Exploration of in vitro drug release testing methods for saquinavir microenvironmental pH modifying buccal films

He, Shaolong; Jacobsen, Jette; Nielsen, Carsten Uhd; Genina, Natalja; Østergaard, Jesper; Mu, Huiling

Published in:
European Journal of Pharmaceutical Sciences

DOI:
10.1016/j.ejps.2021.105867

Publication date:
2021

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Exploration of in vitro drug release testing methods for saquinavir microenvironmental pH modifying buccal films

Shaolong He\textsuperscript{a}, Jette Jacobsen\textsuperscript{a}, Carsten Uhd Nielsen\textsuperscript{b}, Natalja Genina\textsuperscript{a}, Jesper Østergaard\textsuperscript{a,}\textsuperscript{*}, Huling Mu\textsuperscript{a}

\textsuperscript{a}Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark
\textsuperscript{b}Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

\textbf{A R T I C L E   I N F O}

\textbf{Keywords:}
Drug release testing method
UV/Vis imaging
Franz diffusion cell
Microenvironmental pH
Saquinavir buccal film

\textbf{A B S T R A C T}

Buccal films containing a pH modifying excipient may be able to increase bioavailability of drugs with pH-dependent solubility such as saquinavir. Access to suitable in vitro drug release testing methods may facilitate buccal formulation development. This study aimed to explore two release testing methods for characterising buccal films and to elucidate the relationship between microenvironmental pH (pH\textsubscript{b}) and saquinavir release. The Franz diffusion cell method was applicable to investigate the effect of hydroxypropyl methylcellulose (HPMC) grade on saquinavir release. Films containing HPMC K100 LV had a faster saquinavir release than films containing HPMC K100 LV. A UV/Vis imaging method was developed to visualise saquinavir release and pH\textsubscript{b} changes during the initial dissolution. Within 5 min, the pH\textsubscript{b} decreased from 6.8 to around 5.4 for HPMC K100 LV-based films containing 11.1 % or 16.6 % (w/w) malic acid. Subsequently, the pH\textsubscript{b} increased due to increasing concentrations of saquinavir. An increase in malic acid content led to a faster saquinavir release. The combination of methods may be broadly applicable for excipient screening in development of buccal formulations. The imaging approach holds promise for characterizing other pH modifying formulation principles.

1. Introduction

Saquinavir is an anti-retroviral drug with low oral bioavailability due to its limited aqueous solubility\textsuperscript{(Branham et al., 2012)} and extensive first-pass metabolism in the intestine and in the liver\textsuperscript{(Pal and Mitra, 2006; Usansky et al., 2008; Wang et al., 2012)}. Generally, drugs administered via buccal administration could be directly delivered to the systemic circulation. Buccal delivery of saquinavir is a potential strategy to avoid the possible extensive metabolism associated with oral administration. However, the slow release of saquinavir from buccal formulations caused by its poor solubility in human saliva at physiological pH constitutes an obstacle for attaining a high bioavailability. Previous studies showed that saquinavir has a pH-dependent aqueous solubility, i.e. decreased solubility as pH increased in the range of 3.0 to 7.3\textsuperscript{(Buchanan et al., 2008; Obonga et al., 2013; Takano et al., 2016)}. Studies have shown that a low pH (< 5.5) in the mouth caused erosion of dentine and enamel\textsuperscript{(Hans et al., 2016; West et al., 2001)} and a pH lower than 2.5 induced acute effects such as irritation and toxicity on the oral mucosa\textsuperscript{(Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2004)}. Thus, a well-controlled local decrease of the microenvironmental pH (pH\textsubscript{b}) at site of administration might increase the solubility of saquinavir, and consequently enhance saquinavir release, and at the same time have a minimal destructive effect for the oral tissues and teeth. Previously, saquinavir release testing has been conducted using an in vitro perfusion model allowing perfusion media to flow on top of buccal films, which were packed with filter paper to diminish film erosion. The results showed that the addition of organic acids such as malic acid to buccal films led to an increase in saquinavir release from buccal films due to the decreased pH\textsubscript{b}\textsuperscript{(He et al., 2020)}. However, the effect of the organic acids (pH modifier in the buccal films) on enhancement of saquinavir release was negligible because the low pH\textsubscript{b} in the vicinity of the swelling buccal films only persisted for a short time\textsuperscript{(He et al., 2020)}. The short-lived pH change might be attributed to a concurrent fast release of the acids. Increasing the concentration of organic acid in buccal formulations might be an effective way to extend the duration of the low pH\textsubscript{b}\textsuperscript{(Siepe et al., 2006)}.

\textsuperscript{*} Corresponding author.
\textit{E-mail address: jesper.ostergaard@sund.ku.dk (J. Østergaard).}

https://doi.org/10.1016/j.ejps.2021.105867
Received 1 March 2021; Received in revised form 22 April 2021; Accepted 26 April 2021
Available online 2 May 2021
0928-0987/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
To elucidate the relationship between the dose of acid, pH, and saquinavir release behaviour as well as to attain knowledge to guide the formulation development and optimization, appropriate drug release testing methods are needed.

For drug dissolution and release testing of oromucosal dosage forms in vitro, compendial (USP and Ph. Eur.) methods and apparatuses for solid or semi-solid dosage forms, such as tablets and gels, have been widely employed. Basket dissolution apparatus, paddle dissolution apparatus and paddle over disk dissolution apparatus as well as Franz diffusion cells have been applied to test sublingual and buccal formu-
lations (Adekele et al., 2014; El-Mahrouk et al., 2014; Kassem et al., 2014; Urbanova et al., 2016). Most of these methods do not mimic sufficiently the physiological conditions present in the oral cavity. Although, only around 1.1 ml of saliva with a normal secretion rate of 1-2 ml/min is present in the oral cavity (Proctor, 2016), relatively large volumes of dissolution medium with stirring have been used to create sink conditions, especially in the cases of poorly water soluble drugs. For semisolid buccal gels and thin buccal polymer-based films with high erosion rate, the disintegration or erosion of these polymer matrices has been the rate-limiting step during the dissolution process. These rapidly disintegrating formulations would disintegrate and/or dissolve within a few minutes upon contact with large volumes of dissolution medium during stirring. Thus, the dissolution process of these formulations in vitro using the above mentioned apparatuses might be different from that occurring in the oral cavity. To avoid the fast disintegration and erosion of oromucosal formulations during testing, flow through cells equipped with an acetate cellulose membrane (0.45 μm) have been applied to study the release of salbutamol from buccal hydrogels (Zeng et al., 2014). The release of lidocaine from thiolated pectin based buccal gels has been investigated using Franz diffusion cells with a cellulose acetate filter having 0.2 μm pore size mounted between the donor and acceptor compartment (Hauptstein et al., 2014). The application of a membrane is an effective way to decrease the erosion rate of oromucosal formulations during the dissolution testing. However, the resistance to drug diffusion provided by a membrane might have a crucial effect on the observed drug release profile (Jug et al., 2018). Alternative methods capable of circumventing these disadvantages should be pursued.

The Franz diffusion cell is a FDA-recommended method to assess the acceptability of minor process and/or formulation changes in approved semisolid formulations (Grantome, 2015), and it might be an appropriate drug release testing method for HPMC-based buccal films with high erosion rate. UV/Vis imaging has advantages of real-time monitoring of drug absorbance and visualization of drug transport, and has been applied in characterization of drug diffusion, drug dissolution/release and pH measurement in hydrogel matrices (Jensen et al., 2016, 2015; Ye et al., 2012, 2011). Thus, a UV/Vis imaging setup might be used to investigate saquinavir release from the buccal films and pH₃₄ during dissolution, which would be conducive to elucidating the relationship between saquinavir release and pH₃₄. In the present study, pH₃₄ modifying buccal films with different compositions were prepared. Two different setups, (1) a Franz diffusion cell with a filter paper (particle retention size: 5-13 μm) as a holder for the films and (2) a UV/Vis imaging system with an agarose hydrogel mimicking the human saliva pH and buffer capacity, were employed to investigate saquinavir release from the films. The aims of the present research were twofold: (1) to develop a Franz diffusion cell method and a UV/Vis imaging in vitro platform with an agarose hydrogel matrix for saquinavir release studies of the pH₃₄ modifying buccal films, and (2) to investigate the effect of pH modifier and film-forming polymer, namely malic acid and HPMC, on saquinavir release behavior and elucidate the relationship between pH₃₄ and saquinavir release.

### Table 1

Compositions of the buccal films.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>HPMC* K100LV</th>
<th>HPMC* K3LV</th>
<th>Malic acid</th>
<th>Glycerol</th>
<th>Saquinavir mesylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.3 (66.3%)</td>
<td>–</td>
<td>0.05 (11.1%)</td>
<td>0.09 (19.9%)</td>
<td>0.012 (2.7%)</td>
</tr>
<tr>
<td>F2</td>
<td>–</td>
<td>0.3 (66.3%)</td>
<td>0.05 (11.1%)</td>
<td>0.09 (19.9%)</td>
<td>0.012 (2.7%)</td>
</tr>
<tr>
<td>F3</td>
<td>0.3 (82.9%)</td>
<td>–</td>
<td>0.05 (13.8%)</td>
<td>–</td>
<td>0.012 (3.3%)</td>
</tr>
<tr>
<td>F4</td>
<td>0.3 (55.4%)</td>
<td>–</td>
<td>0.05 (9.2%)</td>
<td>0.18 (33.2%)</td>
<td>0.012 (2.2%)</td>
</tr>
<tr>
<td>F5</td>
<td>0.3 (62.2%)</td>
<td>–</td>
<td>0.08 (16.6%)</td>
<td>0.09 (18.7%)</td>
<td>0.012 (2.5%)</td>
</tr>
<tr>
<td>F6</td>
<td>0.3</td>
<td>–</td>
<td>0.02 (4.7%)</td>
<td>0.09 (21.3%)</td>
<td>0.012 (2.8%)</td>
</tr>
<tr>
<td>F7</td>
<td>0.3 (71.2%)</td>
<td>–</td>
<td>0.09 (22.4%)</td>
<td>0.09 (22.4%)</td>
<td>0.012 (3.0%)</td>
</tr>
<tr>
<td>F8</td>
<td>0.3</td>
<td>–</td>
<td>0.05 (11.4%)</td>
<td>–</td>
<td>(20.5%)</td>
</tr>
</tbody>
</table>

*HPMC: hydroxypropyl methylcellulose. The mass percentage was obtained by

### 2. Materials and methods

#### 2.1. Chemicals

Saquinavir mesylate (SQVM) was obtained from Hoffmann-La Roche Ltd (Basel, Switzerland). Malic acid, glycerol and agaro (type I) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydroxypropyl methylcellulose (HPMC) K100 LV (viscosity: 80-120 mPa·s, 2% (w/v) in water at 20°C) and HPMC K3 LV (viscosity: 2.4-3.6 mPa·s, 2% (w/v) in water at 20°C) Dow Chemical, MI, USA) were used as film forming polymers. Bromothymol blue (a pH indicator), monopotassium dihydrogen phosphate, dipotassium hydrogen phosphate anhydride and sodium chloride were obtained from Merck (Darmstadt, Germany). Purified water was prepared using a SG ultra-pure water system (SG water, Barsbuttel, Germany).

#### 2.2. Preparation of buffer solution and agarose hydrogel

A buffer solution consisting of 13 mM potassium phosphate buffer and 145 mM sodium chloride (adjusted to pH 6.8 using 0.1 M sodium hydroxide or hydrochloric acid solution if necessary) was used to simulate human saliva pH and buffer capacity as previously described (Gittings, S, 2017; He et al., 2020). An agarose hydrogel (0.5%, w/v) was prepared by suspending agarose powder in the buffer to form an agarose suspension. The agarose suspension was kept in a water bath at 95 ± 1°C for 20 min to dissolve the agarose followed by transfer to a quartz cell (internal dimensions 5.0 mm × 8.0 mm × 38.0 mm (H × W × L) from Starna Scientific Ltd. (Essex, UK). The quartz cell was left at ambient temperature for at least 1 h for gelation of the agarose gel. The agarose hydrogel in the quartz cell was cut into a cuboid shape prior to the measurements. Upon the preparation of the agarose hydrogel for the pH₃₄ measurements, the appropriate amount of bromothymol blue was dissolved in 2 ml sodium hydroxide solution (0.1 M) in a 100 ml volumetric flask followed by addition of water to 100 ml to form the stock solution of the pH indicator (9.61 × 10⁻⁵ M). A suitable volume of the stock solution was diluted using the buffer to form the bromothymol blue solution (6.73 × 10⁻⁵ M). Agarose powder was dissolved in the bromothymol blue solution following by gelation to form hydrogel as described above. The pH of the hydrogel containing bromothymol blue was 6.8 (the hydrogel was squashed followed by measuring using a pH meter), and the addition of bromothymol blue has no obvious effect on the pH due to its low concentration.
2.3. Preparation of buccal films

Exact amounts as given in Table 1 of SQVM, glycerol as a plasticizer and malic acid as a pH modifier were dissolved in 6 ml preheated water (70-75 °C) to form the drug solution. HPMC was added gradually to the drug solution while stirring at 70-75 °C until a homogeneous white dispersion formed. A volume of 4 ml cold water (4-6 °C) was added to the dispersion followed by vortexing until a transparent solution was formed at ambient conditions. The obtained casting solution was stored in a cold room (4-6°C) for 24 h to remove air bubbles. The bubble free casting solution was carefully casted onto polystyrene Petri dishes placed on a horizontal surface, and dried at room temperature for 36 h to achieve thin films. The films were packed and stored in a desiccator over silica at room temperature. The compositions of the buccal films are summarized in Table 1. Formulations, F1 and F2, were designed to investigate the effect of HPMC type on the properties of the films. Formulations, F1, F3 and F4, were designed to investigate the effect of glycerol content on the properties of the films. Formulations, F1, F5, F6 and F7, were designed to investigate the effect of malic acid content on the properties of the films.

2.4. Evaluation of buccal films

2.4.1. Visual appearance

The prepared films were inspected visually for transparency and colour. The ease of manual removal from the Petri dish was also evaluated.

2.4.2. Thickness and drug content

The thickness of the buccal films was measured using a digital caliper (Linear tools Limited, Middlesex, UK) at six different places, and the mean value was calculated. The buccal films were cut into small squares (1 cm × 1 cm) for drug content measurement. The drug content was determined by dissolving these in 100 ml of purified water. The solution was assayed for saquinavir spectrophotometrically (Cary 60, Agilent Technologies, CA, USA) at 300 nm using an appropriate calibration curve.

2.4.3. Mechanical properties

A TA.XTplus texture analyser (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 30-kg load cell was used to investigate the mechanical properties of the buccal films. Briefly, rectangular film strips (2 cm × 4 cm) were cut, and 1 cm on each end was clipped between clamps attached to the texture analyser, leaving a testing area of 0.5 cm × 2 cm for determination. The upper clamp moved upwards at a rate of 3 mm/s until the film break. Tension force versus elongation distance curves were recorded. From the resulting plots, elongation at break (E, %), the tensile strength at break (TS, N/mm²) and Young’s modulus (YM, N/mm²) were calculated by using the equations below:

\[
\text{Tensile strength (TS)} = \frac{F_i}{S_0} \quad (1)
\]

\[
\text{Elongation (E, %)} = \left( L_f - L_0 \right) / L_0 \times 100 \quad (2)
\]

\[
\text{Young’s modulus (YM)} = \frac{\sigma_f}{\epsilon_f} \quad (3)
\]

where \(F_i\) (N) is the tensile force at break and \(S_0\) (mm²) is the initial cross-sectional area of the film, \(L_i\) (cm) is the initial length of the samples and \(L_f\) is the final length (cm) at rupture, \(\sigma_f\) (N/mm²) and \(\epsilon_f\) are the stress and strain in the elastic region, respectively.

2.4.4. Moisture absorption

Dry buccal films pieces (1 cm × 1 cm) were weighed accurately using a digital balance (Mettler AE240, Mettler Toledo, OH, USA). Upon weighing, the films were kept in a desiccator containing a saturated sodium chloride solution at ambient temperature (RH, 75%). After 24 h, the films were taken out and weighed immediately. The moisture absorption (%) was determined using the equation:

\[
\text{Moisture absorption (\%)} = \left( \frac{W_f - W_i}{W_i} \right) \times 100 \quad (4)
\]

where \(W_f\) (mg) is the final weight of the film and \(W_i\) (mg) is the initial weight of the film.

2.4.5. Visual inspection of saquinavir precipitation in buccal films

To investigate the effect of the excipients on saquinavir precipitation during the swelling process of the buccal films, dry buccal film pieces (1 cm × 1 cm) with different compositions were put in 1.5 ml tubes, and 400 µl buffer solution (prepared in section 2.2, pH was adjusted to 7.40 using 0.1 M sodium hydroxide solution) was added to the tubes. After 10 min of film swelling, the films were visually inspected, and pH of the buffer solution in the tubes was measured using a pH meter (744 pH meter, Mettler Toledo, OH, USA) with a micro-electrode (Biotrode, Metrohm AG, Herisau, Switzerland).

2.4.6. Saquinavir release study using Franz diffusion cells

Saquinavir release experiments were conducted using Franz diffusion cells with 7 ml receptor chambers (Fig. 1). To achieve sink conditions, a volume of 7 ml buffer solution at pH 4.5 (prepared in section 2.2, pH was adjusted to 4.5 using 0.1 M hydrochloric acid) was added to the receptor chamber under magnetic stirring. Small pieces of buccal films (2 cm × 2 cm) were mounted between the donor chamber and the receptor chamber in a cell. A filter paper (Qualitative filter paper 413, particle retention: 5-13 µm, Cat. No. 516-0815, VWR international BVBA, Leuven, Belgium) was cut into small square pieces (2.1 cm × 2.1 cm), which were used as holders for the buccal film pieces in the Franz diffusion cells. A volume of 300 µl sample was withdrawn from the receptor chamber followed by replenishing with the same volume of fresh buffer solution (pH 4.5) at different time points (15 min, 30 min, 45 min, 1 h, 1.5 h, 3 h, 4 h and 5 h). Upon suitable dilution, the sample solution was measured using a UV/Vis spectrophotometer (Cary 60, Agilent Technologies, CA, USA) at 300 nm. The saquinavir concentration was calculated using a standard curve. The cumulative percentage of saquinavir release was calculated, and the release profiles from the buccal films were graphed.

2.4.7. Saquinavir release study and pH<sub>M</sub> measurement using UV/Vis imaging

Saquinavir release experiments and pH<sub>M</sub> measurements were performed at ambient temperature using an Actipix D100 dissolution imaging system (Paraytec Ltd, York, UK) with a tailor-made cartridge (Fig. 2). A quartz cell with internal dimensions 5.0 mm × 8.0 mm × 38.0 mm (H × W × L) (Type: 49/Q/S, Starna Scientific Ltd., Essex, UK) was
placed in the cartridge. The imaging area was 9.0 × 7.0 mm²; the pixel size was 7 × 7 µm², and pixels were binned 2 × 2. Images were recorded at a rate of 2.8 frames/s. In the experiments, the buccal film was attached below the agarose hydrogel containing the buffer solution, simulating the human saliva pH and buffer capacity in the quartz cell. Images recorded at a wavelength of 300 nm provided information on saquinavir release. To investigate pH₅₀, the pH indicator bromothymol blue was added to the hydrogel and images were recorded at a wavelength of 610 nm. Actipix D100 software version 1.4 and version 1.9 (Paraytec. Ltd.) were used to record and analyse the images, respectively.

### 2.4.8. Statistical analysis

An independent t-test was employed to compare the data (such as thickness, saquinavir content, mechanical properties and moisture absorption) between two formulations. One-way analysis of variance (ANOVA) and Turkey’s multiple comparisons test was performed to determine the significant variance between three or more formulations. Data were presented as mean with standard deviation (SD) and experiments were performed in triplicate unless otherwise stated. A p value of ≤ 0.05 was considered statistically significant. SigmaPlot 13.0 (SYSTAT, USA) was used for all statistical analysis.

### 3. Results and discussion

#### 3.1. Characterization of buccal films

The results of visual inspection, thickness, saquinavir content and mechanical properties are listed in Table 2. Buccal films containing malic acid were transparent and colourless (F1 to F6). In contrast, the HPMC K3 LV-based films were stronger to resist deformation under the HPMC K3 LV-based films were stronger to resist deformation under

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Visual appearance</th>
<th>Thickness (mm)</th>
<th>Saquinavir content (µg/cm²)</th>
<th>Tensile strength (N/mm²)</th>
<th>Elongation (%)</th>
<th>Young’s modulus (N/mm²)</th>
<th>Moisture absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Transparent, colourless</td>
<td>0.20 ± 0.04</td>
<td>491 ± 48</td>
<td>8.3 ± 1.9</td>
<td>45.6 ± 14.1</td>
<td>131.6 ± 14.5</td>
<td>14.5 ± 1.6</td>
</tr>
<tr>
<td>F2</td>
<td>Transparent, colourless</td>
<td>0.19 ± 0.03</td>
<td>477 ± 39</td>
<td>15.5 ± 3.6</td>
<td>57.3 ± 9.7</td>
<td>179.5 ± 55.8</td>
<td>12.5 ± 0.6</td>
</tr>
<tr>
<td>F3</td>
<td>Transparent, colourless</td>
<td>0.15 ± 0.04</td>
<td>443 ± 28</td>
<td>16.5 ± 1.1</td>
<td>34.1 ± 1.9</td>
<td>282.0 ± 22.1</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>F4</td>
<td>Transparent, colourless</td>
<td>0.19 ± 0.05</td>
<td>491 ± 68</td>
<td>9.6 ± 4.7</td>
<td>35.2 ± 15.4</td>
<td>175.5 ± 53.8</td>
<td>14.9 ± 4.3</td>
</tr>
<tr>
<td>F5</td>
<td>Transparent, colourless</td>
<td>0.17 ± 0.03</td>
<td>493 ± 55</td>
<td>10.6 ± 3.1</td>
<td>40.7 ± 21.3</td>
<td>162.2 ± 8.8</td>
<td>17.0 ± 0.6</td>
</tr>
<tr>
<td>F6</td>
<td>Transparent, colourless</td>
<td>0.18 ± 0.02</td>
<td>468 ± 54</td>
<td>9.4 ± 2.5</td>
<td>46.2 ± 15.6</td>
<td>137.9 ± 10.7</td>
<td>17.3 ± 7.6</td>
</tr>
<tr>
<td>F7</td>
<td>Transparent, colourless</td>
<td>0.16 ± 0.04</td>
<td>458 ± 57</td>
<td>8.6 ± 2.9</td>
<td>21.0 ± 7.7</td>
<td>202.9 ± 67.7</td>
<td>18.3 ± 1.0</td>
</tr>
</tbody>
</table>

Note 1: F1 and F2 contained HPMC K100 LV and HPMC K3 LV respectively. The wt% of glycerol in F3, F4 and F5 was 0.0%, 19.9% and 33.2% respectively. The wt% of malic acid in F2, F4 and F7 was 4.7%, 11.1% and 16.6% respectively.

Note 2: The superscript symbol “F” indicated the value has significant difference from one or several specific formulations in the same row.
The films. F1 and F2 were designed to investigate the effect of HPMC grade. F1, F3, and F4 were designed to investigate the effect of glycerol concentration. F1, F5, F6, and F7 were designed to investigate the effect of acid concentration. The composition of the formulations is shown in Table 1.

The visual appearance of the buccal films and buffer pH after 10 min of incubation in the buffer (initial pH 7.40) at ambient temperature are shown in Fig. 3. White precipitation was observed in the films containing 0% (wt%, F7) and 4.7% (wt%, F6) malic acid due to the relatively high pH (achieved pH 7.38 and 6.94, respectively). In contrast, the buffer pH with films containing 11.1% (F1) and 16.6% (F5) malic acid decreased from 7.40 to a pH in the range of 3.98 to 5.76. The pH of the buffer (pH 7.40) decreased because the dissolved malic acid in the films increased the concentration of hydrogen ions. A high amount of malic acid in the films led to a low pH and close to the swelling films, which prevented precipitation of saquinavir because of its pH-dependent solubility. White precipitate was not observed in the films containing different levels of glycerol (F1, F3 and F4), which suggests that the glycerol content had little effect on the precipitation. HPMC with moderate mucoadhesion properties has been widely used in buccal dosage forms (Nafee et al., 2004; Nair et al., 2019; Russo et al., 2016). A previous study showed that the drug release rate of HPMC-based solid dosage forms generally increased with decreasing viscosity grade of HPMC polymers (Karthikeyan et al., 2015). To achieve fast saquinavir release from the buccal films, low viscosity grade HPMC K3 LV and HPMC K100 LV were applied. Precipitation was observed in the film containing HPMC K3 LV with 11.1% malic acid (F2), but not in the film containing HPMC K100 LV with the same level of malic acid (F1). According to the manufacturer’s information, the viscosity of HPMC K100 LV aqueous solution (2%, w/v) is 22-50 folds higher than that of HPMC K3 LV aqueous solution (2%, w/v) (DOW chemical company, 2013). The low viscosity grade of HPMC K3 LV led to a faster release of malic acid from the buccal films, which might shorten the duration of low pH level in the HPMC K3 LV matrix. Therefore, precipitation was found in the film (F2) despite this film contained 11.1% malic acid. In our preliminary study, the films were wetted by water using a continuous flow perfusion model, and pH changes of the effluent from the films were investigated as described in Supplementary data section 1. The results also indicated that malic acid released faster from the films with HPMC K3 LV than from the film containing HPMC K100 LV, because the duration of a low medium pH of the effluent upon the films containing HPMC K3 LV was shorter than the pH decrease observed for the films containing HPMC K100 LV (supplementary data Fig. S1A).

3.3. The effect of excipients on saquinavir release

3.3.1. Investigation of the effect of hydroxypropyl methylcellulose (HPMC) grade and malic acid content on saquinavir release using the Franz diffusion cell method

The effect of malic acid and HPMC grade on saquinavir release behaviour was investigated in a preliminary study using the Franz diffusion cell method with a receiver medium pH of 6.8. A faster saquinavir release from the films containing malic acid was observed, but no major effect of HPMC grade on saquinavir release was observed (Fig. S2). However, only around 20% saquinavir was released from the films containing malic acid during 4 h, which might be caused by the low drug solubility at pH 6.8. To obtain sink condition, the saquinavir release study for the buccal films was conducted again using the same setup, however, at pH 4.5. The HPMC K3 LV-based films exhibited a faster saquinavir release than HPMC K100 LV-based films due to the relatively low viscosity grade of HPMC K3 LV, and the addition of malic acid led to a faster saquinavir release from the HPMC-K100 LV-based
films (Fig. 4). Generally, increasing the amount of the acidic modifier in formulations is an effective way to facilitate the release behaviour of weakly basic drugs having a pH-dependent solubility (Taniguchi et al., 2014). However, a significant increase in saquinavir release was not observed after 45 min of dissolution despite the increased malic acid content in the films. Besides, the films containing 4.7% (w/w) malic acid showed a faster saquinavir release as compared to the films containing 11.1% (w/w) malic acid. The effect of malic acid on saquinavir release might be weakened by the following factors: 1) a relatively large volume of dissolution media (7 ml) led to a fast release of malic acid for all the HPMC K100 LV films containing different amounts of malic acid, and 2) the pH of dissolution media (4.5) is much lower than pKa value of saquinavir (7.1). The solubility of saquinavir was increased to a maximum level by the acidic pH and a further decrease in pH caused by malic acid had minimal effect on saquinavir solubility.

The Franz diffusion cell method with filter papers acting to prevent
However, no clear malic acid concentration-dependent effect on saquinavir release was observed. The effect of malic acid concentration on saquinavir release was investigated in section 3.3.1 using the Franz diffusion cell method. A UV/Vis imaging system with an agarose hydrogel containing buffer simulating human saliva pH and buffer capacity was used (Fig. 2). The concentration of saquinavir at the detection interface for the films was monitored within 10 min upon attaching the films to the hydrogel to avoid the physical blocking of light. The absorbance of saquinavir solution at 300 nm was found to be pH-independent (Fig. S3). Thus, the absorbance of hydrogels containing different concentrations of saquinavir (at pH 4.5) was measured at 300 nm, and the calibration curve was calculated using this calibration curve and the iso-concentration lines (Fig. S4). The saquinavir concentration in the hydrogel was determined through the relationship between absorbance and concentration shifts within 10 min upon attaching the films to the hydrogel to avoid the physical blocking of light. The absorbance of hydrogels containing different concentrations of saquinavir (at pH 4.5) was measured at 300 nm, and the concentration of saquinavir at the detection interface was around 65 µg/ml (Fig. 5).

Changes in saquinavir concentration (absorbance) in the hydrogel were followed by UV imaging and a representative video can be found in the supplementary video S1. Selected absorbance images for saquinavir release are presented in section 3.3.1 using the Franz diffusion cell method. However, no clear malic acid concentration-dependent effect on saquinavir release could be observed during 5 h. To investigate the effect of malic acid content on saquinavir release during the initial dissolution process, a new setup mimicking the environment in the oral cavity for saquinavir release from buccal films was investigated.

3.3.2. Investigation of the effect of malic acid content on saquinavir release using UV/Vis imaging

The effect of malic acid concentration on saquinavir release was investigated in section 3.3.1 using the Franz diffusion cell method. However, no clear malic acid concentration-dependent effect on saquinavir release was observed during 5 h. To investigate the effect of malic acid content on saquinavir release during the initial dissolution, a UV/Vis imaging system with an agarose hydrogel containing buffer simulating human saliva pH and buffer capacity was used (Fig. 2). The dissolved ingredients of the buccal films in the agarose hydrogel did not absorb light at 300 nm, hence absorbance changes in the hydrogel during dissolution were solely due to the shifts in saquinavir concentration. However, there was a tiny cavity between the quartz cell and the hydrogel, the semisolid matrix of the swelling film slowly went into the cavity over time, leading to blocking of the light thereby interfering with saquinavir quantification. The buccal film was attached below the hydrogel to prevent the film matrix entering into the cavity. A detection interface close to the film/hydrogel interface was defined (indicated in Fig. 5, the distance between the two interfaces was 1.1 mm) and a detection area without physical blocking of light in the hydrogel was selected for drug release measurements as previously described (He et al., 2020). Physical light blocking caused by the semisolid matrix was not found at the detection interface during the first 10 min of the dissolution. After 10 min, the blocking of light was observed at the detection interface. The absorbance changes caused by saquinavir concentration changes were monitored within 10 min upon attaching the films to the hydrogel to avoid the physical blocking of light. The absorbance of saquinavir solution at 300 nm was found to be pH-independent (Fig. S3). Thus, the absorbance of hydrogels containing different concentrations of saquinavir (at pH 4.5) was measured at 300 nm, and the concentration of saquinavir against absorbance was plotted to obtain the calibration curve (Fig. S4). The saquinavir concentration in the hydrogel was calculated using this calibration curve and the iso-concentration lines (contour lines in the images) were indicated in the selected images (Fig. 5). A concentration of 10 µg/ml saquinavir in the hydrogel led to an absorbance of ~20 mAU, and was defined as the detection limit for saquinavir measurement.

Changes in saquinavir concentration (absorbance) in the hydrogel were followed by UV imaging and a representative video can be found in the supplementary video S1. Selected absorbance images for saquinavir release from the buccal films are shown in Fig. 5. After 5 min of dissolution of the film containing 16.6% (w/w) malic acid, the saquinavir concentration at the detection interface was around 65 µg/ml (Fig. 5). The concentration of saquinavir at the detection interface for the films containing 0.0%, 4.7% and 11.1% (w/w) malic acid was around 34 µg/ml. However, the area between the detection interface and the iso-concentration line (34 µg/ml) for the films containing 4.7% and 11.1% malic acid was larger than that for the film without malic acid, indicating that the amount of saquinavir released from the films containing 4.7% and 11.1% malic acid was larger than that from the film without malic acid. The images obtained at 10 min suggest that the concentration of saquinavir at the interface increased with the increasing content of malic acid in the films.

The drug concentration-distance profiles (Fig. 6) after 5 min and 10 min also indicate that the addition of malic acid led to an increase in local concentration of saquinavir at the interface due to the decrease in

![Fig. 6. Saquinavir concentration-distance profiles for agarose hydrogel during dissolution of the hydroxypropyl methylcellulose (HPMC) K100 LV-based films at 300 nm. A. Profiles at 5 min. B. Profiles at 10 min. The concentration was presented as mean ± SD, n = 3]. MA: malic acid.

![Fig. 7. Saquinavir release from hydroxypropyl methylcellulose (HPMC) K100 LV-based films containing malic acid (MA) at pH 6.8 and at ambient temperature. Results were presented as mean ± SD, n=3. Different letters above the bars (a, b, c) indicate statistical significance within the group, with a same letter being not different from each other. One-way analysis of variance (ANOVA) was employed and the level of significance was α = 0.05.](https://example.com/figure7.png)
pH. The larger area under the concentration-distance curves indicate a faster saquinavir release from the buccal film (Fig. 6). The increased amount of malic acid in buccal films led to an increase in saquinavir release during the initial dissolution (Fig. 7). However, the difference in saquinavir release from buccal films containing 4.7 % and 11.1 % malic acid was relatively small at 10 min. Nevertheless, the UV/Vis imaging method provided data on local saquinavir concentration and release percentage of saquinavir during the initial dissolution, which was difficult to obtain using the Franz diffusion cell method. The current UV imaging setup was limited to short term experiments due to the migration of the formulation eventually blocking transmission of light through the matrix. Optimization of the prototypic cell design was out of scope.
but with suitable alterations, it should be possible to accommodate measurements at the formulation interface for longer periods.

3.4. The effect of malic acid content on microenvironmental pH during dissolution

To investigate the effect of malic acid content on pH\textsubscript{M} at a model of the buccal mucosa, a UV/Vis imaging system with an agarose hydrogel simulating human saliva pH and buffer capacity, was used in the pH\textsubscript{M} measurements during the initial dissolution of the films. A low concentration of bromothymol blue (6.73 × 10\textsuperscript{-5} M) was included in the agarose hydrogel to monitor pH changes in the gel at 610 nm. During dissolution, the buccal film was attached on the hydrogel (Fig. 2). To prevent the interference from the blocking of light and to obtain information on pH\textsubscript{M} changes at the detection interface, absorbance changes were monitored during the initial dissolution within 10 min similar to the measurements presented in section 3.5.2. The pH\textsubscript{M} in the hydrogel was obtained using a pH-absorbance profile (calibration curve, Fig. S5). The calibration curve shows that the absorbance of bromothymol blue is sensitive to pH changes in the range of 5.3 to 7.6. Thus, the pH measuring range for this method was limited from 5.3 to 7.6. Detailed information related to the pH imaging assay has been described previously (He et al., 2020).

Absorbance images for the buccal films containing malic acid obtained at 5 min and 10 min were selected for the analysis. After 5 min of dissolution, the pH\textsubscript{M} for the saquinavir films without malic acid (F7) was around 6.8 (Fig. 8A). The presence of malic acid in the films reduced the pH\textsubscript{M} from 6.8 to the range of 5.4 to 6.2, depending on the concentration of malic acid (Fig. 8B, C, D). This indicates that the addition of malic acid decreased the pH\textsubscript{M}. After 10 min of dissolution, the pH\textsubscript{M} for saquinavir films containing 11.4 % and 16.6 % malic acid was to 6.2 and 5.9, respectively, that was higher than their pH\textsubscript{M} at 5 min dissolution (pH around 5.4) (Fig. 8 C and D). The increase in pH\textsubscript{M} was most likely caused by the release of saquinavir monovalent cations from the films, because the pH\textsubscript{M} for the films containing 11.4 % malic acid without saquinavir stayed around 5.4 after 10 min dissolution (Fig. 8E). Different ionization constants have been reported for the weak base saquinavir, including pK\textsubscript{a} 7.0 and 5.5 (Branham et al., 2012) and pK\textsubscript{a} 7.1 and 1.1 (Kushaba et al., 2000; Pathak et al., 2010). The reported pK\textsubscript{a} values also indicate the saquinavir could accept protons when the pH\textsubscript{M} was in the range of 5.4 to 6.8. The pH\textsubscript{M} for the film containing 11.1 % malic acid increased from 5.4 to 6.2 during the time period from 5 min to 10 min (Fig. 8C), the corresponding local saquinavir concentration at the detection interface increased from 34 µg/ml to 103 µg/ml (Fig. 5). Therefore, an increasing content of malic acid in formulations could achieve a lower pH\textsubscript{M} level and a longer duration of low pH\textsubscript{M} level. However, the release of saquinavir was associated with a subsequent increase in pH\textsubscript{M}.

4. Conclusion

In the present study, two in vitro drug release testing methods were applied for characterizing saquinavir containing pH modifying buccal films. Key formulation variables, HPMC grade, pH modification and glycerol, content were identified using the Franz diffusion cell method. The UV/Vis imaging method was capable of monitoring microenvironmental pH (pH\textsubscript{M}) changes and saquinavir release, thereby facilitating elucidation of the relationship between pH\textsubscript{M} and saquinavir release as function of time and position relative to the buccal film. The incorporation of malic acid into the films led to a low pH\textsubscript{M} and hence enhanced saquinavir release. Upon as transient pH decrease, the released saquinavir increased the pH\textsubscript{M} due to a high local concentration of the basic compound. Therefore, the combination of the Franz diffusion cell method and the UV/Vis imaging-method can provide beneficial information to drug release studies of pH\textsubscript{M} modifying buccal formulations.

The UV/Vis imaging approach taken for in vitro monitoring of the drug release and microenvironmental pH is likely to be of wider utility and of interest in relation to a range of formulation types, e.g., tablets, where pH modification is applied to control release or dissolution. The current prototypic was limited in terms of film application to the gel (physical blocking of the light) and the limited time span over which pH changes could be monitored. Future work should aim at refining cells and matrices for imaging, for instance by additive manufacturing, which should allow experiments of longer duration and to increase further the physiological relevance in relation to buccal application or other sites of administration.

CRediT authorship contribution statement

Shaolong He: Methodology, Investigation, Data curtion, Formal analysis, Writing – original draft, Writing – review & editing, Project administration.
Jette Jacobsen: Methodology, Supervision, Writing – review & editing.
Carsten Uhd Nielsen: Supervision, Writing – review & editing.
Natalja Genina: Writing – review & editing.
Jesper Østergaard: Conceptualization, Methodology, Writing – review & editing.
Huling Mu: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgment

Shaolong He acknowledges the China Scholarship Council (201708510087) for financial support and the technical support from Søren Michael Nielsen, Rita Wulff Rasmussen, Jens Graff, Mette Frand sen and Dorthe Ørskov.

Supplementary materials


References

Hauptstein, S., Hintzen, F., Müller, C., Ohm, M., Bernkop-Schnürch, A., 2014. Development and in vitro evaluation of a buccal drug delivery system based on...


