and efavirenz respectively. Calibration curves were constructed in the concentration ranges expected from dissolution of the drug in saliva. Calibration curves all had correlation coefficient (r²) values of > 0.99.

2.6. Statistical analysis

Results are expressed as mean ± standard deviation (SD). pH, buffer capacity and surface tension were analysed with unpaired t-test. Dissolution and viscosity results were analysed with one-way ANOVA and Sidak’s multiple comparisons test. A p < 0.05 was considered to represent a significant difference.

3. Results

3.1. Characterisation of rat and human saliva

Stimulated rat (RS SS) and human saliva (HS SS) were characterised for: pH, buffer capacity, viscosity, and surface tension (Fig. 2). The characterisation results for human saliva were in agreement with our previously reported works [28,29]. All characterisation parameters of stimulated rat saliva were observed to be different from stimulated human saliva. Stimulated rat saliva had dramatically higher pH compared to stimulated human saliva (Fig. 2A), but substantially lower buffer capacity (Fig. 2B) and surface tension (Fig. 2C). Stimulated rat saliva had also statistically significantly lower (p < 0.05) viscosity at shear rates from 1.33 to 10.0 s⁻¹ compared to stimulated human saliva, whilst no significant differences between rat and human saliva were found at 1 s⁻¹ nor in the range of 13.33–1000 s⁻¹ shear rates (Fig. 2D).

3.2. Dissolution of sildenafil citrate in saliva

Dissolution of sildenafil citrate API powder (50 mg dose equivalent) was assessed in pooled stimulated rat and human saliva (equivalent to 1.0 mL residual volume of saliva in humans). Concentrations of sildenafil in human saliva were profoundly higher than the concentrations of sildenafil in rat saliva, as shown in Fig. 3. Changes in pH of saliva over
sildenafil dissolution time course are shown in Fig. 4. In both HS and RS saliva pH was shown to reduce to pH 4.5 within the first time point (1 min).

3.3. Dissolution of efavirenz in saliva

Dissolution of efavirenz API powder (200 mg dose equivalent) was assessed in pooled stimulated rat and human saliva (equivalent to 1.0 mL residual volume of saliva in humans). Concentrations of efavirenz in human saliva were substantially lower than in rat saliva, as shown in Fig. 5. Changes in saliva pH over efavirenz dissolution time course are shown in Fig. 6. In both HS and RS saliva pH was shown to increase over 2 min with substantial differences between HS and RS throughout the dissolution time course.

4. Discussion

Most currently used taste evaluation methods are designed primarily for the assessment of liquid dosage forms [13]. However, these methods designed for determining taste of liquid dosage forms with taste-masking agents may not be optimal for solid oral dosage forms and suspensions. Particulate-based taste-masked formulations aim to prevent an aversive taste response by reducing the release of the poorly tasting drug into the oral cavity. Thus, the dissolution properties of the API in saliva become critical. There is a lack of in vivo preclinical models that incorporate biorelevant dissolution and thus expose a realistic concentration to the receptor for particulate-based formulations. To assess the applicability of rat as an in vivo taste evaluation model for particulate-based formulations, rat saliva was characterised in this work for parameters important for drug dissolution and compared to these parameters in human saliva. Moreover, dissolution of model bitter APIs were assessed in rat saliva and compared head to head to dissolution in human saliva. Rat saliva was found to be dramatically different to human saliva for all tested parameters. The pH of rat saliva was substantially more alkaline compared to human saliva, as shown in Fig. 2A. The rat saliva and human saliva pH levels recorded in this study are also supported by similar findings in literature [28,29,45]. The difference in pH between rat and human saliva can significantly affect the dissolution profile of drugs, especially of weakly acidic ionisable APIs and excipients due to their higher aqueous solubility at higher pH. For example, acidic drugs clinically relevant for paediatric use and taste aversiveness such as efavirenz, diclofenac and chloral hydrate can achieve higher concentrations in rat oral cavity compared to the human oral cavity due to this pH effect on solubility. In addition, taste-masking strategies such as the use of weak base anion exchange resins will not function in a manner similar to human oral cavity at the higher pH of rat saliva [10]. The different pH could also affect other pH dependent coatings and suspensions. Therefore, this difference in pH between rat and human saliva could impact the extrapolation of particulate-based formulation rat taste studies to humans.

The buffer capacity of rat saliva was significantly lower than human saliva, as displayed in Fig. 2B. As saliva is a buffered aqueous medium the dissolution rate of sparingly soluble weak acid or weak base drugs can be affected due to ionic interactions at the microenvironment solid-liquid interface [46]. The impact the buffer has on drug dissolution is dependent on several factors, such as ionisation constants of the buffer and drug, molar concentration of the buffer, buffer capacity, and the concentration of buffer species reacting with the drug [46]. Therefore, the results suggest that it is likely that the dissolution of drugs could be affected by this difference in buffer capacity which could then impact the correlation between rat and human particulate-based formulation taste studies.

Previous studies have shown the effect of surface tension on drug dissolution [47,48]. The surface tension of rat saliva was dramatically lower than the surface tension of human saliva. The Washburn equation explains that the penetration of dissolution media is the rate limiting
step to solid dosage form disintegration, and is directly influenced by media surface tension [49]. Therefore, the disintegration of particulate-based formulations in the oral cavity will be affected by saliva surface tension.

From shear rates 1.33–10.0 s⁻¹ saliva viscosity was significantly lower than the viscosity of human saliva. The relationship of viscosity on drug dissolution rate is shown to be inversely proportional [50]. Using the Noyes-Witney dissolution model, an increase in dissolution media viscosity would increase the thickness of boundary layers and decrease the diffusion coefficient [51]. In contrast, the shear rate experienced in humans from the initial perception of solids within the oral cavity has shown to be 50 s⁻¹ [52]. At 50 s⁻¹ no significant differences in viscosity were observed between rat and human saliva. Conversely, the concentration of efavirenz was shown to be significantly higher in rat saliva compared to human saliva. These differences in dissolution profiles between the two saliva sources are likely to be due to the differences in physicochemical properties of saliva. For sildenafil citrate dissolution, the measurements of dissolution chamber pH for both saliva types was shown to remain at pH 4.5 after the one-minute time point as shown in Fig. 4. The immediate reduction in saliva pH likely suggests that the introduction of sildenafil citrate into saliva had caused dissociation of the salt back into the sildenafil free base [53]. The pH over the
In order to have a reliable rat taste model to assess particulate-based taste-masked formulations, and to have direct correlation to human taste procedures the characteristics of rat saliva should be to some extent similar to human saliva. The dissolution of drugs would have to be similar in both rat and human saliva as the media of the oral cavity would dictate the release of drug from taste-masked formulations. However, this study has shown that the physicochemical characteristics of rat saliva are very different from human saliva. Moreover, this was further confirmed by the dissolution of two model bitter drugs being completely different in rat versus human saliva. Therefore, the data suggest that a rat taste model for the assessment of particulate-based taste-masked formulations would not likely be representative of the taste response from human taste panels. This is likely to have implications for the adaptation of currently existing BATA models used for liquid taste masked formulations to particulate formulations. Looking at other common laboratory animals, previous comparisons have also shown that canine saliva is different to human saliva as it has higher pH, buffer capacity, and concentration of minerals [54]. These considerable changes in saliva characteristics between species suggest that particulate-based taste-masked formulations should be for now assessed in vivo utilising human taste panels. Further work is needed to find another suitable species for a preclinical in vivo model with similar saliva characteristics to humans. Moreover, in vitro biorelevant oral cavity dissolution models, mimicking physicochemical...

Fig. 3. Dissolution-time profile of sildenafil citrate API powder in pooled stimulated rat saliva (RS SS) and pooled stimulated human saliva (HS SS). Values are expressed as mean ± SD (n = 3). **** Significantly lower (p < 0.0001) concentration in rat compared to human saliva.

Fig. 4. pH of saliva over the course of sildenafil citrate dissolution in pooled stimulated human saliva (HS SS), and pooled stimulated rat saliva (RS SS). Values expressed as mean ± SD (n = 3). ***** Significantly higher (p < 0.0001) pH in rat compared to human saliva.

remaining time course in both rat and human saliva was maintained around pH 4.5, this suggests the differences seen in sildenafil concentrations over the time course between the two saliva sources was not primarily due to pH. Instead, these differences in dissolution for sildenafil between the two saliva types may be caused by other saliva parameters such as mucin content, proteome composition, or ionic exchange from the salivary pellicle. For efavirenz the difference in dissolution profile from rat and human saliva was likely due to the differences in saliva pH. Efavirenz is known to have a pH-dependent relationship with regards to solubility. Previous studies have shown that when media pH exceeds pH 8.0, the solubility of efavirenz increases 6-fold compared to the solubility at pH 7.4 [26]. Fig. 6 shows that the differences in saliva pH are likely to be a main contributing factor to the differences in EFV concentration. However, in this study the difference in efavirenz concentration was much greater than 6-fold, suggesting that other characteristics of rat saliva were also contributors to the dissolution profile observed.

Fig. 5. Dissolution-time profile of efavirenz API powder in pooled stimulated rat saliva (RS SS) and pooled stimulated human saliva (HS SS). Values are expressed as mean ± SD (n = 6). ******Significantly higher (p < 0.0001) concentration in pooled stimulated rat saliva compared to pooled stimulated human saliva.

Fig. 6. pH measurements taken from saliva over the course of efavirenz dissolution in pooled stimulated human saliva (HS SS), and pooled stimulated rat saliva (RS SS). Values expressed as mean ± SD (n = 3). ******Significantly higher (p < 0.0001) pH in rat compared to human saliva.
parameters of human saliva and fluid dynamics of oral cavity could provide a useful alternative to human panels in the future.

5. Conclusion

In this study it has been found that stimulated rat saliva is significantly different from stimulated human saliva in terms of pH, buffer capacity, surface tension, and viscosity. In addition, the dissolution of two model bitter drugs, dandelin citrate and efavirenz, gave very different concentrations in stimulated rat saliva compared to stimulated human saliva. These differences suggest that the fate of the particulate-based dosage forms in the rat oral cavity could be quite different compared to human oral cavity. This discrepancy in saliva parameters and dissolution of model drugs suggests that a rat preclinical taste evaluation method of particulate-based taste-masked formulations could not be representative of the taste of these particulate-based taste-masked formulations in humans. Alternative preclinical in vivo models in other species, or improved biorelevant in vitro models should be considered instead.

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