International Journal of Pharmaceutics 555 (2019) 228–236

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Application of biorelevant saliva-based dissolution for optimisation of orally disintegrating formulations of felodipine

Joseph Ali[a], Atheer Zgair[a,b], Ghaidaa S. Hameed[a,c], Martin C. Garnett[a], Clive J. Roberts[a], Jonathan C. Burley[a], Pavel Gershkovich[a,*]

[a] School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom
[b] College of Pharmacy, University of Anbar, Ramadi, Anbar, Iraq
[c] College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq

ARTICLE INFO

Keywords:
Biorelevant dissolution
Human saliva
Oral cavity
Felodipine
Orally disintegrating tablets

ABSTRACT

The oral cavity is of great importance to the performance of orally retained formulations, including: orally disintegrating tablets, taste-masked formulations, and buccal/sublingual delivery systems. With regards to *in vitro* dissolution assessment of these dosage forms, human saliva should be represented by the dissolution media. Currently there is no general consensus regarding oral cavity dissolution. In this study pooled human saliva was characterised and utilised as dissolution media for biorelevant oral cavity dissolution studies and to assess drug release. Lipophilic drug felodipine with challenging biopharmaceutical properties was selected for assessment in oral cavity dissolution studies. These saliva dissolution studies investigated for the first time how biorelevant dissolution can be implemented as a screening tool to guide the formulation development process and to predict dosage form performance within the mouth. In this study a combination of three dissolution enhancement strategies (cryomilling, solid dispersion, and inclusion complexation) were employed to eventually increase the concentration of felodipine in saliva 150-fold. Using this successful formulation strategy orally disintegrating tablets of felodipine were produced. Interestingly, the percentage release of felodipine in compendial dissolution apparatus was shown to be over 80% after 10 min. On the other hand, saliva-based dissolution showed that percentage release of felodipine was only 0.2% after 10 min using the same formulation. This discrepancy in drug release between dissolution media highlights the need for biorelevant dissolution apparatus for the oral cavity to reliably assess performance of relevant dosage forms *in vitro*.

1. Introduction

The oral cavity is a site for drug dissolution that is generally overlooked in pharmaceutical development. Following the oral administration of drugs there are several processes which take place over a relatively short period of time. These processes include: disintegration, dissolution, taste perception, drug absorption, and drug removal via swallowing. Compared to conventional solid oral dosage forms, such as tablets and capsules, orally retained formulations can be greatly impacted by the time spent within the oral cavity. The performance of orally retained formulations such as sublingual and buccal tablets, orally disintegrating tablets (ODTs), and oral films in the oral cavity rely on disintegration and dissolution in saliva (Bartlett and van der Voort Maarschalk, 2012). On the other hand, there are orally retained formulations that aim to prevent disintegration and dissolution within saliva as a method of taste-masking. Therefore, a robust method of determining dissolution of dosage forms and drug release in saliva is important.

In development of orally retained formulations numerous tests can be used to assess the performance of the dosage form in the oral cavity, including: wetting time, disintegration, moisture uptake, and dissolution tests. With regards to dissolution testing, current approaches used for orally retained formulations are usually the same as the methods used for conventional tablets. The dissolution of conventional tablets is typically done with United States Pharmacopeia (USP) apparatuses 1–4 (Azarmi et al., 2007; Kraemer et al., 2012). Typical dissolution media used in USP dissolution include: 0.1 M HCl, buffers at pH from 1.2 to 7.5, simulated gastric/intestinal fluid, water, and surfactant solutions.

Abbreviations: US, Unstimulated saliva; SS, Stimulated saliva; βCD, Beta-cyclodextrin; MβCD, Methyl-beta-cyclodextrin

* Corresponding author at: Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.
E-mail address: pavel.gershkovich@nottingham.ac.uk (P. Gershkovich).

https://doi.org/10.1016/j.ijpharm.2018.11.051
Received 9 July 2018; Received in revised form 22 October 2018; Accepted 17 November 2018
Available online 19 November 2018

The conventional dissolution procedures, such as USP apparatuses, were not initially designed to be biorelevant for the oral cavity (Gittings et al., 2014). Therefore, these compendial dissolution methods might not accurately reflect how a formulation will perform within the oral cavity. Therefore, dissolution method conditions different from the current conventional procedures will be required for biorelevant evaluation of drug performance within the oral cavity (Hermans et al., 2017).

There have been several dissolution tests that have aimed for biorelevant dissolution of the oral cavity (Yajima et al., 2002; Hoang Thi et al., 2012; Tietz et al., 2018). However, there is no general consensus on what parameters should be used in oral cavity dissolution (Gittings et al., 2014). What is agreed, is that a biorelevant dissolution testing would be of great value and would allow for a robust assessment method with better predictions of *in vivo* behaviour and dosage form performance (Dressman and Reppas, 2000; Kostewicz et al., 2002; Okumu et al., 2008; Shono et al., 2009; Sunesen et al., 2005; Vertzoni et al., 2005; Hermans et al., 2017). However, currently available methods have limited biorelevance. Current approaches for oral cavity dissolution testing have not yet formed a clear basis on what the dissolution media should be used as a surrogate for human saliva. Several different approaches have been taken to simulate saliva for use in pharmaceutical development (Gal et al., 2001; Guhmann et al., 2012). In addition, there have been reports in the fields of oral hygiene and food sciences of using of actual human whole saliva as dissolution media (Saxegaard et al., 1988; Buettner, 2002; Manarelli et al., 2017). To use human saliva in a reliable and reproducible manner for purposes of dissolution, pooling methods were previously employed to reduce inter-individual variability (Schipper et al., 2007). In addition, for a better prediction of the formulation performance in the oral cavity, dissolution media volumes were adjusted to realistic volume to better reflect resident volume of saliva in the mouth (Lagerlof and Dawes, 1984; Rudney et al., 1995). In this study, dissolution exposure time was set to 10 min to be used as a formulation screening trade-off time end point for various orally retained formulations, such as ODTs and taste-masked formulations. While this exposure time is longer than the average disintegration time of ODTs (European Pharmacopeia, 2014; United States Pharmacopeia, 2014), it is shorter than the time required to prevent dissolution for effective taste masking (Lopez et al., 2018).

Saliva is composed of 99% water, therefore compounds with poor aqueous solubility will require dissolution enhancement strategies for successful ODT development. There are various techniques available for increasing drug solubility and therefore dissolution (Savjani et al., 2012; Sharma and Baldi, 2016; Bavishi and Borkhataria, 2016; Alkassas et al., 2017; Khames 2017; Repka et al., 2018). The solubility enhancing techniques used in this study included: particle size reduction, solid dispersions, and inclusion complexes. Firstly, cryomilling was used to enhance dissolution by reducing particle size and by creating amorphous drug particles. Then a polymeric solubilizer, Soluplus® was used to aid the solubilisation of poorly soluble drugs in aqueous media and to stabilise the amorphous milled drug material. Lastly, complexation with cyclodextrins was used as it can improve the aqueous solubility of lipophilic compounds.

ODTs have been shown to be attractive dosage forms due to their ease of use for the patient. Another benefit to dosage forms fated for dissolving or disintegrating in the oral cavity is that it allows for direct absorption through the oral mucosa allowing drugs to reach systemic circulation bypassing the gastrointestinal tract and hepatic first-pass metabolism. For some compounds, the ability to decrease irritation to the subsequent compartments of the gastrointestinal tract can be advantageous, for example, piroxicam can be used in an ODT formulation to avoid its side effect of gastric irritation (Lai et al., 2011). ODTs have also shown potential when rapid onset of drug action is required, for example, headache relief and freedom from pain with rizatriptan ODTs (Cameron et al., 2015). There have been a number of lipophilic compounds that have been formulated as ODTs for commercial use, such as: loratadine, ondansetron, tepoxalin, loperamide, and cisapride (Fu et al., 2004). In the present study felodipine was used as a model lipophilic compound to assess the suitability of biorelevant human saliva dissolution as a screening tool to guide the development and optimisation of an ODT formulation.

Felodipine is a 1,4-dihydropyridine derivative that acts as a calcium antagonist and is primarily used in the treatment of angina pectoris and hypertension. Felodipine is a lipophilic, crystalline powder with very low water solubility of around 3 μg/mL (Kim et al., 2006; Mielcarek et al., 2006). According to the Biopharmaceutics Classification System (BCS), felodipine is a class II drug, as it has low aqueous solubility and high permeability. The oral bioavailability of felodipine is low (15%) mostly due to poor aqueous solubility and extensive first-pass metabolism both in the intestinal wall and the liver (Dunselman and Edgar, 1991). In addition, patients with hypertensive crisis and angina sometimes require drugs with quick onset of action. Therefore, formulating felodipine as an ODT could be advantageous to achieve more rapid onset of action, and buccal absorption could avoid first pass metabolism effects. There have been previous reports of formulating felodipine as an ODT (Basalious et al., 2013; Tung et al., 2014), however, previous *in vitro* dissolution testing for felodipine ODTs used USP 2 methods as described by the U.S. Pharmacopeia monograph (United States Pharmacopeia, 2009). This USP 2 approach has very limited similarity to the oral cavity, therefore it is unlikely to give a useful indication of ODT performance in the mouth.

Therefore, the aim of the present work was to use pooled human stimulated saliva in dissolution as a screening tool to guide the development and optimisation of ODT formulation of a model lipophilic compound, felodipine.

2. Materials and methods

Felodipine API powder was kindly donated by AstraZeneca (Macclesfield, UK), Soluplus® and Kollidon CL (polyvinylpyrrolidone, crosslinked) by Baden Aniline and Soda Factory (BASF) company (Germany) and spray dried lactose by Foremost Farms (USA). Magnesium stearate, sodium dodecyl sulphate (SDS), methyl-beta-cyclodextrin (MβCD) and beta-cyclodextrin (βCD) were purchased from Sigma Aldrich (Gillingham, UK), and colloidal silicon dioxide 2% was purchased from JRS (Germany). 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

All human saliva samples were collected in accordance with Ethics Reference Number: R12122013 from Faculty of Medicine and Health Sciences, Nottingham University Hospitals. Participation was voluntary and informed written consent was obtained. All data was held in accordance with the Data Protection Act. Exclusion criteria included chronic or acute illness in the past 3 months, cold or flu symptoms, oral health concerns, and taking medication (except contraceptives). Participants were asked to not eat, smoke, drink or use oral hygiene at least 2 h before collection of saliva. To avoid differences in saliva due to circadian rhythms, all saliva was collected between 14:00 and 16:00 h.

Participants first donated unstimulated (US) saliva before being asked to chew on 5 cm × 5 cm square of Parafilm® for stimulated (SS) saliva, which is a known inert material that is widely used for mechanical stimulation of saliva (Aiuchi et al., 2008; Bardow et al., 2000; Christersson et al., 2000; Inoue et al., 2008). To donate saliva, participants were asked to lean forward and drain saliva into sterile polypropylene graduated centrifuge tubes via sterile disposable funnels (Grenier Bio-One, UK). Saliva samples were then flash frozen in liquid nitrogen and stored at −80 °C. In total, four volunteers donated unstimulated and stimulated saliva (demographic data are shown in Appendix A. Supplementary Data). The donated saliva was pooled,

characterised, and used in dissolution studies.

2.2. Characterisation of pooled human saliva

Pooled saliva was characterised for: pH, buffer capacity, viscosity, and surface tension. An S220 seven compact pH/ion meter was used with InLab Science Pro electrode (Mettler Toledo, Switzerland) to measure pH. For buffer capacity each pooled saliva sample (4 mL) was preheated to 37 °C. The sample was titrated with 0.01 M HCl at 37 °C until pH had decreased by 1 unit. The buffer capacity in mmol H^+/L was calculated by measuring the volume of acid added. An Anton Paar Modular Compact Rheometer (MCR) 302 (Anton Paar GmbH, Germany) was used with a cone-plate set up to measure viscosity. The cone 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 Rheoplus software (Anton Paar GmbH, Germany) for analysis. A DSA 100 Drop Shape Analyser using a pendant drop method with DSA 4 software (Kruss GmbH, Germany) was used to measure surface tension. Temperature was set to 37 °C using an MB-5 heat circulator and water bath (Julabo GmbH, Germany).

2.3. Oral cavity dissolution studies of felodipine and its formulations in pooled stimulated human saliva

The volume of saliva used in this oral cavity dissolution method was based on mimicking the resident saliva volume within the adult human mouth (Lagerlof and Dawes, 1984; Rudney et al., 1995). Multiple glass tubes (16 × 100 mm) were used as dissolution vessels and were kept at 37 °C each with a magnetic stirrer. One mL of pooled stimulated human saliva was added to 2.5 mg felodipine, as depicted in Fig. 1. After the addition of saliva to the drug, dissolution run time was set to 10 min with constant 200 rpm stirring speed to allow adequate mixing of solid material in relatively small volumes of media, and for direct comparisons between different formulations. As the dissolution procedure in this study aimed to rapidly screen and rank-order multiple formulations, a single time point was used. At the 10-minute time point the whole dissolution sample was directly transferred to Costar Spin-X centrifuge tubes with 0.22 µm pore cellulose acetate (CA) filters (Corning Life Sciences, UK) and centrifuged for 5 min at 17,000 × *g*.

The following formulations components were tested as powders in human saliva dissolution: felodipine API powder (as received), cryomilled felodipine, co-cryomilled felodipine and Soluplus®, cryomilled felodipine complexed with βCD or MβCD, co-cryomilled felodipine and Soluplus® complexed with βCD or MβCD.

The following formulations were tested as ODTs in human saliva dissolution: felodipine API powder (as received), co-cryomilled felodipine and Soluplus®, co-cryomilled felodipine and Soluplus® complexed with MβCD.

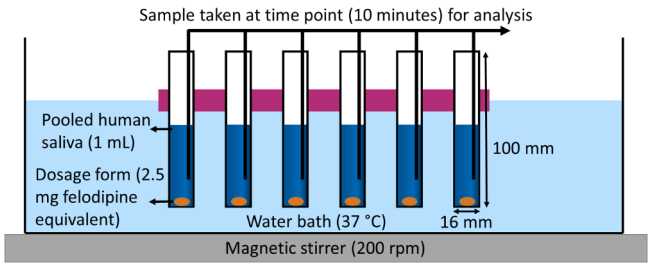

Fig. 1. A schematic representation of the oral cavity dissolution set up utilising human saliva as dissolution medium. Pooled human saliva (1 mL) was added to 2.5 mg of felodipine and stirred at 200 rpm for 10 min. At the 10-minute time point the whole sample was taken for sample preparation and analysis via HPLC-UV.

2.4. Compendial dissolution studies of felodipine formulations in USP 2 apparatus

Compendial dissolution was performed using USP 2 apparatus (Erweka Dissolution Tester, Germany) with phosphate buffer at pH 6.5 containing 1% SDS, as recommended by the felodipine monograph designed for extended release tablets (United States Pharmacopeia, 2009). The volume of media used was 600 mL, and was maintained at 37 °C. A paddle speed of 50 rpm was employed. For comparisons to be made to oral cavity dissolution, at 10 min 5 mL samples were withdrawn, and underwent the same sample preparation steps used for saliva dissolution samples. The ODTs containing co-cryomilled felodipine and Soluplus® complexed with MβCD were assessed.

2.5. Determination of felodipine in pooled stimulated saliva by HPLC-UV

Hundred µL of the saliva sample was transferred to a glass test tube, along with: 10 µL of internal standard (IS), and 300 µL of acetonitrile (stored at −20 °C), then vortex mixed for 2 min. Next, 200 µL of water was added to each tube, and vortex mixed for a further 2 min. Then, 4 mL of methyl-tert-butyl ether (MTBE) was added to each test tube, vortex mixed at 1200 min^{-1} for 10 min in a multi-tube vortexer (VWR VX-2500), and then 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 (60% ACN, 40% 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.

A Waters (Milford, USA) 2695 separations module HPLC system and Waters 996 diode array UV detector was used. Samples in the autosampler were maintained at 4 °C and the column oven was set to 50 °C. Empower 2 software was used for data processing.

Separation of the extracted felodipine sample was achieved with Supleco LC-18 4.6 × 150 mm, 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 60% ACN and 40% HPLC grade water, eluted at isocratic conditions at 0.6 mL/min. Cannabidiol was used as the internal standard (IS). Felodipine and cannabidiol were detected at 360 nm and 220 nm at 7.4 and 15.4 min respectively. The accuracy and precision of the method gave acceptable values of ± 15% relative error (RE), and ≤15% relative standard deviation (RSD) respectively from both intra-day and inter-day analyses (US Food and Drug administration (FDA), 2001). The lowest limit of quantification (LLOQ) for felodipine (lowest determined concentration of felodipine in spiked pooled saliva samples that had acceptable accuracy and precision of RE ± 20%, and RSD ≤ 20% respectively) was determined to be 25 ng/mL in both saliva states. Calibration curves were constructed in the concentration ranges expected from dissolution of the drug in saliva. Calibration curves all had correlation coefficient (r^2) values of > 0.99. The recovery of felodipine from pooled stimulated saliva dissolution samples was 100.10 ± 0.56% (mean ± SD, n = 3).

2.6. Cryomilling of felodipine and co-milling with Soluplus®

Cryomilling was performed on felodipine and Soluplus® using a 6870 Freezer/Mill (SPEX, USA) as described previously (Hameed, 2017). One gram of total weight of either felodipine API alone, Soluplus® alone or their mixture (1:1 ratio) was subjected to cryomilling for 150 min. The starting material was pre-cooled for 3 min and then milled for 2 min. The material was then cooled and milled on a cycle until 150 min was reached. Cryomilled material was then vacuum sealed in an argon filled environment with relative humidity of 7% to minimise as far as possible moisture uptake. Production of amorphous material

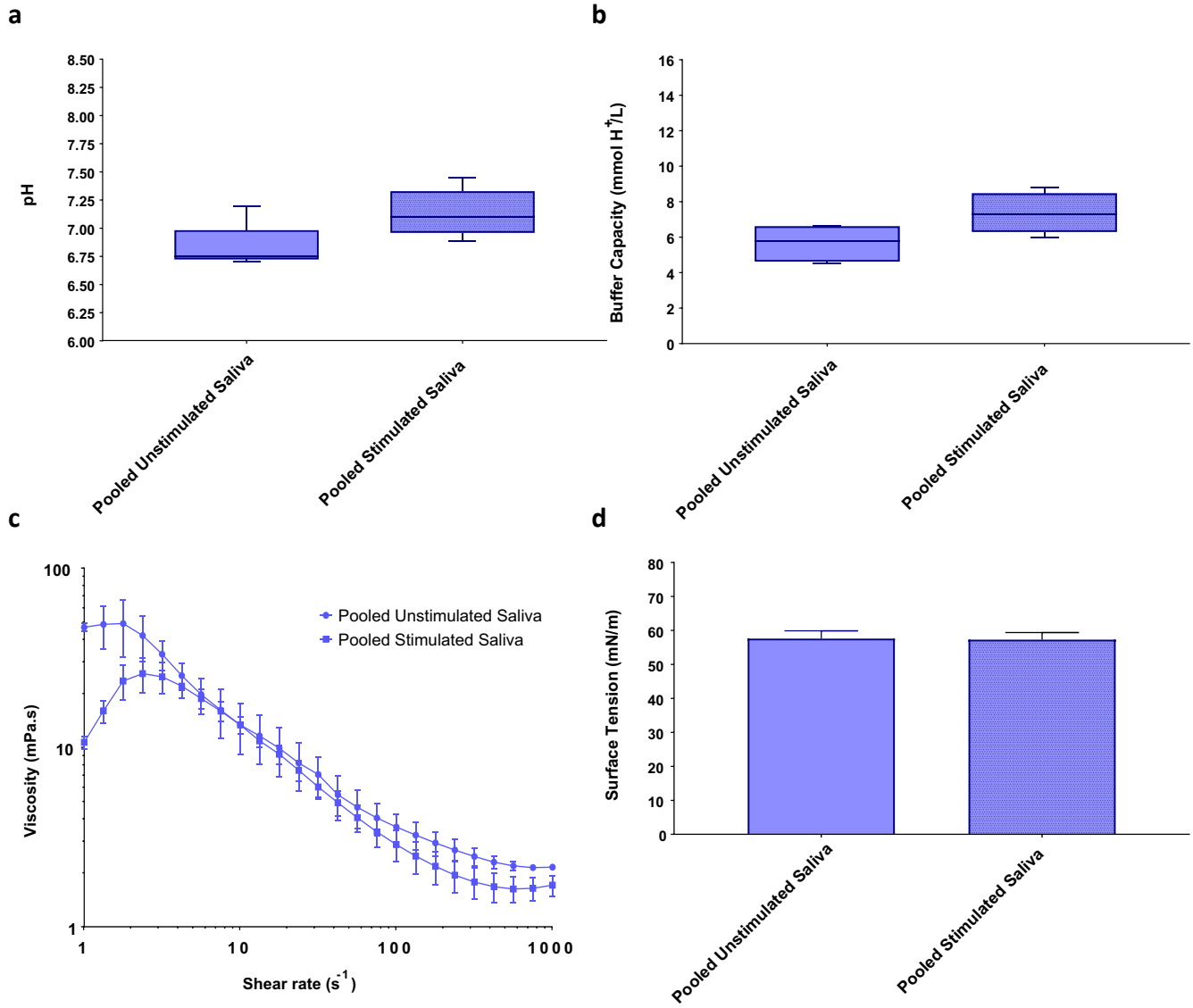

Fig. 2. Characterisation of pooled human saliva samples. (a) pH of pooled unstimulated and stimulated human saliva (n = 12, triplicate). Box represents median value, 25th and 75th percentile. Whiskers represent maximum and minimum values. (b) Buffer capacity of pooled unstimulated and stimulated human saliva (n = 4, triplicate). Box represents median value, 25th and 75th percentile. Whiskers represent maximum and minimum values. (c) Viscosity of pooled unstimulated and stimulated human saliva (n = 3, duplicate), at different shear rates. (d) Surface tension of pooled unstimulated and stimulated human saliva (n = 5, duplicate).

was confirmed by X-ray powder diffraction (XRPD) as described in Appendix A. Supplementary Data.

2.7. Preparation of felodipine mixtures and complexation with cyclodextrins

A physical mixture of cryomilled felodipine with cryomilled Soluplus® was mixed thoroughly in the dry state at 1:1 ratio. The mixture was then passed through a No. 60 sieve and stored in a desiccator. Complexation with cyclodextrins was undertaken with two methods; kneading and lyophilisation. The kneading method was used with MβCD and βCD, cyclodextrins were placed into a mortar, moistened with 50% ethanol and kneaded to a paste consistency. Felodipine was then added at equimolar ratios to cyclodextrins and kneaded into the mixture whilst adding successively 50% ethanol. The kneaded mixtures were then dried at 50 °C for 8 h. The dried powder was then passed through a No. 60 sieve and stored in a desiccator.

For the lyophilisation method both MβCD and βCD was used to make 50 mL of respective cyclodextrin solutions in water, then felodipine was added and stirred at 500 rpm to form a stable suspension. Methanol was then added to the solution at room temperature and was stirred for 12 h. Then the solution was flash frozen in liquid nitrogen and lyophilised in a Scanvac CoolSafe Touch 110-4 freeze dryer (Labogene, Denmark) to give a solid complex. Freeze-dried samples were ground in a mortar, then passed through a No. 60 sieve and stored in a desiccator.

2.8. Production of felodipine orally disintegrating tablets (ODTs)

Blends containing cryomilled felodipine and cryomilled Soluplus® (1:1) were mixed with excipients to give 0.8% drug loading resulting in 2.5 mg felodipine in a 300 mg tablet (8 mm diameter, 5 mm height). The following excipients were added to the formulations to give a 300 mg tablet: Kollidon CL (15 mg), spray dried lactose (271 mg), colloidal silicon dioxide (6 mg), magnesium stearate (3 mg). 60 g batches were produced in a blender. The tablets were then compressed using concave punches on the Piccola multi-station press (Riva SA, Argentina) using 8 mm flat, round tooling at 6 kN compression force.

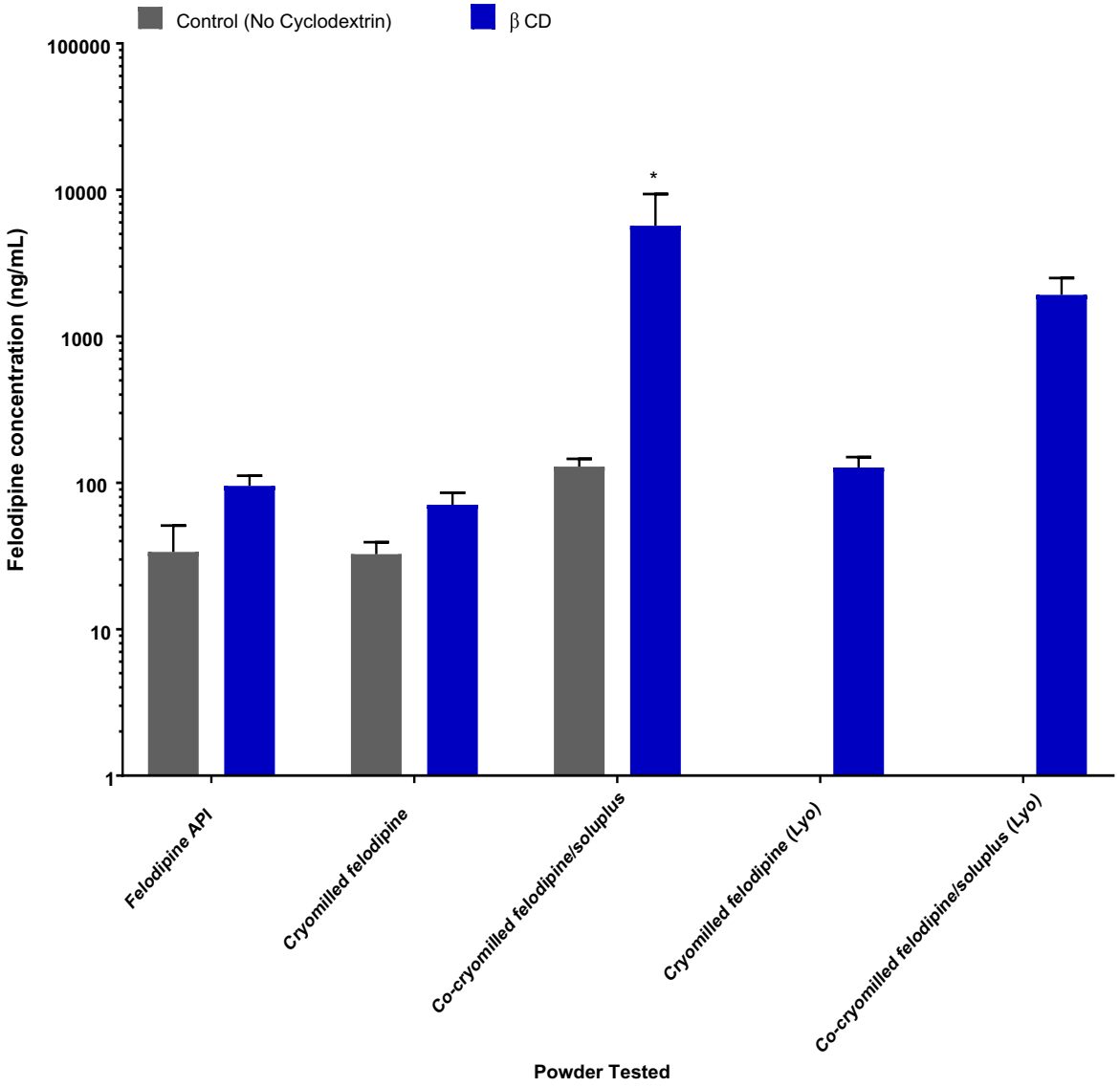

Fig. 3. The effect of dissolution enhancement strategies including cryomilling, addition of Soluplus®, and inclusion with beta-cyclodextrin (βCD) on the concentration of felodipine post 10-minute dissolution in pooled stimulated human saliva. Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using Kruskal-Wallis with Dunn's multiple comparisons test. Statistical differences compared to felodipine API and cryomilled felodipine without cyclodextrins; $^{*}p < 0.05$.

2.9. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical differences between data sets were assessed using Kruskal-Wallis with Dunn's multiple comparisons test. A p value < 0.05 was considered to represent a significant difference.

3. Results and discussion

3.1. Characterisation of pooled human saliva

The physicochemical parameters tested for pooled human saliva were: pH, buffer capacity, viscosity, and surface tension (Fig. 2). All physicochemical properties of saliva were comparable to saliva parameters measured for a wider population of healthy adult volunteers (Gittings et al., 2015). This suggests that the pooling approach had not significantly altered saliva characteristics and therefore was suitable to use for biorelevant dissolution testing. Additional potential approaches to take the use of pooled human saliva dissolution studies further would be to characterise saliva within different age groups and disease states that are applicable for the treatment/medication of interest.

3.2. Dissolution of felodipine powder in pooled stimulated human saliva

The concentrations of felodipine from various formulations after pooled stimulated human saliva dissolution are shown in Figs. 3 and 4. The initial dissolution testing of felodipine API powder demonstrates that felodipine has a very low concentration post-dissolution within pooled stimulated human saliva (34 ng/mL), which is 7-fold lower than reported concentration in pH 7.4 buffer (Alelyunas et al., 2009), 100-fold lower than the reported concentration in water (Kim et al., 2006; Mielcarek et al., 2006), and 600-fold lower than reported for pH 6.8 buffer (Bhole and Patil, 2009). The low concentration of felodipine post 10-minute dissolution in pooled stimulated saliva demonstrates that this compound and probably other drugs with similar physicochemical properties would require solubility enhancement for successful buccal/sublingual delivery.

In this study, the effect of three different solubility enhancement

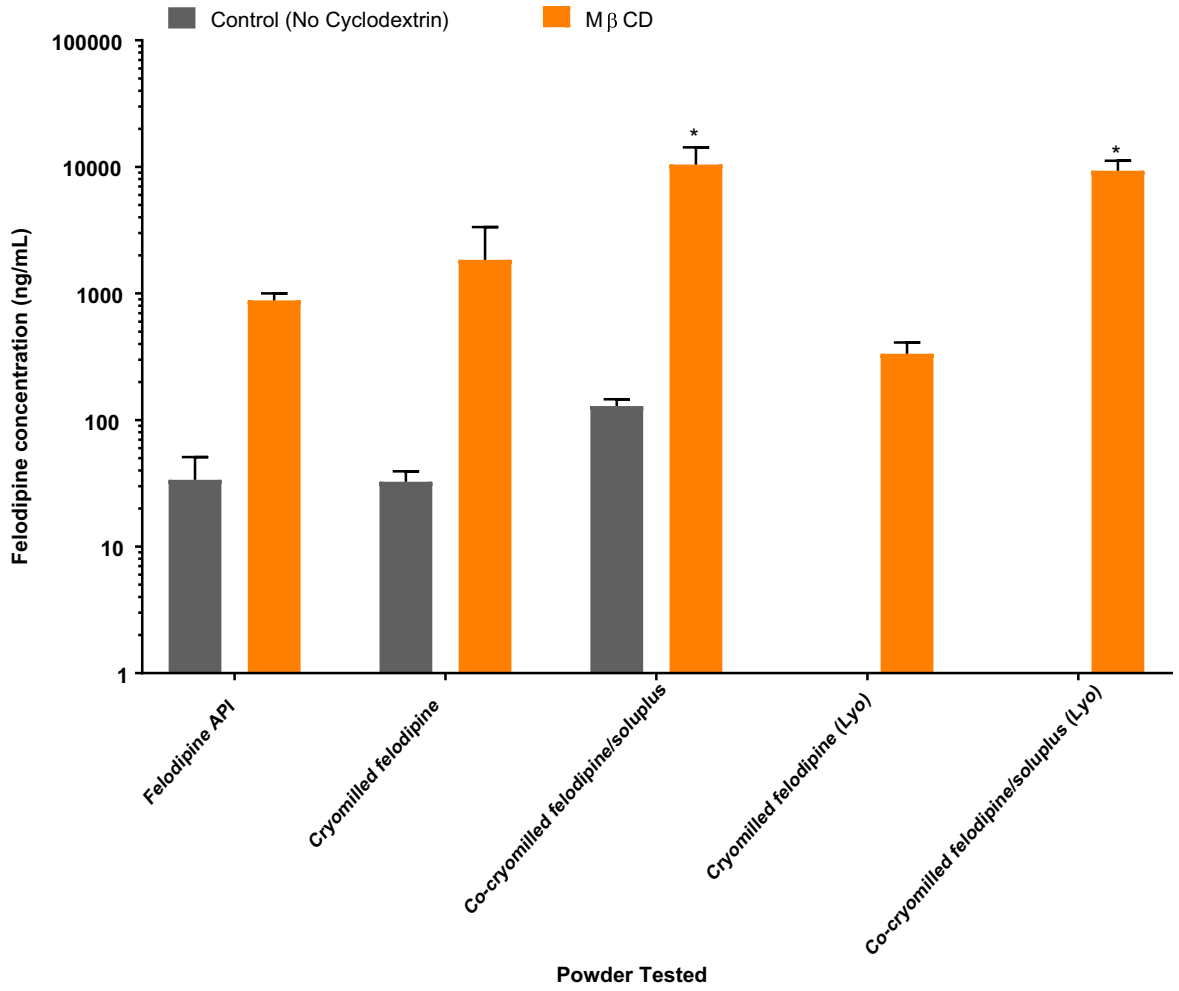

Fig. 4. The effect of dissolution enhancement strategies including cryomilling, addition of Soluplus®, and inclusion with methyl-beta-cyclodextrin (MβCD) on the concentration of felodipine measured after 10-minute dissolution in pooled stimulated human saliva. Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using Kruskal-Wallis with Dunn's multiple comparisons test. Statistical differences compared to felodipine API and cryomilled felodipine without cyclodextrins; $^{*}p < 0.05$.

strategies on the concentration of felodipine in pooled stimulated human saliva was shown. Firstly, felodipine was cryomilled to enhance dissolution by reducing particle size and by creating amorphous drug particles. The next step was to add a polymeric solubilizer Soluplus®, aiding the solubilisation of poorly soluble drugs in aqueous media. Next was the inclusion of felodipine within cyclodextrins. Complexation with cyclodextrins was achieved with two different methods, kneading and lyophilisation. Figs. 3 and 4 show that significantly higher levels of felodipine were observed using either βCD or MβCD, when coupled with co-cryomilled felodipine and Soluplus® (Kruskal-Wallis with Dunn's multiple comparisons test). MβCD when complexed by the kneading method or the lyophilisation (Lyo) method in the presence of co-cryomilled felodipine and Soluplus® gave significantly higher concentrations of felodipine in stimulated saliva when compared to felodipine API and cryomilled felodipine alone (Kruskal-Wallis with Dunn's multiple comparisons test). The use of these strategies separately did not significantly increase drug concentration. Importantly it was found that the combination of all three strategies: cryomilling, addition of Soluplus®, and complexation with cyclodextrins gave a significant increase in felodipine concentration in saliva (5.69 μg/mL for βCD, 10.41 μg/mL for MβCD) when compared to felodipine API powder.

Commercially available ODTs of other lipophilic compounds have been produced primarily with the Zydis® technology, and one account using the Quicksolv® technology (Fu et al., 2004). The Zydis® technology physically traps the drug within a water-soluble sugar/polymer matrix which is then lyophilised. However, the use of lipophilic compounds with Zydis® is not attributed to a direct increase in the water solubility of the drug. Instead it is due to the fast disintegration times associated with this technology, and the indirect benefit that compounds that are practically insoluble in water will have less stability issues due to moisture when compared to water soluble compounds (Amipara and Gupta, 2013). This demonstrates that current commercially available ODTs containing lipophilic compounds do not usually employ drug solubility enhancement strategies such as those presented in this study. However, there are previous reports of solid dispersions and micronisation strategies for felodipine ODTs specifically (Raghavendra Rao et al., 2010). Whilst there are several formulation development procedures available to use in the development of ODTs there are little to no biorelevant *in vitro* dissolution studies that can determine the performance of ODTs within the oral cavity and therefore guide rational design strategies.

3.3. Dissolution of felodipine orally disintegrating tablets (ODTs) in pooled stimulated human saliva

Based on the results from the dissolution of different felodipine powders (as described in Section 3.2) the inclusion of methyl-beta-cyclodextrin and co-milling with Soluplus® was utilised as the ODT formulation. It was observed that 300 mg ODTs with methyl-beta-cyclodextrin with cryomilled felodipine, and cryomilled Soluplus®

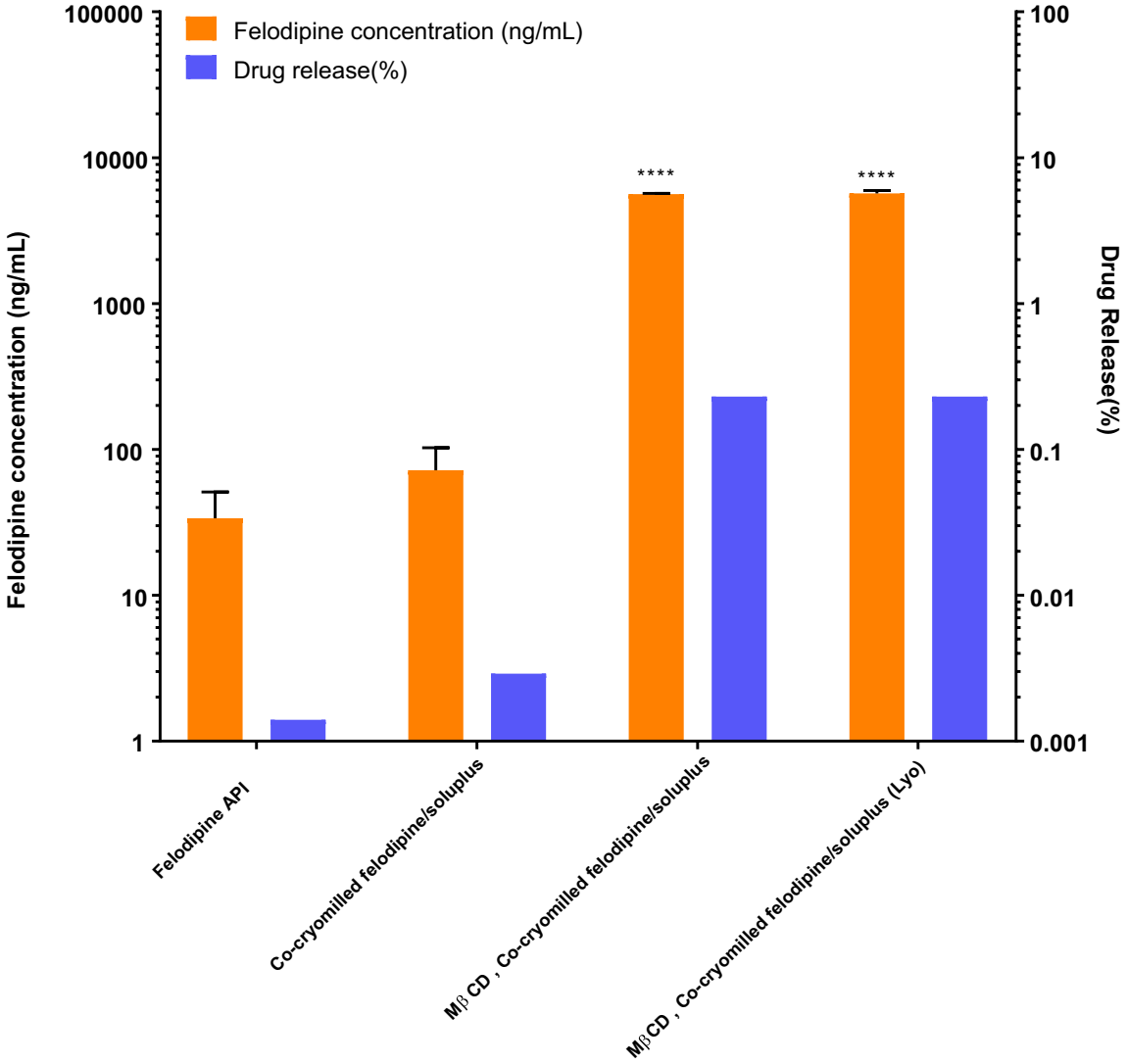

Fig. 5. The concentration of felodipine measured and percentage drug released after 10-minute dissolution of formulated tablets in pooled stimulated human saliva. Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using Kruskal-Wallis with Dunn's multiple comparisons test. Statistical differences compared to felodipine API alone; $^{****}p < 0.0001$.

significantly ($p < 0.0001$, Kruskal-Wallis with Dunn's multiple comparisons test) increased the concentration of felodipine when compared to felodipine 300 mg tablets without cryomilling and without the addition of Soluplus®/cyclodextrins (Fig. 5).

3.4. Comparison between saliva-based dissolution and compendial dissolution

Solid dosage forms are routinely evaluated within compendial dissolution apparatuses. For oral dosage forms, the compendial apparatuses used are USP 1–4. These dissolution apparatuses were designed for conventional dosage forms (designed to be quickly swallowed) and are capable of representing areas of the GI such as the stomach and small intestine with appropriate selection of dissolution media (Culen and Dohnal, 2013). However, the conventional dissolution set ups are not suitable for oral cavity dissolution purposes, especially for orally retained formulations such as ODTs for which their effectiveness is primarily affected by the dissolution and solubility in mouth and saliva.

Percentage drug release was calculated at the 10 min dissolution of formulated felodipine ODTs in the stimulated saliva dissolution method and in compendial USP 2 monograph dissolution. It was observed that there is a significant difference in percentage drug release between saliva-based dissolution, giving a 0.2% drug release, and compendial dissolution, giving an 83% drug release from formulated ODTs (Fig. 6).

This study has shown that biorelevant pooled stimulated human saliva dissolution allowed to guide the development of ODT formulation of a model lipophilic compound and eventually to achieve over a 150-fold increase in concentration within saliva. Whilst this formulation development has provided a substantial increase in felodipine concentration, the percentage of felodipine released from ODTs in pooled stimulated saliva still equates to only 0.2%. Interestingly, the low drug release of felodipine in saliva in this oral cavity dissolution set up is not reflected in compendial USP 2 dissolution of felodipine ODTs and felodipine API. Compendial USP 2 dissolution resulted in felodipine API release ranging from 30 to 40% as reported by others (Basalious et al., 2013) and supported by our own data presented here, as well as in our previous studies (Hameed, 2017). Furthermore, compendial USP 2 dissolution tests on formulated felodipine ODTs following production as stated in this study resulted in 83% drug release after 10 min (Hameed, 2017). Another report has also shown that percentage release of felodipine from ODTs was over 80% after 5 min dissolution in compendial USP 2 dissolution apparatus (Tung et al., 2014). The USP 2 dissolution studies for felodipine mentioned were all adapted from the felodipine monograph designed for extended release tablets (United

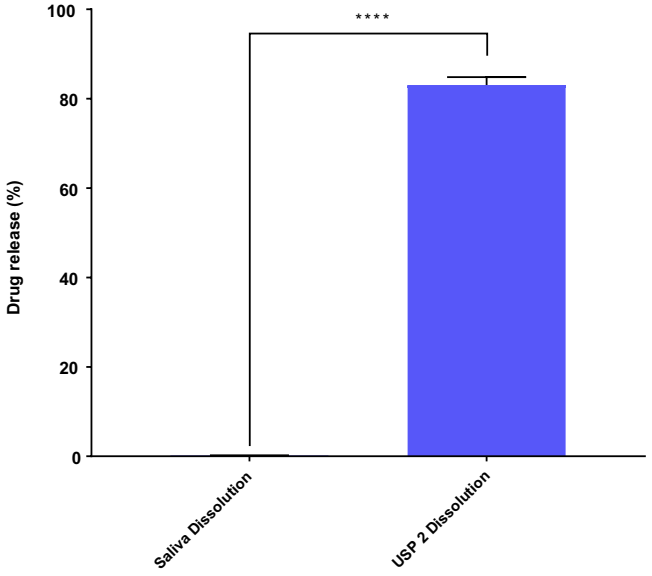

Fig. 6. Percentage drug release of felodipine after 10-minute dissolution of formulated orally disintegrating tablets in two different dissolution set ups; pooled stimulated saliva dissolution, and compendial USP 2 dissolution. Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using Kruskal-Wallis test. Statistical difference; $^{****}p < 0.0001$.

States Pharmacopeia, 2009).

The felodipine monograph method (United States Pharmacopeia, 2009) recommends the use of phosphate buffer (pH 6.5) with 1% SLS as dissolution media. It is likely that the large differences seen in percentage drug release between USP 2 dissolution and oral cavity dissolution is due to the differences between the two dissolution media used. This highlights the benefit of using human saliva as dissolution media to avoid the potentially skewed picture of predicted ODT performance generated by standard compendial dissolution media approaches.

Further studies will be needed to assess if the proposed biorelevant pooled saliva dissolution approach could to be used successfully for rational development of wide range of compounds with poor aqueous solubility.

4. Conclusion

In this study it has been found that biorelevant pooled stimulated human saliva dissolution approach guided rational design of orally disintegrating tablets of a model poorly water soluble compound, felodipine. With the use of cryomilling, solid dispersion, and inclusion complexation strategies together as much as a 150-fold increase in concentration of felodipine in human saliva was achieved. Whilst these formulation strategies greatly increased felodipine concentration in saliva, the percent of dose released was still very low (0.2%). The drug release from the same formulation using a compenidal phosphate buffer was shown to be as high as 83%. The discrepancy in drug release between dissolution media highlights the importance of dissolution apparatus and biorelevant media selection for efficient assessment of performance of orally retained formulations. Further studies will be needed to assess if the proposed biorelevant pooled saliva dissolution approach could be successfully used for rational development of wide range of compounds with poor aqueous solubility.

Acknowledgments

This work was supported by the Engineering and Physical Sciences Research Council [grant number EP/L01646X]; the University of Nottingham CDT in Advanced Therapeutics and Nanomedicine; and Pfizer Ltd. The authors would also like to thank Joanne Bennett and Anne Cram for their advice in preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2018.11.051.

References

Aiuchi, H., Kitasako, Y., Fukuda, Y., Nakashima, S., Burrow, M.F., Tagami, J., 2008. Relationship between quantitative assessments of salivary buffering capacity and ion activity product for hydroxyapatite in relation to cariogenic potential. Aust. Dent. J. 53, 167–171. https://doi.org/10.1111/j.1834-7819.2008.00027.x.

Alelyunas, Y.W., Liu, R., Pelosi-Kilby, L., Shen, C., 2009. Application of a Dried-DMSO rapid throughput 24-h equilibrium solubility in advancing discovery candidates. Eur. J. Pharm. Sci. 37, 172–182. https://doi.org/10.1016/j.ejps.2009.02.007.

Al-kassas, R., Bansal, M., Shaw, J., 2017. Nanosizing techniques for improving bioavailability of drugs. J. Control. Release. 260, 202–212. https://doi.org/10.1016/j.jconrel.2017.06.003.

Amipara, L.V., Gupta, M., 2013. Oral disintirating tablet of antihypertensive drug. J. Drug Deliv. Ther. 3, 85–92. https://doi.org/10.1111/head.12009.

Azarmi, S., Roa, W., Löbenberg, R., 2007. Current perspectives in dissolution testing of conventional and novel dosage forms. Int. J. Pharm. 328, 12–21. https://doi.org/10.1016/j.ijpharm.2006.10.001.

Bardow, A., Moe, D., Nyvad, B., Nauntofte, B., 2000. The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. Arch. Oral Biol. 45, 1–12. https://doi.org/10.1016/S0003-9969(99)00119-3.

Bartlett, J.A., van der Voort Maarschalk, K., 2012. Understanding the oral mucosal absorption and resulting clinical pharmacokinetics of asenapine. AAPS PharmSciTech 13, 1110–1115. https://doi.org/10.1208/s12249-012-9839-7.

Basalious, E.B., El-Sebaie, W., El-Gazayerly, O., 2013. Rapidly absorbed orodispersible tablet containing molecularly dispersed felodipine for management of hypertensive crisis: development, optimization and in vitro/in vivo studies. Pharm. Dev. Technol. 18, 407–416. https://doi.org/10.3109/10837450.2012.659258.

Bavishi, D.D., Borkhataria, C.H., 2016. Spring and parachute: how cocrystals enhance solubility. Prog. Cryst. Growth Char. Mater. 62, 1–8. https://doi.org/10.1016/j.pcrysgrow.2016.07.001.

Bhole, P., Patil, V., 2009. Enhancement of water solubility of felodipine by preparing solid dispersion using poly-ethylene glycol 6000 and poly-vinyl alcohol. Asian J. Pharm. 3, 240. https://doi.org/10.4103/0973-8398.56305.

Buettner, A., 2002. Influence of human salivary enzymes on odorant concentration changes occurring in vivo. 1. Esters and thiols. J. Agric. Food Chem. 50, 3283–3289. https://doi.org/10.1021/jf011586r.

Cameron, C., Kelly, S., Hsieh, S.C., Murphy, M., Chen, L., Kotb, A., Peterson, J., Coyle, D., Skidmore, B., Gomes, T., Clifford, T., Wells, G., 2015. Triptans in the acute treatment of migraine: a systematic review and network meta-analysis. Headache J. Head Face Pain 55, 221–235. https://doi.org/10.1111/head.12601.

Christersson, C.E., Lindh, L., Arnebrant, T., 2000. Film-forming properties and viscosities of saliva substitutes and human whole saliva. Eur. J. Oral Sci. 108, 418–425. https://doi.org/10.1034/j.1600-0722.2000.108005418.x.

Culen, M., Dohnal, J., 2013. Advances in dissolution instrumentation and their practical applications. Drug Dev. Ind. Pharm. 9045, 1–6. https://doi.org/10.3109/03639045.2013.841184.

Dressman, J.B., Reppas, C., 2000. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. Eur. J. Pharm. Sci. 11, 73–80. https://doi.org/10.1016/S0928-0987(00)00181-0.

Dunselman, P.H.J.M., Edgar, B., 1991. Felodipine clinical pharmacokinetics. Clin. Pharmacokinet. 21, 418–430. https://doi.org/10.2165/00003088-199121060-00003.

European Pharmacopeia (Ph Eur), 2014. Strasbourg, France: European Directorate for the Quality of Medicines, Council of Europe.

Fu, Y., Yang, S., Jeong, S.H., Kimura, S., Park, K., 2004. Orally fast disintegrating tablets: developments, technologies, taste-masking and clinical studies. Crit. Rev. Ther. Drug Carrier Syst. 21, 433–476. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v21.i6.10.

Gal, J.Y., Fovet, Y., Adib-Yadzi, M., 2001. About a synthetic saliva for in vitro studies. Talanta 53, 1103–1115. https://doi.org/10.1016/S0039-9140(00)00618-4.

Gittings, S., Turnbull, N., Henry, B., Roberts, C.J., Gershkovich, P., 2015. Characterisation of human saliva as a platform for oral dissolution medium development. Eur. J. Pharm. Biopharm. 91, 16–24. https://doi.org/10.1016/j.ejpb.2015.01.007.

Gittings, S., Turnbull, N., Roberts, C.J., Gershkovich, P., 2014. Dissolution methodology for taste masked oral dosage forms. J. Control. Release 173, 32–42. https://doi.org/10.1016/j.jconrel.2013.10.030.

Guhmann, M., Preis, M., Gerber, F., Pöllinger, N., Breitkreutz, J., Weitschies, W., 2012. Development of oral taste masked diclofenac formulations using a taste sensing system. Int. J. Pharm. 438, 81–90. https://doi.org/https://doi.org/10.1016/j.ijpharm.2012.08.047.

Hameed, G.S., 2017. Investigation of Cryomilling as a Potential Tool for the Production of Amorphous Solid Dispersions. University of Nottingham Thesis (PhD).

Hermans, A., Abend, A.M., Kesisoglou, F., Flanagan, T., Cohen, M.J., Diaz, D.A., Grady, H., 2017. Approaches for establishing clinically relevant dissolution specifications for immediate release solid oral dosage forms. AAPS J. 19. https://doi.org/10.1208/

s12248-017-0117-1.
Hoang Thi, T.H., Morel, S., Ayouni, F., Flament, M.P., 2012. Development and evaluation of taste-masked drug for paediatric medicines – Application to acetaminophen. Int. J. Pharm. 434, 235–242. https://doi.org/10.1016/j.ijpharm.2012.05.047.
Inoue, H., Ono, K., Masuda, W., Inagaki, T., Yokota, M., Inenaga, K., 2008. Rheological properties of human saliva and salivary mucins. J. Oral Biosci. 50, 134–141. https://doi.org/10.2330/joralbiosci.50.134.
Khames, A., 2017. Investigation of the effect of solubility increase at the main absorption site on bioavailability of BCS class II drug (risperidone) using liquisolid technique. Drug Deliv. 24, 328–338. https://doi.org/10.1080/10717544.2016.1250140.
Kim, E.J., Chun, M.K., Jang, J.S., Lee, I.H., Lee, K.R., Choi, H.K., 2006. Preparation of a solid dispersion of felodipine using a solvent wetting method. Eur. J. Pharm. Biopharm. 64, 200–205. https://doi.org/10.1016/j.ejpb.2006.04.001.
Kostewicz, E.S., Brauns, U., Becker, R., Dressman, J.B., 2002. Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media. Pharm. Res. 19, 345–349.
Kraemer, J., Gajendran, J., Guillot, A., Schichtel, J., Tuereli, A., 2012. Dissolution testing of orally disintegrating tablets. J. Pharm. Pharmacol. 64, 911–918. https://doi.org/10.1111/j.2042-7158.2012.01473.x.
Lagerlof, F., Dawes, C., 1984. The volume of saliva in the mouth before and after swallowing. J. Dent. Res. 63, 618–621. https://doi.org/10.1177/00220345840630050201.
Lai, F., Pini, E., Angioni, G., Manca, M.L., Perricci, J., Sinico, C., Fadda, A.M., 2011. Nanocrystals as tool to improve piroxicam dissolution rate in novel orally disintegrating tablets. Eur. J. Pharm. Biopharm. 79, 552–558. https://doi.org/10.1016/j.ejpb.2011.07.005.
Lopez, F.L., Mistry, P., Batchelor, H.K., Bennett, J., Coupe, A., Ernest, T.B., Orlu, M., Tuleu, C., 2018. Acceptability of placebo multiparticulate formulations in children and adults. Sci. Rep. 8, 9210. https://doi.org/10.1038/s41598-018-27446-6.
Manarelli, M.M., Pessan, J.P., Delbem, A.C.B., Amaral, J.G., Paiva, M.F., Barbour, M.E., 2017. Protective effect of phosphates and fluoride on the dissolution of hydroxyapatite and their interactions with saliva. Caries Res. 51, 96–101. https://doi.org/10.1159/000452716.
Mielcarek, J., Czernielewska, A., Czarczyńska, B., 2006. Inclusion complexes of felodipine and amlodipine with methyl-β- cyclodextrin. J. Incl. Phenom. 54, 17–21. https://doi.org/10.1007/s10847-005-2603-9.
Okumu, A., DiMaso, M., Löbenberg, R., 2008. Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. Pharm. Res. 25, 2778–2785. https://doi.org/10.1007/s11095-008-9642-z.
Raghavendra Rao, N.G., Kulkarni, U., Setty, C.M., 2010. Comparative study on effect of diferent techniques used in the formulation of felodipine fast dissolving tablets. Int. J. Pharm. Pharm. Sci. 2, 152–159.
Repka, M.A., Bandari, S., Kallakunta, V.R., Vo, A.Q., McFall, H., Pimparade, M.B., Bhagurkar, A.M., 2018. Melt extrusion with poorly soluble drugs – An integrated review. Int. J. Pharm. 535, 68–85. https://doi.org/10.1016/j.ijpharm.2017.10.056.
Rudney, J.D., Ji, Z., Larson, C.J., 1995. The prediction of saliva swallowing frequency in humans from estimates of salivary flow rate and the volume of saliva swallowed. Arch. Oral Biol. 40, 507–512. https://doi.org/https://doi.org/10.1016/0003-9969(95)00004-9.
Savjani, K.T., Gajjar, A.K., Savjani, J.K., 2012. Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012, 1–10. https://doi.org/10.5402/2012/195727.
Saxegaard, E., Lagerlöf, F., Rølla, G., 1988. Dissolution of calcium fluoride in human saliva. Acta Odontol. Scand. 46, 355–359. https://doi.org/10.3109/00016358809004788.
Schipper, R.G., Silletti, E., Vingerhoeds, M.H., 2007. Saliva as research material: biochemical, physicochemical and practical aspects. Arch. Oral Biol. 52, 1114–1135. https://doi:10.1016/j.archoralbio.2007.06.009.
Sharma, N., Baldi, A., 2016. Exploring versatile applications of cyclodextrins: an overview. Drug Deliv. 23, 739–757. https://doi.org/10.3109/10717544.2014.938839.
Shono, Y., Jantratid, E., Janssen, N., Kesisoglou, F., Mao, Y., Vertzoni, M., Reppas, C., Dressman, J.B., 2009. Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling. Eur. J. Pharm. Biopharm. 73, 107–114. https://doi.org/10.1016/j.ejpb.2009.05.009.
Sunesen, V.H., Pedersen, B.L., Kristensen, H.G., Müllertz, A., 2005. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. Eur. J. Pharm. Sci. 24, 305–313. https://doi.org/10.1016/j.ejps.2004.11.007.
Tietz, K., Gutknecht, S.I., Klein, S., 2018. Bioequivalence of locally acting lozenges: evaluation of critical in vivo parameters and first steps towards a bio-predictive in vitro test method. Eur. J. Pharm. Biopharm. 123, 71–83. https://doi.org/10.1016/j.ejpb.2017.11.011.
Tung, N.T., Hung, M. Van, Vo, X.M., Nguyen, T.H., Pham, T.M.H., 2014. Formulation optimization of orally disintegrating tablets containing solid dispersion of felodipine and hydroxypropyl methylcellulose using face-centered central composite design. J. Pharm. Investig. 44, 111–118. https://doi.org/10.1007/s40005-013-0106-z.
United States Pharmacopeia, 2009. U.S. Pharmacopeia-National Formulary [USP 33 NF 28]. Felodipine Extended-Release Tablets Monograph. United States Pharmacopeial Conv, Rockville, Md.
United States Pharmacopeia, 2014. Rockville, Md: United States Pharmacopeial Conv.
US Food and Drug administration (FDA), 2001. Guidance for Industry: Bioanalytical Method Validation 4–10.
Vertzoni, M., Dressman, J., Butler, J., Hempenstall, J., Reppas, C., 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. Eur. J. Pharm. Biopharm. 60, 413–417. https://doi.org/10.1016/j.ejpb.2005.03.002.
Yajima, T., Fukushima, Y., Itai, S., Kawashima, Y., 2002. Method of evaluation of the bitterness of clarithromycin dry syrup. Chem. Pharm. Bull. 50, 147–152. https://doi.org/10.1248/cpb.50.147.