In vitro and ex vivo evaluation of bi-layered effervescent microenvironmental pH modifying buccal films with saquinavir

He, Shaolong; Nielsen, Carsten Uhd; Mu, Huiling; Jacobsen, Jette

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**Abstract**

Buccal delivery of saquinavir has the advantage to bypass the hepatic first-pass metabolism associated with oral administration. Local microenvironmental pH (pH₉M) modification at the buccal mucosa might increase absorption of saquinavir by balancing the solubility and partition of saquinavir into the buccal mucosa. The present study aimed to evaluate a novel saquinavir pH₉M modifying buccal film using effervescence, as well as to elucidate the relationship between pH₉M and permeation of saquinavir released from the buccal films. Hydroxypropyl methylcellulose-based films were prepared: 1) a bilayered effervescent film composed of an alkaline layer and a layer containing saquinavir and malic acid, 2) a monolayered film composed of saquinavir and malic acid (pH₉M modifying film), and 3) a monolayered film composed of saquinavir (control). The release of saquinavir from these films and permeation of saquinavir across porcine mucosae were evaluated. The monolayered pH₉M modifying film led to a decrease in pH₉M from pH 6.8 to 3.0 after 5.5 min, while the effervescent film had an initial decrease in pH₉M (from pH 6.8 to 4.0) caused by the co-release of malic acid and a subsequent pH₉M increase (from pH 4.0 to 5.9) caused by the release of carbonate from the alkaline layer within 15 min. Saquinavir released faster from the pH₉M modifying film than from the effervescent film. However, a higher permeation of saquinavir and mucosal accumulation was observed for the effervescent film. This could be attributed to the higher concentration of ionized specie and a faster tissue partitioning of unionized saquinavir, respectively. These results suggest that effervescent pH₉M modifying film is a potential formulation strategy for buccal delivery of saquinavir.

**Keywords:** pH modification, Buccal delivery, Saquinavir, Effervescence

1. Introduction

Saquinavir is a protease inhibitor used for the prevention and treatment of human immunodeficiency viruses. Clinical studies have shown that the oral bioavailability of saquinavir is very low (1%–9%) due to the extensive hepatic first pass metabolism and poor water solubility [1–3]. Buccal transmucosal administration delivering drugs into the systemic circulation via the jugular veins is capable of circumventing the hepatic first pass metabolism [4,5]. Thus, buccal delivery of saquinavir might be a promising strategy to increase systemic bioavailability of saquinavir. However, the solubility of saquinavir is pH-dependent [2,6], and saquinavir is poorly soluble in human saliva (pH 6.5–7.4 [7]), which hamper the approach. Therefore strategies to circumvent these limitations are needed. It has been reported that decreasing pH from 7.0 to 3.0 led to a significant increase in saquinavir solubility due to the increased ionization of saquinavir [2,6]. However, a pH lower than 2.5 induced acute local mucosal irritation [8]. Therefore, a well-controlled short-period decrease of the microenvironmental pH (pH₉M) at the site of administration might improve saquinavir release, and have a minimal destructive effect for the oral tissues.

Incorporating pH modifiers such as acidifying agents, alkalizing agents and buffering agents in formulations is a direct and effective way to adjust pH₉M [9,10]. Previous studies have demonstrated that the addition of organic acids, namely malic acid, citric acid and succinic acid in hydroxypropyl methylcellulose (HPMC)-based saquinavir buccal films led to a low pH₉M and improved saquinavir release from the films in simulated saliva [11]. Since drug solubility and permeability can be inversely related due to the ionization state of the molecule [10,12], it is also important to evaluate the permeation of drugs released from such formulations. Among various other pH₉M modification strategies, effervescence of carbon dioxide (CO₂) could lead to a short-period decrease in pH₉M followed by an increase in pH₉M [13]. Such formulations typically...
contain a base that is capable of releasing CO2 (i.e. sodium carbonate or sodium bicarbonate) and an acid that induces the release of CO2 [14]. The pHH might be reduced due to the formation of carbonic acid caused by the effervescence reaction and the release of acidic excipients from the formulations. Subsequently, the pHH increases along with the dissociation of carbonic acid (into CO2 and water) and the dissipation of the CO2 as well as the release of basic ingredients from the formulations during the dissolution process. The transient shifts in pHH might increase the buccal absorption of saquinavir by compromising the solubility and the tissue partitioning of saquinavir.

In the present study, HPMC K3 LV and HPMC K100 LV were used as the film-forming polymers, malic acid and sodium carbonate were chosen as pH modifiers in the buccal formulations. It was hypothesized that bilayered effervescent saquinavir pHH modifying films containing malic acid and sodium carbonate can lead to an initial decrease and subsequently a gradual increase in pHH during the dissolution of the saquinavir, and that the effervescent film can improve the permeation of saquinavir across the buccal mucosa. The aims of this study were to formulate and prepare saquinavir pHH modifying buccal films using different pHH modulation methods, to investigate the pHH as well as the ex vivo permeation of saquinavir across porcine buccal tissues, and to elucidate the relationship between pHH and permeation of saquinavir released from the buccal films.

2. Materials and methods

2.1. Chemicals

Saquinavir mesylate (SQM) was obtained from Hoffmann-La Roche Ltd (Basel, Switzerland). Malic acid, glycerol (> 99%) and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MN, USA). Hydroxypropyl methylcellulose (HPMC) K3 LV and HPMC K100 LV (Dow Chemical, Michigan, USA) were used as film forming polymers. Sodium carbonate, ammonium acetate monopotassium dihydrogenphosphate anhydrate, dipotassium hydrogenphosphate anhydrate, phosphoric acid (85%) and sodium chloride were obtained from Merck KGaA (Darmstadt, Germany). Acetonitrile was purchased from VWR International, LLC (PA, USA). Purified water (water) from a SG ultra-pure water system (Barsbuttel, Germany) was used.

2.2. Formulation and film preparation methods

Three types of saquinavir buccal films were designed and prepared, i.e., the monolayered saquinavir film without malic acid (control film), monolayered saquinavir film with malic acid (pHH modifying film), and the effervescent saquinavir pHH modifying film (Fig. 1). The composition of the films is listed in Table 1. To achieve unidirectional drug release, ParaFIlm® (Bemis Manufacture Company, WI, USA) was temporarily used as a backing membrane in the drug release study and drug permeation study. The films containing saquinavir and malic acid were prepared using a solvent evaporation method as previously described [11]. Briefly, exact amounts of SQM, glycerol and malic acid (Table 1) were dissolved in 12.0 ml preheated water (70–75 °C) to form a drug solution. HPMC K 3 LV was added gradually to the drug solution while stirring at 70–75 °C until a white dispersion formed. A volume of 8.0 ml cold water (4–6 °C) was added to the dispersion followed by vortexing to obtain a transparent solution at ambient conditions. The solution was stored in a cold room (4–6 °C) for 24 h to remove air bubbles followed by film casting in polyethylene Petri dishes placed on a horizontal surface, and drying at room temperature for 36 h. To prepare the HPMC-based film containing sodium carbonate, suitable amounts of sodium carbonate and glycerol, as given in Table 1, were dissolved in 20.0 ml preheated water at 70–75 °C followed by gradually adding HPMC K100 LV while stirring until a white dispersion formed, which was stored in a cold room (4–6 °C) for 24 h to form alkaline HPMC solution and to remove air bubbles. The bubble free alkaline HPMC solution was poured onto a horizontal aluminum plate, and then scraped using a thickness adjustable KTQ-III film applicator (Yingxu Chemical Machinery Co., Ltd., Shanghai, China) and dried gradually at ambient temperature for 24 h. Effervescent films were prepared using a paste method. Briefly, a suitable amount of ethanol (96%) as a binder was manually sprayed onto the surface of the film containing saquinavir and malic acid, to slightly moisten the film and obtain stickiness. Immediately adhering the film with sodium carbonate on the sticky film followed by putting a metal block on top to compress the films for 30 min to obtain the effervescent saquinavir pHH buccal film.

2.3. Quantification of saquinavir

Content of saquinavir was analyzed using an Elite LaChrom HPLC system (VWR International, Tokyo, Japan) equipped with a L-2130 pump, a L-2450 diode array detector and a L-2200 autosampler. Reversed phase chromatography was performed using a Aerus® C18 column (100 x 4.6 mm, 3.6 μm, Phenomenex, Torrance, CA, USA) at 30 °C and a mobile phase of 10 mM ammonium acetate buffer:
acetonitrile 60:40 (v/v) (pH 4.9). A flow rate of 0.5 mL/min and an injection volume of 20 μL were applied resulting in a retention time of 5.5 min. Saquinavir was detected at 240 nm. The chromatograms were analyzed using EZChrom Elite software Version 3.1.3. Calibration curves were prepared by diluting a stock solution of saquinavir in water and phosphate buffer solutions. For drug content measurement and drug release study, a linear calibration curve was used in the range of 0.45–165 μg/mL (R² = 0.9987). For permeation study, a linear calibration curve was used in the range of 25–850 ng/mL (R² = 0.9964). The detection limit was 25 ng/mL.

2.4. Visual inspection

The prepared films were visually inspected for transparency and color at daylight. The ease of removal from the casting plate and Petri dish was also evaluated.

2.5. Saquinavir content in the buccal films

One manually cut film square (1 cm × 1 cm) was fully dissolved in 100 mL water followed by 10 folds dilution used for saquinavir quantification by UV-HPLC. The quantification was triplicated for each formulation.

2.6. Swelling

One manually cut film square (1 cm × 1 cm) was placed in a glass Petri dish followed by adding 200 μL of phosphate buffer solution (PBS, 13 mM potassium phosphate and 145 mM sodium chloride, pH 6.8) simulating human saliva pH and buffer capacity on the top of the film, and the PBS was prepared as previously described [15]. The swelling process of the film pieces was monitored over time using a digital microscope (Dino-lite microscope (USB), Taiwan, China) equipped with a backlight laptop.

2.7. pHx investigation of the films

One manually cut film square (1 cm × 1 cm) was placed in a 1.5 mL Eppendorf tube followed by adding 400 μL of PBS. Immediately, a micro pH electrode (Biotrode, Metrohm AG, Herisau, Switzerland) was positioned close to the surface of the drug layer of the film piece at ambient temperature. The pH values were recorded at different time intervals. The measurement was triplicated for each formulation.

2.8. Saquinavir release study

Saquinavir release from films was conducted using glass Franz diffusion cells (orifice diameter 5 mm, diffusion area: 0.2 cm²). A volume of 3.00 mL PBS was added to the receptor chamber and using magnetic stirring. A buccal film (1.0 cm × 1.0 cm) on top of a filter paper (Qualitative filter paper 413, particle retention: 5 μm, Cat. No. 516–0815, VWR international BVBA, Leuven, Belgium) (1.1 cm × 1.1 cm) was mounted between the donor and the receptor chamber. Samples (250 μL) were withdrawn from the receptor chambers at predetermined time intervals (5 min, 15 min, 30 min, 1 h, 2 h and 3 h). Air bubbles induced were removed via the side arm by carefully tilting the Franz cell after each withdrawal. The removed samples were immediately replaced with the same volume of pre-warmed water followed by sealing the receptor chamber using small Parafilm® pieces to prevent water evaporation. Samples were centrifuged (13500 rpm, 10 min, at ambient temperature), and subsequently analyzed by UV-HPLC. After the permeation study, each buccal mucosa was flushed with water to remove the residual film matrix or saquinavir suspension on the tissue surface, and then dried under ambient condition for 24 h. The dried mucosae were kept in 1 mL of the mixture of phosphoric acid solution (phosphoric acid: water 10:3, v/v) at 65 °C for 10 min followed by disruption and homogenization with a pestle. The homogenized tissue suspension was diluted using 9 mL of water. The diluted suspension was centrifuged (13500 rpm, 10 min, at ambient temperature) and the supernatant was subsequently analyzed by UV-HPLC.

2.10. Statistics

Sigma Plot version 14 (Systat Software Inc., USA) was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to determine statistically significant difference between three or more means. Tukey’s test was then performed. The level of significance was α = 0.05. A p value below 0.05 was considered statistically significant. Data were presented as mean ± standard deviation (SD) and experiments were performed in triplicate unless otherwise stated.

3. Results and discussions

3.1. Visual inspection

The visual appearance of the buccal films were evaluated (Table 2). All of the films could easily be removed from the casting plate and Petri dish. The saquinavir films containing malic acid (F2) were transparent and colorless. The saquinavir films without malic acid (F1) were...
partially transparent and slightly yellow, which was caused by the precipitation of saquinavir during the film preparation. The alkaline film (F3) was white and opaque due to the precipitation of sodium carbonate.

### 3.2. Saquinavir content in buccal films

Saquinavir content in the film without malic acid (F1) was lower than that in the film with malic acid (F2). This could be attributed to the precipitation of saquinavir during the preparation process of the film. Indeed, saquinavir precipitation was observed on the bottom of the glass flask for F1 after transferring the saquinavir-polymer suspension to the film applicator. Additionally, manually cutting of the films might be a major factor leading to the variation in saquinavir content of the films.

### 3.3. Swelling

Even though the saquinavir film with malic acid (pH$_M$ modifying film) was transparent visually inspected at daylight (Table 2), some white spots were observed in the films using the digital camera (Fig. 2), which might be the dust left on the surface of the films or saquinavir precipitations formed during the drying process. During the initial swelling process (within 2 min), saquinavir film without malic acid (control film) became white. The color of the alkaline film faded over time. Obvious changes in color were not observed for the saquinavir film with malic acid and the effervescent saquinavir film. The addition of malic acid in the film suppressed saquinavir precipitation due to the low pH microclimate in and around the swollen film. White sodium carbonate precipitation dissolved gradually in the alkaline film without saquinavir resulting in a color fading. Air bubbles were not observed in the effervescent saquinavir film during the swelling experiment, because the white precipitation made the air bubbles difficult to be recognized, or no sodium carbonate was released from the alkaline film within 2 min for the reaction with the malic acid in the saquinavir layer. Accordingly, the background light was turned off. Small air bubbles were observed in the effervescent films after 6 min and the number and size of air bubbles gradually increased over time due to the reaction of malic acid with sodium carbonate (Fig. 3). This phenomenon indicates that there is a lag time for the release of sodium carbonate from the alkaline film. The pH in the layer containing saquinavir and malic acid might be low during the initial swelling process. The tested films gradually lost their integrity due to the high aqueous solubility of HPMC (after 2 min), and gels were formed when they absorbed enough PBS (Supplementary videos). Among the test films, the alkaline film had the fastest disintegration rate.

### 3.4. Investigation of microenvironmental pH for the films

The results of pH$_M$ measurement (Fig. 4) showed that the three different films decreased pH$_M$ to different extents. Saquinavir films with malic acid (pH$_M$ modifying film) and effervescent saquinavir film (effervescent pH$_M$ modifying film) led to a lower pH$_M$ than the saquinavir film without malic acid (control film) within 6 min. The pH$_M$ around saquinavir films with and without malic acid decreased from 6.8 to 3.3 and 6.6, respectively, after 3 min, and no major changes in the pH$_M$ were observed in the time period of 3–6 min. The slight decrease in pH$_M$ around the saquinavir films without malic acid was most likely caused by the dissociation of monocationic saquinavir, which could provide hydrogen ions. The pH$_M$ around effervescent saquinavir films decreased from 6.8 to 4.0 in 2 min, and subsequently increased, and the

### Table 2

Visual inspection and saquinavir content in the mono-layered films.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Visual inspection</th>
<th>Saquinavir content (μg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Partially transparent</td>
<td>1028 ± 160</td>
</tr>
<tr>
<td>F2</td>
<td>Transparent</td>
<td>1163 ± 74</td>
</tr>
<tr>
<td>F3</td>
<td>White and opaque</td>
<td>–</td>
</tr>
</tbody>
</table>


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**Fig. 2.** Selected images of physical appearance of the films during the swelling process in the phosphate buffer solution simulating saliva at ambient temperature (with background light). Saquinavir film without MA (control film), saquinavir film with MA (pH$_M$ modifying film). Effervescent saquinavir film (effervescent pH$_M$ modifying film). MA: malic acid.
pH was around 5.9 after 15 min. The initial decrease in pH (within 2 min) was mainly caused by the release of malic acid from the layer containing saquinavir. Additionally, the CO$_2$ induced by the effervescent reaction could also be dissolved in water, forming carbonic acid, and the effect of CO$_2$ on pH lowering might depend on the amount of dissolved CO$_2$. The subsequent gradual increase in pH was caused by the release of sodium carbonate from the alkaline layer.

3.5. Saquinavir release from buccal films

To mimic the dissolution environment for buccal films in the oral cavity, a limited volume (3 ml) of PBS simulating the saliva was employed as the dissolution medium in the in vitro drug release test. The concentration of the saquinavir mesylate in the receptor chambers for the saquinavir films with MA (pH$_M$ modifying film) was around 60 μg/ml. The reported solubility of saquinavir mesylate at pH 6.8 is around 38 μg/ml (read from the figure in the reference) [17]. The results showed that released accumulative percentage of saquinavir was below 50% for the tested films after 2h of dissolution (Fig. 5). This could be attributed to the fact that the solubility of saquinavir in the receiver medium (pH 6.8) was low. The release of saquinavir from the films containing malic acid (pH$_M$ modifying film) was much faster than that from the films without malic acid (control film) and the effervescent films within 2h, which was likely caused by the low pH$_M$ around the acid-containing films. Additionally, the released malic acid from the films might decrease the pH of the dissolution medium, leading to a faster saquinavir release. Besides, our previous study showed that the addition of malic acid...
Fig. 5. Saquinavir release profiles in phosphate buffer at pH 6.8 at 37 °C. Results were presented as mean ± SD, n = 3. To make the symbols easy to be recognized, only positive errors are displayed. Different letters on the error bars indicate statistical significance among the data at the same time point. One-way analysis of variance (ANOVA) using the Turkey test was employed and the level of significance was α = 0.05. MA: malic acid.

Acid in the HPMC-based films could convert crystalline saquinavir to its amorphous form, which might increase the saquinavir release \( [11] \). The effect of malic acid on saquinavir release from the effervescent films was minor because the low pH only persisted for a short period due to the presence of carbonate.

### 3.6. Ex vivo permeation of saquinavir

Cumulative permeation of saquinavir across the porcine buccal mucosa after exposure to the saquinavir suspension (pH 6.8) and different films are shown in Fig. 6. The appearance of saquinavir in the receiver chamber was faster after application of the films than the suspension. While no difference was observed in saquinavir appearance between films with and without malic acid, the effervescent film resulted in more saquinavir on the receiver side. Yet, all conditions resulted in a low amount of saquinavir permeating the tissue (≤ 0.55%). For the films, the appearance of saquinavir in the receiver side was highest within the first 15 min, and hereafter limited appearance was observed. The initial faster appearance of saquinavir in the receptor medium from the effervescent film might be due to the effervescence of CO\(_2\). It has been reported that CO\(_2\) transiently affected the structural integrity of tight junctions in ideal mucosal membrane and without indication of membrane disruption \([18,19]\). The release of CO\(_2\) may initiate a faster wetting and erosion of a solid formulation in conjunction with a local pH favor of the unionized species of the drug, which may result in a steeper concentration gradient across the mucosa, hence increased the permeation enhancement of saquinavir. Interestingly, the permeation of saquinavir across the porcine buccal mucosa upon application of saquinavir suspension was slower than saquinavir buccal films, despite the suspension contained above 3 × higher amount of saquinavir than the films. This could be attributed to that the parafilm\(®\) used as the backing membrane led to a unidirectional saquinavir release from the films toward the mucosa, providing a site-specific high saquinavir concentration gradient across the mucosa and driving passive diffusion of saquinavir into and across the buccal tissue.

Charged drug molecules are less permeable across the lipid-rich epithelium and pH changes might affect the degree of ionization of a weakly dissociable drug \([12,20,21]\). In the present study, a shift in pH\(_M\) affected the release of saquinavir and saquinavir ionization, and consequently the permeation of saquinavir across porcine buccal mucosa. The saquinavir films with malic acid (pH\(_M\) modifying film) had a fast saquinavir release (Fig. 5) due to the low pH\(_M\) (around 3.0) (Fig. 4). The low pH\(_M\) kept the released saquinavir (pK\(_a\) = 7.1 and 5.5) in its bi-cationic form, decreasing saquinavir permeation across the lipid-rich epithelium of buccal mucosa. The slow saquinavir release from the films without malic acid limited the permeation of saquinavir as the formulation did not modify the pH\(_M\). Therefore, pH at the administration site might have a crucial effect on the amount of released saquinavir ready for permeation, additionally the degree of ionization of saquinavir may affect the uptake. The effervescent films (effervescent pH\(_M\) modifying film) initially decreased pH\(_M\) and facilitated the release of saquinavir, and they subsequently increased the pH\(_M\) and reduced the ionization degree of saquinavir, leading to enhanced drug permeation across the mucosa.

The amount of saquinavir accumulated in the tissue was quantified after the permeation study. The tissue exposed to the effervescent film contained more saquinavir than the tissues exposed to the saquinavir suspensions and the non-effervescent films (p < 0.05, Fig. 7). The result is in a good agreement with the result of permeation, i.e. the effervescent films led to a higher permeation of saquinavir. After the 3h of permeation study, the amount of saquinavir permeated across the mucosa tissue was much lower than that accumulated in the tissue upon application of different films (Figs. 6 and 7). This phenomenon was most likely caused by the retention/precipitation of saquinavir in the tissues.

### 4. Conclusion

In the present study, effervescent saquinavir films were designed and evaluated for the potential to improve buccal delivery of saquinavir. Addition of malic acid in the films decreased the microenvironmental pH (pH\(_M\)) leading to a faster release of saquinavir than the films without malic acid. However, addition of the acid in the formulations did not affect the permeation of saquinavir across the porcine buccal mucosa. A higher permeation of saquinavir was observed for the effervescent saquinavir films, even though the release rate of saquinavir from the effervescent films was slower than that from the monolayered
Fig. 7. Amount of saquinavir in porcine buccal tissues after permeation study. Buccal tissues were exposed to saquinavir suspensions (containing 790 ± 70 μg saquinavir), saquinavir films with MA, saquinavir films without MA and effervescent saquinavir film contained 1163 ± 74 μg, 1028 ± 160 μg and 1163 ± 74 μg of saquinavir, respectively. For the films, the percentage is calculated by dividing the amount of saquinavir in a tissue with the amount of saquinavir in the film with a size of 0.2 cm² (the size of diffusion area in Franz cells). Values are mean ± SD, n = 4–6. MA: malic acid. Different letters above the bars (a, b, c) indicate statistical significance. Bars with similar letters are not significantly different from each other. One-way analysis of variance (ANOVA) was employed and the level of significance was α = 0.05.

saquinavir films with malic acid. The effervescent films led to an initial decrease and subsequently an increase in pH, facilitating initial saquinavir release and subsequent saquinavir permeation across the lipid-rich buccal epithelium. Therefore, buccal delivery of saquinavir using microenvironmental pH modifying films with effervescence is a potential strategy to improve buccal absorption of saquinavir and to avoid hepatic first-pass metabolism.

CRediT authorship contribution statement

Shaolong He: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. Carsten Udh Nielsen: Methodology, Data curation, Writing – review & editing. Huiling Mu: Conceptualization, Methodology, Funding acquisition, Writing – review & editing. Jette Jacobsen: Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2022.103954.

References