Permeability of Triamcinolone Acetonide, Released from Mucoadhesive Films, Through a Buccal Mucosa-Mimetic Barrier; Permeapad™

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Declaration of interests: none

Abstract

Objectives: The permeability of triamcinolone acetonide (TA), from bilayer mucoadhesive buccal films, through a biomimetic membrane, Permeapad™, was investigated employing Franz diffusion cell. The delivery systems composition and ethyl cellulose (EC) backing layer, on drug permeability, were assessed.

Methods: Three TA-loaded films were tested; hydroxypropyl methylcellulose (HPMC K4M; bilayer [F1] and monolayer), HPMC K4M/Polyvinylpyrrolidone (PVP): 90/10 [F2], and HPMC K15M film [F3]. All films contained propylene glycol (PG-plasticiser). TA solution alone was used as a control. TA permeability via a Permeapad™ barrier, simulating buccal mucosa, was assessed over 8h using a Franz diffusion cell. TA permeated into the receptor compartment, released in the donor compartment, and located on/within the Permeapad™ barrier were analysed using UV-spectrophotometer.

Results: 45.78% drug retention within the Permeapad™ barrier was delivered from F1 (highest). F1, F2, and F3 significantly improved the TA's permeability through Permeapad™, compared to TA solution alone (e.g., 8.54% TA-solution, 21.58%-F1), attributed to the synergy effect of HPMC and propylene glycol acting as penetration enhancers. F1 displayed a significant increase in drug permeability (receptor compartment; 21.58%) compared to F3 (17.05%). PVP significantly enhanced drug permeability (27.55%). Impermeable EC backing layer controlled unidirectional drug release and reduced drug loss into the donor compartment (e.g., ~28% for monolayer film to ~10% for bilayer film, F1).

Significance: The mucoadhesive films demonstrated improved TA permeability via Permeapad™. The findings suggest that these bilayer mucoadhesive films, particularly F1, hold promise for the effective topical treatment of oral mucosa disorders, such as recurrent aphthous stomatitis and oral lichen planus.

Highlights:

- Bilayer mucoadhesive buccal films to enhance TA permeability through Permeapad™
- F1 for potentially treating oral mucosa disorders - Highest TA retention within Permeapad™
- EC backing layer minimized the loss of TA into the donor compartment
- The biomimetic membrane, Permeapad™ was successfully used for TA permeation assay
- Franz cell was used to predict the amount of drug permeating across Permeapad™

Keywords:

Triamcinolone acetonide, Bilayer mucoadhesive film/patch, Franz diffusion cell, Permeapad™, Permeability

1 Introduction

The oral mucous membrane has been considered as an effective site for the absorption of drugs. The buccal mucosa offers an attractive and feasible administration route for drug delivery [1, 2], and in the last decade, this route has received significant attention in pharmaceutical technology and the Pharmacy industry. This administration route is not only used to deliver drug molecules quickly when an immediate effect is required, it is also used for controlled drug delivery [3]. Buccal and sublingual mucosal membranes are permeable to various drugs (e.g., nitroglycerin and nicotine) due to their non-keratinized epithelium and relatively small thickness (500-800 μm for buccal mucosa and 100-200 μm for sublingual mucosa) [4]. Drugs can be easily absorbed by a passive transcellular transport mechanism. Furthermore, the buccal mucosa is preferred to other mucous membranes in the body (nasal, ocular, vaginal, or rectal mucosae) because of the heavy blood supply in this area, its high tolerance to allergens, and a low predisposition of irreversible tissue damage. In addition, since the mouth is an accessible site, buccal dosage forms can be applied easily by the patient for either local or systemic effects [5].

Many intra-oral drug formulations have been developed for drug delivery such as buccal tablets, lozenges, sprays, films, patches, gels, pastes, etc. Among these formulations, the mucoadhesive buccal film/patch is reported to be the most promising system for delivery of active species via the oral mucous membrane, because it is thinner than buccal tablets, thus more comfortable for the patients, and it remains on the application site for a longer time than oral gels/pastes. Recently, a bilayer mucoadhesive buccal film loaded with triamcinolone acetonide (TA) for treating recurrent aphthous stomatitis was developed. Its ex-vivo residence time on porcine buccal mucosa was found to be >24h. Furthermore, an in vitro study showed that the film released ~100% of TA over 6h in a controlled manner [6].

Drugs' permeability through the oromucosal membranes is a key factor that affects its absorption and therefore it is essential to be evaluated during the development of the delivery system. In vitro permeation studies employing diffusion cells (e.g., Franz cell) can be used to predict the amount of

drug that permeates across the biological membrane (e.g., porcine mucosa) or biomimetic membrane (e.g., Permapad™ barrier) [7]. Franz diffusion cell is a simple, reproducible, and widely used technique for measuring in vitro drug permeability and drug release from different dosage forms (e.g., films, ointments, gels, etc.). Gajdošová *et al.* [8] investigated the permeability of ciclopirox olamine (CPX; an anti-fungal agent), through porcine buccal mucosa using a Franz diffusion cell. The drug was released from a bilayer mucoadhesive buccal film. The authors found that CPX did not pass the buccal tissue but accumulated within it. Churchman *et al*. [9] employed a Franz diffusion cell and porcine buccal mucosa to investigate the feasibility of iontophoresis to accelerate the delivery of four macromolecular species of different molecular weights (dextrans 3kDa and 10kDa, bovine serum albumin 64kDa, and parvalbumin 12kDa). They found that the permeation of the two dextrans and parvalbumin through the buccal mucosa was enhanced after anodal iontophoresis, and the diffusion coefficients for dextrans (3.13 and 2.10×10^{-13} m^2 s^{-1}, respectively) were significantly higher than for parvalbumin (2.10×10^{-13} m^2 s^{-1}).

Permeapad™ is a biomimetic membrane, that was developed in 2015 at Syddansk University [10], and entered the market in 2018. It consists of two cellulose membranes and a lipid layer (lecithin S-100) in between. It is a 'ready-to-use' membrane and can be used for drug permeation assay without any further preparation. Permeapad™ simulates the tissues in the body (i.e., buccal, nasal, and gastrointestinal tract-GIT), via the passive transcellular route. Due to the unique composition of Permeapad™, this membrane is very robust, resistant, stable against solvents and biorelevant solutions, storable at room temperature, and has a long shelf-life (~1 year). Furthermore, the permeation assays through Permeapad™, sandwiched within Franz diffusion cells, can be performed within a large pH range (from 1 to 10)The drug permeability via Permeapad™ is fast, reproducible, cost effective and easy to perform [11]. Bibi *et al.* [12] reported that Permeapad™ membrane can be used to mimic the buccal permeation of drugs. They used side-by-side diffusion chambers to assess the permeability of metoprolol (0.1mM solution) across Permeapad™, and compared the results with published in vitro, ex vivo and in vivo studies [13] for the same formulation. They reported that the

permeability of metoprolol using Permeapad™ membrane correlated well to both in vitro (TR146 cell culture) and ex vivo (porcine buccal mucosa) studies (R^2 = 0.98 and 0.97, respectively). Furthermore, a remarkable in vitro-in vivo correlation (IVIVC, R^2=0.98) was identified when comparing the apparent permeability coefficient of metoprolol through Permeapad™ to the absolute bioavailability of metoprolol administered buccally to mini pigs. . In 2015, Di Cagno *et al.* [14] revealed that the Permeapad™ barrier was applicable for passive permeation assays for different model drugs (e.g., acyclovir, caffeine, atenolol, metoprolol, hydrocortisone, ibuprofen, and paracetamol). They found a good correlation between the permeability values measured using the Permeapad™ barrier and those measured using other in vitro permeability methods (e.g., parallel artificial membrane permeability assay; PAMPA) for both highly and poorly permeable drugs. In addition, they proved the high integrity of the Permeapad™ barrier at different pH values over time. Bibi *et al* [15] found that the Permeapad™ membrane was compatible with various surfactants and co-solvents including dimethyl sulfoxide (DMSO), polysorbate, and triton-X, even in high concentrations. Furthermore, they also found that the Permeapad™ barrier was compatible with four different biorelevant media; fasted state simulating intestinal fluid (FaSSIF and FaSSIF-V2) and fed state simulating intestinal fluid (FeSSIF and FeSSIF-V2). Therefore, Permeapad™ barrier simulates the passive transcellular transport of biological tissues.

Hence, the aim of this study was to evaluate the permeability of TA released from bilayer mucoadhesive films, based on hydroxymethyl cellulose (HPMC) [6], through the Permeapad™ barrier sandwiched in Franz diffusion cells. These films were developed for potentially treating recurrent aphthous stomatitis and other oral mucosa disorders [6].

The objectives of this study were to: i) evaluate TA permeability through Permeapad™ membrane using Franz diffusion cells; ii) study the effect of the bilayer films' composition on TA permeability. These included two different molecular weight HPMC (K4M and K15M), incorporating polyvinyl pyrrolidone (PVP), an ethyl cellulose (EC) backing layer and HPMC K4M monolayer (with no EC); and iii) determine the permeation parameters (e.g., apparent permeability coefficient, P_{app} and the steady-state flux, J_{ss}).

2 Materials and methods

2.1 Materials

All materials were from Sigma-Aldrich Ltd, UK unless otherwise stated. TA, also known as 9α-fluoro-16α-hydroxyprednisolone 16α,17α-acetonide, ($C_{24}H_{31}FO_6$; MW 434.5 Da), was the active drug. Four film-forming polymers were used to fabricate the films; HPMC K4M (average [av] MW: 86,000 Da, viscosity: 4,000 cP, Colorcon Ltd), HPMC K15M (av MW: 120,000 Da, viscosity: 15,000 cP, Colorcon Ltd), PVP K90 (MW, 360,000 Da), and EC (100 cP). Propylene glycol (PG) and castor oil were used as plasticizers. Phosphate buffered saline (PBS) powder was used for preparing the buffer solution (pH 7.4). The biomimetic barrier (Permeapad™) was bought from innoME GmbH, Germany.

2.2 Methods

2.2.1 Preparation of the films

The films were prepared employing the solvent casting method as described previously [6]. Briefly, the backing layer was produced by dissolving EC (5%) in absolute ethanol (>99%) on a magnetic stirrer (heat – stir, UC152D, Stuart®) at room temperature (RT, 22±1˚C). The ethanolic solution was plasticized with castor oil (1ml), then cast into polystyrene Petri dishes (90×15 mm^2) and stored in an incubator (LMS Cooled Incubator) at 30°C for 24h, until the solid backing layer was formed. The adhesive layer was produced by dissolving 2g of each of HPMC K4M, HPMC K15M, and PVP (see table 1) in distilled water (100ml), and each polymeric formulation was plasticized with PG (1ml). TA (20 mg; 1% w/w of the polymer) was dissolved in absolute ethanol (>99%; 4ml) and added separately to each polymeric solution. Then the solutions were cast onto the impermeable backing layers formed earlier in polystyrene Petri dishes and stored in an incubator (LMS Cooled Incubator) at 30°C, until solvent evaporation appeared complete (~72h). The films were cut into circular pieces (1 cm in diameter, using Chroma Gesellschaft™ Cork Borers, size 6mm, Fisher Scientific). Three different compositions were prepared as shown in table 1.

Table 1. The composition of produced films

Formulation	HPMC K4M 2% (ml)	HPMC K15M 2% (ml)	PVP 2% (ml)	PG (ml)	TA (mg)	EC-backing layer %
F1	100	-	-	1	20	5
F2	90	-	10	1	20	5
F3	-	100	-	1	20	5

2.2.2 Evaluation of drug permeability through Permeapad™ barrier:

The in vitro drug permeability study was performed using a standard unjacketed vertical Franz diffusion cell (6G-02-01-11.28-08, PermeGear Inc, SES GmbH Analysesysteme, USA) with 11.28 mm orifice diameter and 1.00 cm^2 diffusion area. The receptor compartment (representing systemic circulation) volume size was 8 ml, and the donor compartment (representing the oral cavity) volume size was 2 ml. The starting donor and receptor solutions were composed of PBS 7.4, PG, and absolute ethanol ≥ 99%, in a ratio of 80:15:5, to maintain sink conditions in the receptor compartment. The Permeapad™ barrier (25 mm in diameter and 0.05 mm in thickness, biomimetic membrane representing buccal mucosa [17]) was clamped between the donor and receptor compartments. The bilayer polymer film was placed onto the Permeapad™ membrane, with the mucoadhesive layer directly in contact with the barrier, while the backing layer was in contact with the donor solution (Figure 1). A small Teflon coated stirrer bar was placed in the receptor compartment. The whole system was equilibrated in a water bath at 37 ˚C for approximately 1 hour. After, the receptor compartment was filled with 8 ml of pre-warmed receptor solution (37 ˚C), and the donor compartment was filled with 1.8 ml of pre-warmed donor solution (37 ˚C). Both the donor compartment and sampling port were covered with Parafilm® to minimise fluid evaporation. The solution in the receptor compartment was continuously stirred at a rate of approximately 300 rpm using a hotplate stirrer (SB 162-3), supplied with a heater and thermometer.

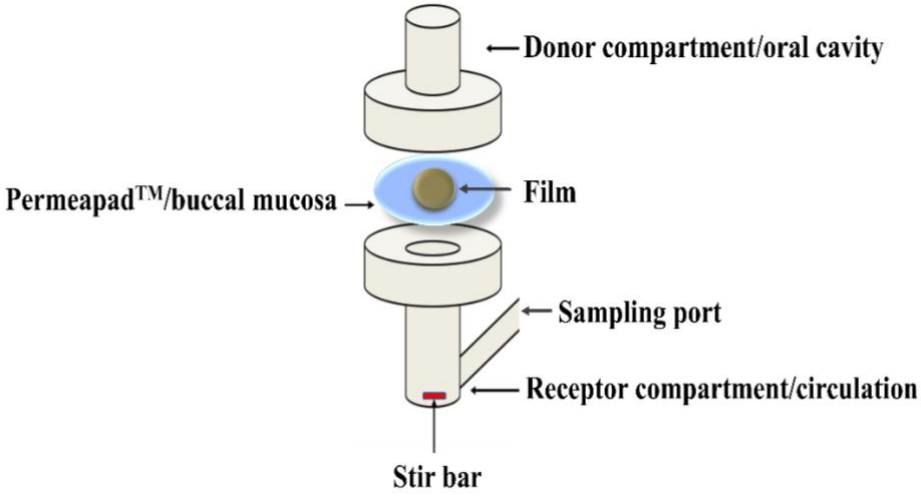


Figure 1. Schematic representation of vertical Franz diffusion cell

A permeation study was also performed using TA solution alone, which was prepared by dissolving TA in the donor solution in a concentration of 100 µg/ml. Thus the 1.8ml of the donor solution in the donor compartment contained the same amount of drug as was present in the film (~180 µg) [6]. This part of the study was conducted to compare the permeability of the drug from a film to a drug solution alone, which used as a control.

To measure the amount of drug permeated through the Permeapad™ membrane to the receptor compartment, samples of 3 ml were withdrawn from the receptor solution at four-time intervals (120, 240, 360, and 480 min) over 8h, using a 5 ml syringe (Luer-Lok™ Tip) equipped with a flexible tube to reach the receptor compartment through the sampling port. The concentration of TA in withdrawn samples was immediately analysed spectrophotometrically, PerkinElmer UV/VIS Lambda 365 double beam UV-visible spectrophotometer provided with a data processing system. UV spectra of reference and sample solutions were recorded in 10 cm path length quartz cells at room temperature (22 ± 1 °C) at a scan rate of 300 nm/min with fixed slit width of 2 nm. The absorbance peak for TA was at a wavelength of 242 nm [18]. Then, the analysed samples were returned to the receptor compartment (to minimize concentration gradient) for further detectable readings using UV spectrometry.

To measure the amount of drug released into the donor compartment over 8h, 1 ml of donor solution was withdrawn, diluted with 3 ml of PBS, PG, absolute ethanol /80:15:5/ solution, filtered through 25 mm syringe filter (cellulose acetate membrane 0.2 µm) to eliminate any undissolved film fragments within the donor compartment, and analysed.

To measure the amount of drug that remained on the Permeapad™ surface (in the delivery system) after 8h, 4 ml of PBS, PG, absolute ethanol (80:15:5) solution was used to wash the Permeapad™ surface and to dissolve any remaining film fragments. A Vortex-2 Genie (Scientific Industries, USA, touch mode at speed control of 6) was used to dissolve the remained film. Then the solution was filtered through a 25 mm syringe filter (cellulose acetate membrane 0.2 µm) and analysed By UV spectroscopy.

The amount of drug located inside the Permeapad™ membrane was calculated theoretically by deducting the amount of TA i) that permeated into the receptor compartment (R), ii) in the donor compartment (D), and iii) remained on the surface of Permeapad™ (S), from the initial quantity of TA present in the film (F), Eq. 1. The initial quantity of TA within each film (n=5 per film) was calculated as previously described [6].

$$M = F - (R + D + S) \qquad \text{(Eq. 1)}$$

Where:

M: is the amount of drug located inside the Permeapad™ membrane

F: is the initial amount of drug in the film

R: is the amount of drug permeated to the receptor compartment

D: is the amount of drug released into donor compartment

S: is the amount of drug that remained on the Permeapad™ surface

The experiment was performed in triplicate for each formulation.

2.2.3 Calculation of permeation kinetics parameters

The cumulative amount of permeated TA per 1 cm^2 (the diffusion area of the Franz diffusion cell) was calculated and plotted against time (min) for all formulations (TA solution, HPMC K4M monolayer film,

F1, F2, and F3). The steady-state flux J_{ss} (µg.cm^2.min^{-1}) and the apparent permeability coefficient, P_{app} (cm.min^{-1}) were calculated from the linear regression of the amount of TA permeated (µg.cm^2) against time (min). The J_{ss} was the slope of the linear regression, and it was calculated using the following equation (Eq. 2) [15, 19, 20]:

$$J_{SS} = \frac{dQ\ /\ dt}{A} \qquad \text{(Eq. 2)}$$

Where:

J_{ss} is the steady-state flux (µg.cm^2.min^{-1})

dQ/dt is the permeation rate (µg.cm^2)

A is the diffusion area (cm^2)

The apparent permeability coefficient (P_{app}) was calculated from the following equation (Eq. 3) derived from Fick's first law of diffusion [15, 21-23]:

$$P_{app} = J_{ss}\ /\ C_I \qquad \text{(Eq. 3)}$$

Where:

P_{app} is the apparent permeability coefficient (cm.min^{-1})

C_I is the initial amount of drug in the film (µg). C_I was calculated as previously described [6].

2.2.4 Statistical analysis:

Student T-test and analysis of variance (one-way ANOVA) were used for statistical comparison of data. The significance level was fixed at $P < 0.05$.

3 Results

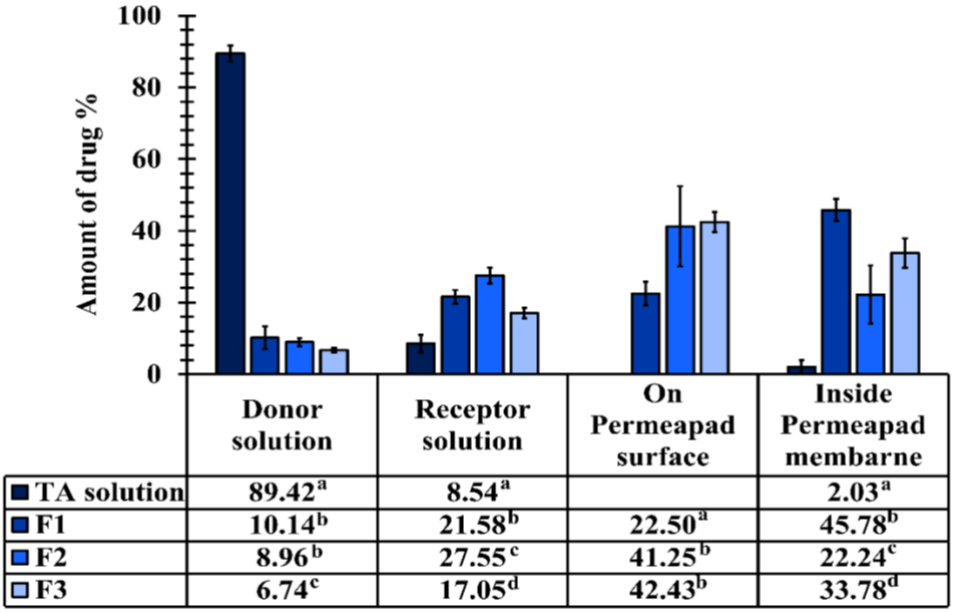
3.1 Effect of the film composition on TA permeability:

The effects of HPMC molecular weight (F1 and F3), and incorporation of PVP in HPMC K4M film (F2) were compared with respect to TA permeability.

Figure 2 shows the amount of TA (%) released i) into the donor compartment, ii) permeated into the receptor compartment, iii) that remained on the surface of the Permeapad™, and iv) the amount located inside the Permeapad™, over 8h for F1, F2, F3, and TA solution. For the drug solution, the

amount of TA located inside the Permeapad™ was surprisingly very small; only 2.03 ± 1.93 %. Furthermore, 89.42 ± 2.24 % of drug remained in the donor compartment, and 8.54 ± 2.47 % permeated to the receptor compartment. Notably, these values exhibit statistically high significant differences when compared to those observed for the films.

F1 showed the highest amount of TA located inside the Permeapad™; 45.78 ± 3.07 %, whereas F2 showed the lowest amount of drug inside the membrane; 22.24 ± 8.10 % ($P < 0.05$). The unreleased amounts of drug (remaining on the Permeapad™ surface) from F1, F2, and F3 over 8h, were 22.50 ± 3.24 %, 41.25 ± 11.20 %, and 42.43 ± 2.79 %, respectively ($P = 0.02$).



	Donor solution	Receptor solution	On Permeapad surface	Inside Permeapad membarne
TA solution	89.42[a]	8.54[a]		2.03[a]
F1	10.14[b]	21.58[b]	22.50[a]	45.78[b]
F2	8.96[b]	27.55[c]	41.25[b]	22.24[c]
F3	6.74[c]	17.05[d]	42.43[b]	33.78[d]

Figure 2. The amounts of TA (%) released into the donor compartment, permeated to the receptor compartment, remained on the surface of the Permeapad™, and located inside the Permeapad™, after 8h, for F1, F2, F3, and TA solution. Note: similar superscript letters indicate no statistically significant difference, in the vertical columns.

Figure 3 shows the cumulative amount of drug (%) permeated through Permeapad™ into the receptor compartment over 8h for the formulations: F1, F2, F3, and TA solution. TA solution showed the lowest drug permeability across the membrane, where only 8.54 ± 2.47 % of drug reached the receptor compartment over 8h. The films demonstrated a high significant enhancement in the permeation of TA. The cumulative amounts of permeated drug over 8h were 21.58 ± 1.92 %, 27.55 ± 2.22 %, and 17.05 ± 1.42 % for F1, F2, and F3, respectively.

The increase in molecular weight of HPMC polymer significantly decreased the drug permeability across the membrane by 4.35 % (from 21.58 % for F1 to 17.05 % for F3; P = 0.02). Addition of PVP polymer to HPMC films significantly increased the drug permeation by ~6 % (from 21.58 % for F1 to 27.55 % for F2; P = 0.01).

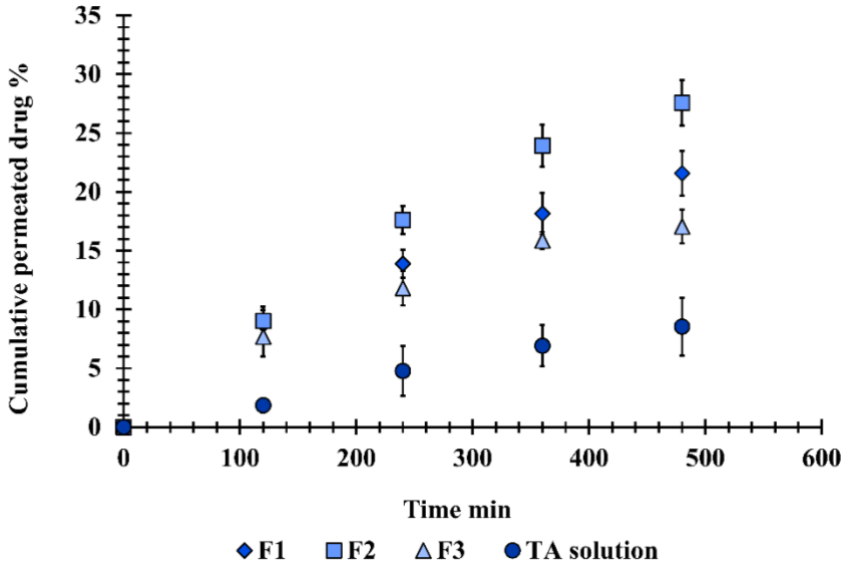


Figure 3. The cumulative amount of drug (%) permeated through Permeapad™ to the receptor compartment over 8h for F1, F2, F3, and TA solution.

3.2 Effect of the backing layer on TA permeability

To study the effect of the backing layer on TA permeability, the permeation study was performed on HPMC K4M monolayer film (prepared according to Alhallak *et al*, [6]) and the data was compared with that obtained for HPMC K4M bilayer film (F1). This film was selected because, from the results of the above study, the amount of drug found inside Permeapad™ was the highest (45.78%). Figure 4 displays the amount of TA (%) released into the donor solution, permeated into the receptor solution, also that remained on the surface of Permeapad™, and located inside the Permeapad™, for HPMC K4M bilayer and monolayer films, over 8h. The EC backing layer significantly reduced the loss of TA into the donor compartment by 17.85 % (from 27.99 ± 4.23 % for monolayer film to 10.14 ± 3.17 % for bilayer film; P < 0.05). The amount of drug located inside Permeapad™ for the bilayer film (45.78 ± 3.07 %) was significantly higher than that for the monolayer film (33.32 ± 6.55 %; p < 0.05. The unreleased amounts

of TA from the bilayer and monolayer films over 8h were virtually similar (22.50 ± 3.24 %, and 22.77 ± 3.56 %, respectively).

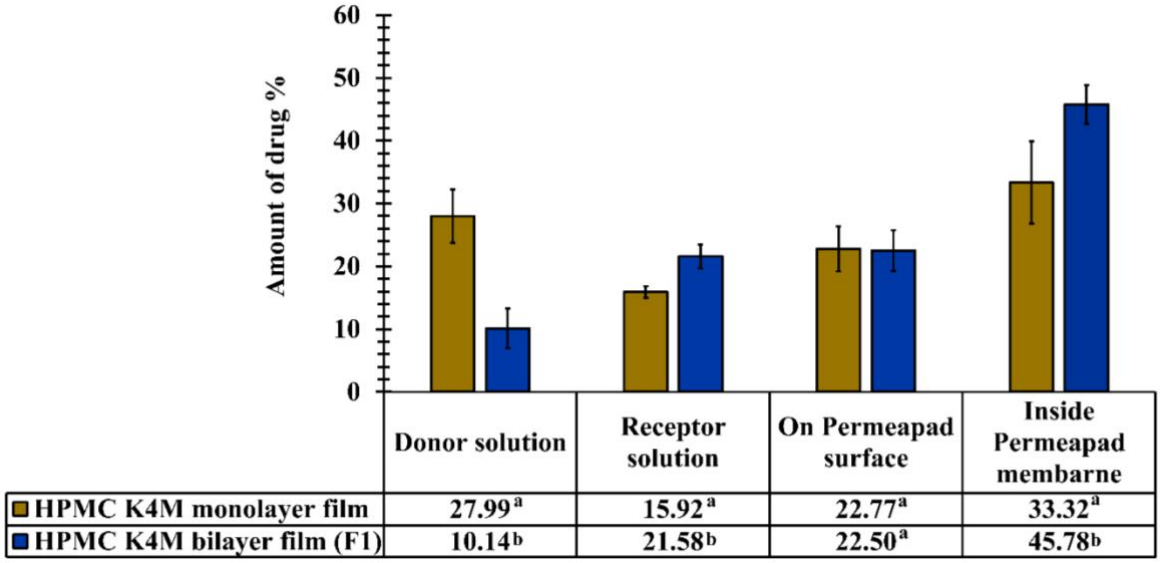


Figure 4. The amount of TA (%) released into the donor solution, permeated into the receptor solution, remained on the Permeapad™, and located inside the Permeapad™ for HPMC K4M bilayer and monolayer films over 8h. Note: similar superscript letters indicate no statistically significant difference, in the vertical columns.

The amount of TA (%) permeated through the Permeapad™ to the receptor compartment over 8h from HPMC K4M bilayer film and HPMC K4M monolayer film is shown in figure 5. The presence of the backing layer significantly increased the permeability of TA through the Permeapad™ by 5.66 % (from 15.92 ± 0.92 % for the monolayer film to 21.58 ± 1.92 % for the bilayer film; P = 0.01).

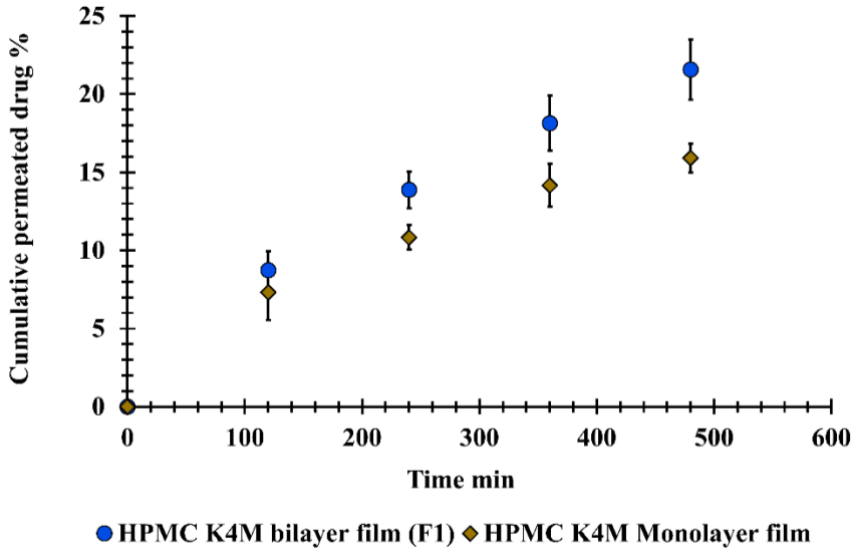


Figure 5. The amount of TA (%) permeated through the Permeapad™ into the receptor compartment, over 8h, from HPMC K4M bilayer film (F1) and HPMC K4M monolayer film.

3.3 Permeation kinetics parameters:

The calculated J_{ss} and P_{app} of TA solution, monolayer film, F1, F2, F3 are shown in table 2. The J_{ss} and P_{app} for F1 were significantly higher than those obtained for TA solution, by 100 % and 93.4 % respectively. They were also significantly higher than those obtained for F3 (by 56.4 % and 32.8 %, respectively). F2 showed a significant increase in J_{ss} and P_{app} compared to the values obtained for F1 (by 33.3% and 44.9%, respectively). The J_{ss} and P_{app} for F1 (HPMC K4M bilayer film) significantly increased by 46.8 % and 47.1 % respectively, in comparison to HPMC K4M monolayer film.

Table 2. The initial amount of drug in the film (C_I), steady-state flux (J_{ss}), and the apparent permeability coefficient (P_{app}) of TA solution, for HPMC K4M monolayer film, and F1, F2, F3 bilayer films. For the J_{ss} and P_{app} columns: different superscript letters indicate statistically significant.

Formulation	C_I (µg)	J_{ss} (µg.cm^2.min^{-1})	P_{app} (cm.min^{-1})
TA solution	180.0	$0.0333^{(a)}$	$1.84 \times 10^{-4(a)}$
HPMC K4M monolayer film	186.6 ± 7.5	$0.0453^{(b)}$	$2.42 \times 10^{-4(b)}$
F1	186.6 ± 7.5	$0.0665^{(c)}$	$3.56 \times 10^{-4(c)}$
F2	172.6 ± 18	$0.0889^{(d)}$	$5.16 \times 10^{-4(d)}$
F3	158.4 ± 9	$0.0425^{(e)}$	$2.68 \times 10^{-4(e)}$

4 Discussion

4.1 Effect of the delivery systems on drug permeability:

This study revealed a noteworthy enhancement in the permeability of TA through the Permeapad™ barrier when the drug was loaded into the polymeric matrices (bilayer or monolayer), as compared to its permeability as a standalone drug solution. For example, the J_{ss} and the P_{app} of F1 were significantly higher than those for TA solution by 100 % and 93.4 % respectively (Table 2). This enhancement in the permeability of drug can be explained by the synergy effect of the hydrophilic mucoadhesive polymer (HPMC) and propylene glycol (PG), the latter which is used as a plasticizer in the formulations. PG is widely used as a penetration enhancer, either on its own or in combination with other penetration enhancers [24, 25]. PG can act as a penetration enhancer by competing with water to form hydrogen

bonds with the polar (head) groups of the lipid layer, which enhance the intracellular penetration of drug [25, 26]. The combination of the HPMC polymer with PG may have affected the permeability of Permeapad™ by establishing chemical interactions (hydrogen bonds) with the barrier lipid layer and generating voids within this layer. These voids allowed more drug i) to remain in the barrier lipid layer or ii) to permeate through this barrier layer. Favacho *et al* [20] reported that combinations of hydrophilic polymers (e.g., HPMC) and surfactants (oleic acid, polysorbate 80 and PG) could affect the permeability of the buccal epithelium. They suggested enhanced drug permeation and retention may arise from different chemical interactions with membrane phospholipids, which modify the drug distribution coefficient and creates distinct intercellular spaces. These factors could contribute to enhanced drug permeation and retention.

4.2 Effect of presence of the backing layer on drug permeability:

HPMC K4M bilayer film (F1) significantly increased J_{ss} and P_{app} by 46.8 % and 47.1 %, respectively, in comparison with HPMC K4M monolayer film (Table 2 and Figure 4). This observation could be attributed to the presence of the impermeable EC backing layer, which controlled the direction of drug release from one side, via the adhesion layer, only. It prevented the loss of drug into the donor compartment and allowed more drug to be permeated through the Permeapad™ membrane [22]. A delivery system with unidirectional release of drug is favoured in the treatment of local mucosal disorders, since it prevents the loss of drug into oral cavity which the patient will ingest, and it maximises the amount of drug at the affected site.

4.3 Effect of the film composition on drug permeability:

The J_{ss} and the P_{app} for F1 (HPMC K4M) were significantly higher than those obtained for F3 (HPMC K15M) by 56.4 % and 32.8 % respectively (Table 2). The higher molecular weight and viscosity of HPMC K15M (120,000 Da, 15,000 cP respectively) compared to HPMC K4M (86,000 Da, 4,000 cP) appear to have played a role in reducing TA release. This observation can be explained by the fact that a thicker gel layer was formed on the HPMC K15M film surface, because this polymer contained more hydrophilic hydroxyl groups (OH) due to its higher molecular weight compared to the HPMC K4M

polymer. This caused more hydration and swelling of the mucoadhesive film [6], thus the drug had to travel via a longer path than that formed on HPMC K4M film surface. Furthermore, the higher viscosity of HPMC K15M (15,000 cP) than that of HPMC K4M (4,000 cP) also decreased the permeability of drug through the Permeapad™ membrane. These findings are in agreement with reported literature [27, 28]. In the former [27], a diffusion cell was used to determine drug release from viscous eye drops, where the diffusion of drug decreased with increasing viscosity. The latter [28] reported on the transdermal delivery of hydrocortisone from eucalyptus oil microemulsion. The viscous delivery agent had a decreased release rate compared to the microemulsion containing PG. The PG reduced the viscosity of the microemulsion resulting increased transdermal drug flux.

Published literature suggests that PVP serves as a permeation enhancer [29]. In the current study films containing PVP (F2) significantly increased the J_{ss} and P_{app} by 33.3% and 44.9%, respectively (Table 2). Therefore, these results could be due to PVP acting as a permeation enhancer, as well as it being a very hygroscopic polymer. The latter led to quick hydration of HPMC, and this increased the flexibility of the films. As a result, the adhesion force of F2 with Permeapad™ increased [6] and enabled more drug molecules to adhere to, and permeate through, the membrane.

F1 released 77.5 % of TA over 8h, whereas F2 and F3 released 58.7 % and 57.6 % of the drug over 8h, the rest remained unreleased within the film on the Permeapad™ surface. These findings can be explained by the gel layer that formed around the drug molecules near the surface of the delivery system. In F1, it is assumed the gel layer was not as thick as that formed in F2 and F3 [30, 31]. Therefore, the drug release path was shorter, and hence the amount of released drug was higher for F1. Thus, F1 showed the highest amount of TA (45.7 %) located inside the Permeapad™ while only 22.2 % and 33.7 % of drug was found in the case of F2 and F3, respectively.

Padula *et al.* [32] developed and characterized microemulsions containing TA for potential buccal administration. To evaluate the permeability of drug, they used pig esophageal epithelium as a model of buccal mucosa. The results showed the flux of TA across the pig esophageal epithelium was 0.022

μg cm^{-2} min^{-1}. The addition of chitosan (1%) increased the drug flux to 0.043 μg cm^{-2} min^{-1}. In our study, the TA flux through Permeapad™ barrier ranged from 0.0425 to 0.088 μg cm^{-2} min^{-1} for F3 and F2, respectively, which reflects that Permeapad™ works well as a tissue barrier membrane (e.g., for buccal mucosa). Further, Di Cagno *et al.* [14] investigated the permeability of low permeable actives (such as calcein) and high permeable actives (such as caffeine) through Permeapad™ barrier, using the Franz diffusion cell. They found that the P_{app} values ranged from 0.7×10^{-4} cm/min for calcein and 12.24×10^{-4} cm/min for caffeine. The P_{app} values that are reported from the current study for TA through Permeapad™ ranged between 1.84×10^{-4} and 5.16×10^{-4} cm/min, which fall within the range reported for low and high permeable actives.

Nicolazzo *et al.* [33] reported that the apparent permeability coefficient of TA through porcine buccal mucosa was 1.52×10^{-4} cm.min^{-1}, which is comparable to the results obtained from this current study. The authors studied the effect of Azone® (a skin penetration enhancer) on the permeability of TA, from Kenalog in Orabase®, across porcine buccal mucosa. They used a diffusion cell, and samples withdrawn from the receptor compartment over 4h were analysed. The results showed that incorporating Azone® 5 % within Kenalog in Orabase® significantly increased the TA flux through porcine buccal mucosa from 0.12 to 0.52 μg cm^{-2} h^{-1} ($P = 0.027$). However, it did not increase the amount of drug located inside the tissue.

Overall, the current research findings provide a comprehensive understanding of the impact of the bilayer delivery systems composition on drug permeability within and across Permeapad™ using a Franz diffusion cell. Permeation of TA was increased, not only by the delivery system itself, but by its composition as well. Unidirectional delivery of TA was achieved in a controlled manner over 8 h. These results pave the way for future advancements in developing such delivery systems for targeted mucosal drug delivery strategies.

5 Conclusion:

From this study, the impact of various factors on the permeability of TA through the Permeapad™ barrier, using a Franz diffusion cell, were demonstrated.

HPMC-based polymer matrices loaded with TA significantly enhanced the drug's permeability compared to the drug solution itself. The synergistic action of the hydrophilic mucoadhesive polymers HPMC, PVP and propylene glycol (PG), acting as penetration enhancers increased the drug's permeation. The impermeable backing layer (to form the bilayer films) provided unidirectional drug release, thus significantly increasing drug permeability compared to the monolayer films. This important finding substantially minimises drug loss in the donor compartment, which refers to drug lost in the oral cavity.

The results demonstrated that the HPMC K4M film (F1) yielded the highest drug retention within the Permeapad™ barrier (45.78 %), indicating that this formulation is the optimal choice for the topical treatment of oral infections (e.g., recurrent aphthous stomatitis), since it maximises the amount of drug at the affected site.

6 References

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