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Characterization of Buccal Mucosa as Barrier for Drug Absorption; A Mechanistic Study via Solvent Uptake and Model Drug Permeation Studies

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ABSTRACT

Porcine buccal mucosa is abundantly used in drug delivery studies. With the emergence of new buccal barrier models, such as cell culture models, a link between these models and traditional porcine models should be established. The examination of buccal mucosa was divided into two experiments: the uptake of different solvents into the buccal mucosa and an infinite dose permeation study of domperidone from different solvent systems through porcine and EpiOral™ tissue culture models; this aims to find a correlation between the two models. The solvent uptake experiment revealed superb uptake of water by the dried mucosa (>300% increase in weight) and a high uptake of solvents with high solubility parameters (24.8 and 16.6% for propylene glycol (PG) and polyethylene glycol 200 (PEG200), respectively). An unexpected decrease in weight was observed for Transcutol P and Isopropyl myristate (IPM), emphasizing the lipid extraction effect of these solvents. The increase in the weight of dried buccal mucosa by hydrophilic solvents was mainly due to the high level of total phospholipids and the low percentage of lipophilic ceramides. Permeation studies of domperidone (as a model drug) depend on single and binary solvent systems using Franz diffusion cells. Transcutol® P (TC), polyethylene glycol 200 (PEG 200), and polyethylene glycol 400 (PEG 400) were used as the single-solvent systems. Binary solvent systems were prepared using binary mixtures of Transcutol® P and water in three different ratios (20, 40%, and 60%). The flux (J) of domperidone from different solvent systems through both models showed the same system rank: 60% TC > 40% TC> TC > PEG 200> PEG 400> 20% TC. Plotting the values of flux (J) and the permeability coefficient (kp) of domperidone from different systems in the porcine buccal model against the EpiOral™ tissue culture model showed a correlation coefficient (r2) greater than 0.8, which confirms good correlation.

KEYWORDS: Permeation; buccal mucosa; domperidone; flux; permeability coefficient; solvent uptake.

1. INTRODUCTION

Buccal drug delivery has received much attention lately. This is mainly because the buccal route provides an alternative to the conventional oral route in terms of avoiding the first-pass effect of drugs and the independence of the GIT factors that affect drug absorption, such as gastric emptying rate and gastric acidity [1]. In addition, the buccal mucosa is relatively permeable, has a rich blood supply, and has substantial resistance to irritants and damage [1-3]. The permeability of the buccal mucosa is 4 to 4,000 times greater than that cross the skin. As a result, a faster onset of action has been observed for several drugs [4]. The application of the oral mucosa as a site for drug delivery has resulted in the presumption that it constitutes a tissue with high permeability; however, in actuality, the permeability characteristics of the oral

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mucosa represent a multifaceted concern that is contingent not merely upon its structural attributes but also on the intrinsic properties of the penetrative agents involved [5]. Squier *et al*. studied the lipid content and water permeability of porcine skin and oral mucosa, and they concluded that water permeability across buccal mucosa is about 10 times more than the skin [6].Ganem *et al*. provided a comprehensive review of the lipid composition of the oral mucosa and its effect on the mucosal permeability barrier [7].

The use of different solvents for buccal drug delivery has been discussed in many studies [8-10]. The majority of enhancers have been documented to exert their influence on intercellular lipids through mechanisms such as fluidization, extraction, or reorganization of the lipid microdomain, consequently disrupting the integrity of the membrane. These occurrences are frequently associated with the thermotropic transitions that lipids experience when exposed to variations in temperature, alterations in hydration, or certain chemical agents. **[7].** The permeability characteristics of tritiated water across porcine buccal mucosa have been demonstrated by Lesch *et al*. to exhibit a high degree of similarity to those observed in human buccal mucosa [11], and more recently, Nielsen and colleagues did not find any noteworthy distinctions in the permeability of mannitol or testosterone across the buccal mucosa of porcine and human origins. [12]. Due to the significant quantities of porcine oral mucosa sourced from abattoirs and their analogous architecture and permeability characteristics to human tissues, a majority of research facilities employ porcine buccal mucosa in the evaluation of mucosal permeability and the impact of diverse chemical penetration enhancers on the delivery of pharmacological compounds via mucosal routes [9, 13-36]. It is consequently advocated that the preclinical assessment of compound permeability across the buccal mucosa is conducted utilizing porcine buccal mucosa, given its structural and permeability attributes that closely resemble those observed in human buccal mucosa. The challenges associated with the extraction of buccal mucosa from porcine sources, coupled with the limited availability of domesticated pigs in certain regions, necessitate the exploration of alternative options.

The utilization of buccal cell cultures for the evaluation of the permeability of the buccal mucosa has garnered significant scholarly interest in recent times. In order to cultivate buccal epithelial cells, it is imperative that they be extracted from a suitable source and maintained under meticulously defined conditions, employing an appropriate growth medium, as well as regulating temperature and humidity [37]. Tavakoli-Saberi *et al*. effectively cultivated cell cultures from the hamster cheek pouch. In contrast to the keratinized hamster cheek pouch mucosa, these cultured cells did not differentiate to form a fully keratinized surface, as observed in the normal hamster cheek pouch. Consequently, they exhibited a higher degree of permeability to compounds [38]. Therefore, cultured hamster cheek cells are more closely mimicked the human buccal mucosa owing to their lack of keratinization, suggesting that this may be an appropriate model for predicting permeability through the human buccal mucosa. Another cell culture model that has been proposed as a model of the human buccal epithelium is the TR146 cell line by Nielsen *et al*. [12, 39-42]. Rupniak *et al*. stated that TR146 cells originate from a human buccal carcinoma [43] when cultured. Jacobsen *et al*. showed that they form an epithelium, resembling the buccal mucosa [40] with the appropriate differentiation patterns observed in human non-keratinized epithelium [44]. Nevertheless, Nielsen *et al*. demonstrated that the TR146 cell culture model has a lower barrier nature than human and porcine buccal epithelium. This was demonstrated by the significantly greater permeability to tritiated water, mannitol, testosterone, dextrans, and nicotine [12, 41, 45, 46], which may be attributed to the cancerous composition of the original cells. Recently, a cell

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culture derived from biopsies of healthy human buccal mucosa was developed by Selvaratnam *et al*., with remarkably similar morphology, membrane-coating granule structure and appearance, and lipid composition to intact buccal tissue [47]. The barrier properties of this cell culture model resemble those of intact buccal mucosa, suggesting that it may serve as an alternative to TR146 cell culture. The advancement of tissue culture techniques suggests the potential development of cell culture models exhibiting morphological and barrier properties akin to those of normal intact buccal mucosa. These models can be valuable for evaluating the buccal permeability and metabolism of various compounds. One of the most commonly used cell culture models is the EpiOral™ model, which represents a highly differentiated, three-dimensional, cultured buccal tissue equivalent. EpiOral™ consists of normal, human-derived epithelial cells cultured to form multilayered, highly differentiated models of human buccal phenotypes. Morphologically, these tissue models closely parallel human tissues, thus providing a useful in vitro model. This model has been used extensively to study the carcinogenic and irritant effects of different agents on the buccal mucosa [48-50]. In addition, this type of tissue culture has been used to assess the buccal drug delivery of different drugs and the effect of different penetration enhancers on the diffusion of these drugs [51-53]. Domperidone is a selective peripheral dopamine antagonist of the D2 receptor and is used for the prevention and symptomatic relief of acute nausea and vomiting. Buccal delivery of domperidone could therefore be convenient to avoid oral administration. The molecular weight of domperidone is 425.92 (gm/mol) with a logP (octanol–water partition coefficient) of 5.95 and Van Krevelen's solubility parameter of 13.35 (cal/cm3)1/2 (calculated using Molecular Modeling Pro® software according to Van Krevelen and Hoftyer [54]).

The aim of this study was to explain the effect of different solvents on heat-separated buccal epithelium using the solvent uptake technique. To find a correlation between the porcine buccal model and the EpiOral™ model using a lipophilic drug, domperidone, in different solvent systems.

2. METHODOLOGY

2.1. Materials

The materials listed next were acquired for this study: Domperidone was provided by Amoun Pharmaceuticals in Egypt, Lauroglycol P (propylene glycol monolaurate), Labrafac PG (propylene glycol dicaprylate/dicaprate), and Transcutol P (diethylene glycol monoethyl ether) were received as complimentary samples from Gattefossé (France). PEG 400 and PEG 200 were acquired from Fluka GmbH, Germany. Miglyol 810 was acquired from Sasol GmbH in Germany. Isopropyl myristate (IPM) was sourced from Avocado (UK). Geraniole and cineole were sourced from Acrōs Organics (USA). Propylene glycol (PG) and D-limonene were sourced from Fisher Scientific (UK). Tissue-engineered human buccal mucosa (EpiOral™) was sourced from MatTek Corporation (Ashland, MA, USA) and maintained under refrigeration (4°C) for a maximum of 24 hours before application. Samples of porcine cheek were obtained from a local ranch. HPLC solvents were sourced from Fisher Scientific (UK).

2.2. Preparation of Porcine Buccal Epithelium

Whole-pig cheeks were supplied by a local ranch. Once the cheeks arrived, they were stored in Krebs-Ringer bicarbonate buffer and frozen until use. On the day of the experiment,

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the cheeks were thawed and the buccal mucosa was separated using scalpels and surgical scissors. The buccal epithelium was separated from the underlying connective tissue by immersing the buccal mucosa in a normal saline solution at 60 ^{o}C for 1 min. The buccal epithelium was carefully peeled off from the connective tissue using surgical forceps.

2.3. Solvent Uptake Experiment

The separated buccal epithelium was cut into square pieces (1 cm^2 area) and dried in a desiccator chamber in the dark until a constant weight was reached. After drying the epithelia, each piece was placed in a test tube containing 2 ml of solvent. The test tubes were kept in a temperature-controlled water bath at 37±0.5o C for 48 h. After incubation, the pieces were removed from the test tubes, and excess solvent was removed using filter paper. The pieces were reweighed, and the percentage of solvent uptake was determined using the following equation:

$$\%solvent\ uptake\ =\ (W_2 - W_1/W_1)\ x\ 100 \quad (1)$$

where W_1 is the weight of dried epithelia and W_2 is the weight of the epithelia after immersion in the solvent. All samples were weighed using a Sartorius Ultramicro microbalance (Satorius, Germany). Each solvent was tested in triplicates.

2.4. Preparation of Porcine Buccal Mucosa for Permeation Studies

Fresh pig cheek samples were obtained from a local ranch and stored in Krebs Ringer bicarbonate buffer at -30°C until use. After thawing, the underlying connective tissue was removed using a scalpel blade, and the remaining buccal mucosa was carefully trimmed to a uniform thickness of approximately 500 µm using surgical scissors. Tissue thickness was measured using a digital micrometer and recorded to ensure consistency across tissue specimens.

2.5. Permeation Studies

The saturated solutions of domperidone prepared in each solvent system were used in this step. The barrier membranes of the porcine buccal mucosa or EpiOral™ membrane samples were mounted in vertical Franz diffusion cells (with a receptor compartment capacity of 5 ml and a diffusion-available surface of 1 cm^2). The receptor compartments were filled with isotonic phosphate buffer saline (1.55 M and pH 7.4) and stirred at 600 rpm in a thermostatically controlled water bath (Grant Instruments, UK).

The biological membranes of porcine buccal mucosa were equilibrated by introducing phosphate-buffered saline into the donor compartment at 37°C and maintained for 30 minutes before the experiment. Subsequent to this period, the phosphate buffer saline was meticulously extracted from the donor compartment. A 1-mL volume of a saturated drug solution in different solvent systems was subsequently added to the donor compartment. Following the application of the donor solution, the donor compartments were sealed with glass slips to replicate the occlusive environment of the oral cavity. Permeation studies were performed over a duration of 12 hours, during which 1-mL samples were extracted from the receptor compartment at designated time intervals and replaced with an equivalent volume of phosphate-buffered saline maintained at 37°C. All experiments were conducted in triplicate. For EpiOral™, the experiments were carried out under the same conditions as for porcine buccal mucosa for 6 h

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(to avoid compromising the barrier properties of the membrane after 6 h). All experiments were conducted in triplicate. The permeation of domperidone was evaluated by plotting the cumulative amount permeated per unit surface area of the membrane (in µg.cm-2) against the collection time (in minutes). The total permeated amount was determined using equation 1, considering the volume of the receptor phase (VR), the volume sampled at each time point (Vs), and the quantified concentration of the sample collected at the nth time point (Cn). The cumulative amount determined at each time point via Eq. (2) was subsequently divided by the diffusion area of the Franz Cells (cm²).

$$cumulative\ amount = V_R \times C_n + V_s\left(\sum C_1 + + C_{n-1}\right) \quad (2)$$

2.6. Data analysis for Permeation Studies

The steady-state flux Jss (µg.cm-2.hr-1) at time t was determined from the slope of the linear segment of the cumulative drug permeation profile per unit area against time. The total drug concentration in the receptor compartment after 6 hours and 12 hours was designated as Qcum (µg.cm-2). The permeability coefficient kp (cm-1. hr-1) of domperidone for each formulation was determined by dividing Jss by the starting concentration of the medication in the donor compartment, represented by the saturated solubility of domperidone in each formulation (Co), as illustrated in Eq. 3. Results are expressed as mean ± standard deviation (SD).

$$k_p = \frac{J_{ss}}{C_o} \quad (3)$$

Data analysis of flux was performed using ANOVA to compare fluxes and cumulative drug amounts in the receptor compartment of different formulations, followed by Tukey's significant difference test using IBM SPSS® version 20 for Windows®. A probability of less than 5% ($p < 0.05$) was considered significant.

2.7. HPLC Analysis

A reverse-phase HPLC column (Supelco LC-18, 5 µm, 150 mm × 4.6 mm) was utilized. The HPLC system utilized was the HP Agilent 1100, which included a binary pump (HP G1312A) and a UV-VIS detector (HP G1314A Variable Wavelength Detector). The mobile phase consisted of methanol, acetonitrile, and 10mm triethylamine (pH 7) in a volume ratio of 20:33:47, with a flow rate of 1 mL/min. The detection wavelength was established at 285 nm, with a retention time of 5.5 minutes. The assay demonstrated linearity (r2 = 0.9997) across a concentration range of 0.05–100 µg/mL, with a minimum detection limit of 0.03 µg/mL. The recovery percentages varied between 98.0% and 101.0%.

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Table 1: Percentage increase in weight of buccal epithelium corresponding to each solvent (mean ± SD) (n=3).

Solvent	Solubility Parameter	% weight increase (±SD)
Miglyol 818	7.82	10.01±2.4
IPM	8.21	-3.42±0.99
Cineole	8.65	9.05±3.71
Labrafac PG	8.72	4.17±0.98
Transcutol P	10.62	-3.69±0.92
PEG 200	11.24	16.61±1.64
PEG 400	12.06	3.52±1.54
Ethanol	12.26	1.62±0.5
PG	14.06	24.8±2.44
Glycerol	17.1	11.67±3.9
Water	22.97	494.47±5.6

3. RESULTS

3.1. Solvent Uptake Experiment

The results of solvent uptake by porcine buccal epithelium are shown in Table 1 and Fig. 1. The increased solvent uptake in the case of solvents with high solubility parameters is higher than that in solvents with low solubility parameters. The highest percentage uptake was recorded in water with almost a 500% increase in weight, while the lowest was in the case of Labrafac PG and PEG 400 (4.17 and 3.52%, respectively). There was a negative uptake in the case of IPM and Transcutol P (-3.42 and -3.69% respectively).

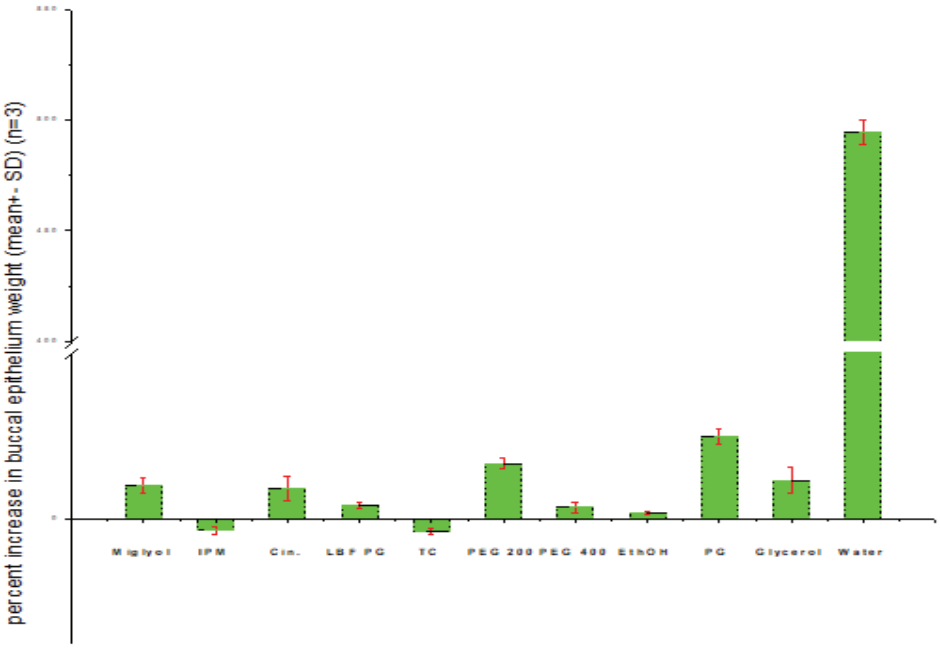

Fig. 1: The uptake of different solvents into porcine buccal epithelium (mean±SD) (n=3).

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3.2. Permeation Studies

This study investigated the permeation of domperidone through porcine buccal tissue and tissue culture models, using various solvent systems. Figs. 2 and 3 illustrate the permeation profiles of domperidone from various solvents, utilizing the EpiOral™ tissue culture model and porcine buccal mucosa. The flux of domperidone across porcine buccal mucosa was maximized with Transcutol, yielding a value of 2.327 µg/cm²·h, which was statistically significantly different from the values obtained with PEG 200 and PEG 400 ($p < 0.05$). PEG 200 exhibited a flux value of 1.091 µg/cm²·h, while PEG 400 a value of 0.947 µg/cm²·h.

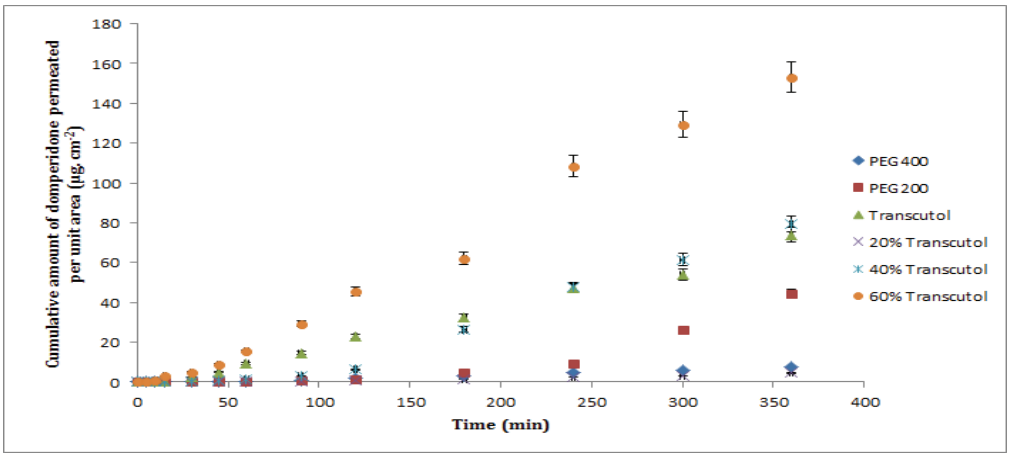

Fig. 2: Permeation profiles of domperidone using the EpiOral™ buccal model from different solvent systems. (Means ± S.D.; n=3).

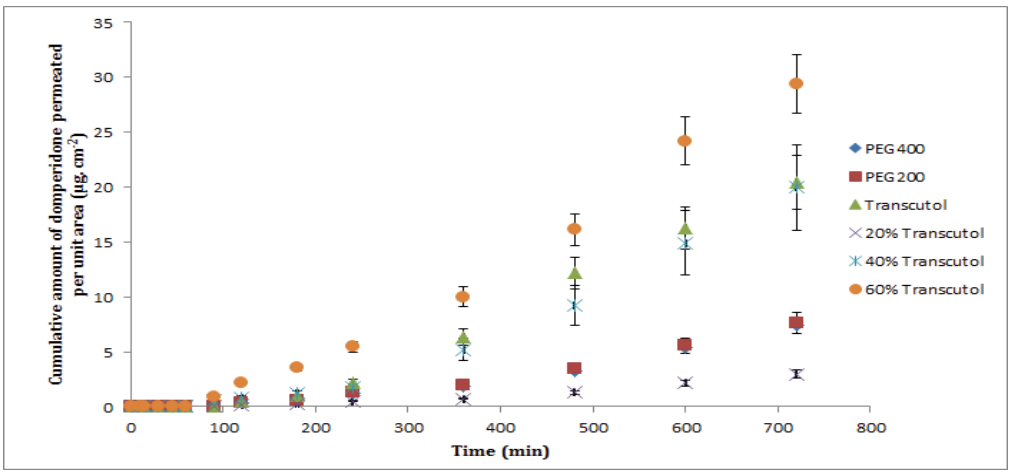

Fig. 3: Permeation profiles of domperidone using a porcine buccal model from different solvent systems. (Means ± S.D.; n=3)

Mixing Transcutol with water at concentrations of 40% and 60% v/v produced significantly higher flux values ($p < 0.05$) compared to Transcutol alone. In contrast, the 20% v/v binary mixture exhibited the lowest flux value of 0.413 µg/cm²·h, which was significantly different from the other formulations ($p < 0.05$), except for PEG 400 ($p > 0.05$). The findings from the EpiOral™ tissue culture model were consistent with those derived from porcine buccal mucosa. The flux data (Table 2) indicated that Transcutol produced the highest flux values at 15.905 µg/cm²·h, which was significantly different from the values obtained with

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PEG 200 and PEG 400 ($p < 0.05$). PEG 200 demonstrated a flux value of 13.367 µg/cm²·h, whereas PEG 400 showed a value of 1.39 µg/cm². The flux values from PEG 200 were not significantly different from those of PEG 400 ($p > 0.05$). Mixing Transcutol with water at concentrations of 40% and 60% v/v produced significantly higher flux values ($p < 0.05$) than Transcutol alone, yielding 17.45 and 29.39 µg/cm²·hr, respectively. The binary mixture of 20% v/v exhibited the lowest flux value (1.087 µg/cm²· h), which was significantly different from the other formulations ($p < 0.05$), with the exception of PEG 400 ($p > 0.05$). The solvent systems were ranked as follows: 60% v/v, 40% v/v, Transcutol, PEG 200, PEG 400, and 20% v/v.

Table (2): permeation parameters of domperidone through different buccal models in different solvent systems (mean ± S.D.)

SOLVENT	Flux, J (µg. cm^{-2}. h^{-1})		Q_{cum} (µg. cm^{-2})		kp (cm. h^{-1})	
	Porcine[a]	EpiOral™[,b]	Porcine	EpiOral™	Porcine	EpiOral™
PEG400	0.948 ± 0.11	1.4 ± 0.18	7.45 ± 0.99	7.6 ± 0.1	0.00012 ± 1.8E-05	0.00019 ± 2.82E-05
PEG200	1.092 ± 0.13	13.368 ± 1.6	7.62 ± 0.96	44.36 ± 5.8	0.00015 ± 2.22E-05	0.0018 ± 0.00027
Transcutol	2.328 ± 0.28*	15.906 ± 1.9*	20.46 ± 3.8*	73.8 ± 9.6*	0.0001 ± 1.52E-05*	0.00069 ± 0.0001*
20% TC (v/v)	0.413 ± 0.05	1.086 ± 0.13	2.98 ± 0.4	4.5 ± 0.6	0.001 ± 0.00015	0.0026 ± 0.00039
40% TC (v/v)	2.67 ± 0.32	17.5 ± 2.1	19.6 ± 2.6	79.26 ± 10.3	0.0017 ± 0.00026	0.011 ± 0.002
60% TC (v/v)	3.306 ± 0.4*	29.4 ± 3.5*	29.3 ± 3.8*	153.1 ± 19.9*	0.0008 ± 0.0001*	0.00675 ± 0.001*

a n=5
b n=3
* p<0.05

By plotting the values of fluxes, $Q^{cum,}$ and k_p of domperidone through porcine buccal mucosa against those through EpiOral™, correlation coefficients (r^2) of 0.8555, 0.9147, and 0.8052, respectively, were obtained, as shown in Figss (4,5 and 6), respectively.

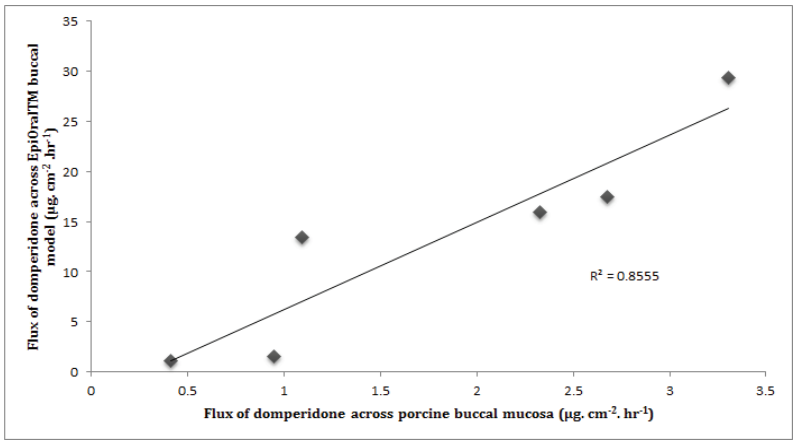

Fig. 4: Plot of flux values obtained from permeation of domperidone through porcine buccal mucosa against values obtained from the EpiOral™ buccal model

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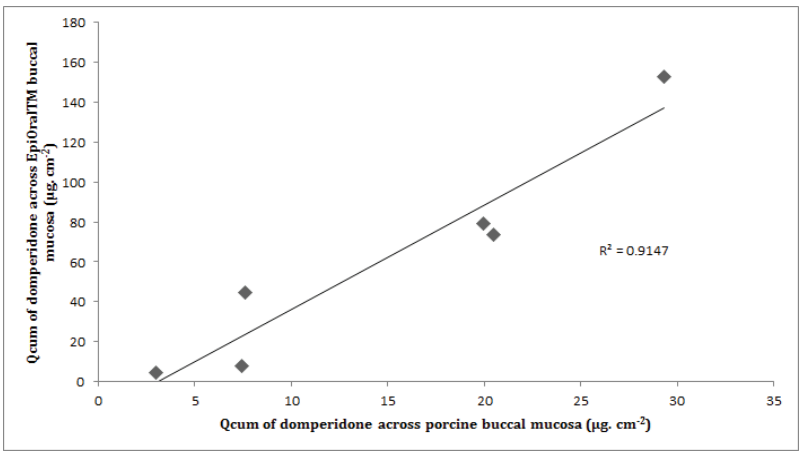

Fig. 5: Plot of cumulative amount values permeated of domperidone through porcine buccal mucosa against values obtained from EpiOral™ buccal model

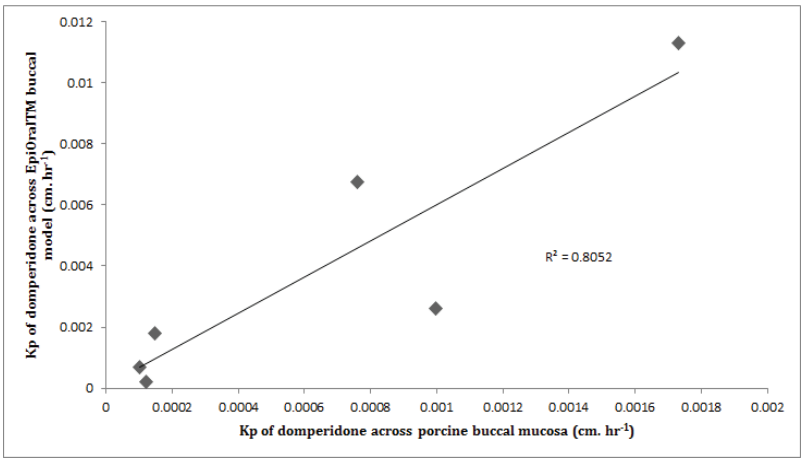

Fig. 6: Plot of permeability coefficient values obtained from domperidone permeation through porcine buccal model against values obtained from the EpiOral™ buccal

4. DISCUSSION

The high uptake of water and other hydrophilic solvents can be attributed to the hydration of buccal epithelial lipids with water and polyhydric alcohols such as PG, Glycerol, PEG 200, and PEG 400. Buccal epithelial lipids (mainly phospholipids and glycosylceramides) undergo thermotropic mesomorphism (interchange between the gel and liquid crystalline phases). Additionally, specific molecules can be prompted to form liquid crystals through the incorporation of a secondary chemical component, such as a solvent like water, a process known as lyotropic mesomorphism [55]. The disorder in lipid structure, induced by thermotropic or lyotropic transitions, is associated with a reduction in the diffusional resistance of the tissue [56]. Hydration significantly affects lipid fluidity, modifying differential scanning calorimetry (DSC) profiles and infrared (IR) spectra. Increased hydration results in a reduction of transition temperature (TM) [57] and thus increased membrane fluidity [56]. A decrease in TM has also been observed with some substances frequently used as oral mucosal permeation enhancers, i.e., fatty acids [58-61], surfactants [62, 63], Azone® [26, 64] and ethanol [65, 66].

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The increase of polar solvent uptake into the buccal epithelium could be explained if we take a look at the work done by Wertz *et al*. and Squier *et al*. to study the difference in lipid content and water permeability between skin and buccal mucosa [6, 67-69]. These differences can be seen in Table (3). From the table, it can be seen that buccal mucosa contains more phospholipids than skin, which is mainly in the form of phosphatidyl ethanolamine. In the case of ceramides, buccal mucosa is very deficient in free ceramides if compared to the skin, while it is rich in the more hydrophilic glycosylceramide due to the deficiency in the glycosidase enzymes in the buccal epithelium and this may result in the higher water permeability of this region [70]. From the previous findings, it is clear why polyhydric alcohols and water had more uptake than the less hydrophilic solvents like oleic acid and Labrafac PG. The highest uptake was recorded in the case of PG after water due to its low molecular weight. The molecular weight effect is well shown in the cases of PEG 200 and 400. Being higher in its molecular weight, PEG 400 shows a lower uptake than PEG 200. In the case of Ethanol, the low uptake of ethanol can be attributed to the lipid extraction mode of action of ethanol as a penetration enhancer [71]. This will have a negative effect in the increase of weight of the epithelium. The lipid extraction mode of action can also be illustrated in the data obtained from both Transcutol® P and IPM. Both solvents are considered powerful fat solubilizers. This extraction mode of action leads to the negative weight difference in the buccal epithelium.

Table 3: composition (weight percent) of lipids from skin and buccal mucosa (adapted from references [6, 67-69])

Lipids	Skin	Buccal mucosa	Model
Total phospholipids	24.1	38.2	Pig epith.
Phosphatidylethanolamine	8.4[a]	14.9[a]	Pig epith.
Cholesterol sulphate	0.2	7.8	Pig epith.
Cholesterol esters	2.6	5.9	Pig epith.
Glycosylceramide	2.3	16.5	Pig epith.
Total ceramide	12.2	0.8	Pig epith.

[a] Expressed as mg/g dry tissue.

The diffusion of a solute across a membrane is chiefly governed by the thermodynamic activity of the solute in the solvent and the solvent's activity within the membrane [72]. This notion clarifies the permeability characteristics of domperidone through biological membranes. In single solvent systems, PEG 200 and PEG 400 exhibit limited diffusivity across both membranes due to their high viscosity, which hinders their passage through the membrane's intricate structure. Furthermore, the amalgamation of these solvents with back-flux water alters the solubility parameters, resulting in an elevated value and a reduction in the thermodynamic activity of the lipophilic medication within the tissue. Transcutol, which can be characterized as a hygroscopic liquid with the remarkable ability to completely mix with both polar and non-polar solvents, exhibits a unique property that facilitates its widespread application in various chemical and pharmaceutical contexts. Recognized for its efficacy as a transdermal permeation enhancer, Transcutol is distinguished by its non-toxic nature, remarkable biocompatibility with both skin and mucous membranes, and its superior capacity for solubilization, which collectively contribute to its utility in enhancing the delivery of therapeutic agents. Furthermore, it is noteworthy that Transcutol significantly amplifies the accumulation of compounds that are applied topically to the skin, while avoiding any increase in the rate of transdermal permeation, thus ensuring a targeted delivery without compromising safety [73].

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Transcutol demonstrated a significantly higher flux of domperidone when compared to both PEG 200 and PEG 400, an outcome that can be attributed to its notably reduced viscosity, which facilitates easier movement, its enhanced diffusivity that allows for a more rapid and effective distribution, and its superior solubilizing capacity, which is particularly effective for the lipids present in the buccal mucosa. The solubilizing characteristics of Transcutol with respect to buccal mucosal lipids were rigorously investigated through a detailed solvent uptake experiment, which effectively illustrated a pronounced extraction effect on the lipids found within the buccal mucosa, thereby providing substantial evidence of its efficacy in this context. However, the hygroscopic characteristics exhibited by Transcutol lead to the concurrent diffusion of water molecules from the receptor side of the biological membrane as the Transcutol compound traverses this membrane barrier, effectively altering the equilibrium of moisture on either side. This opposing flux of water molecules significantly impacts the rate at which a particular pharmaceutical agent permeates through the membrane by modifying its partitioning behavior within the membrane itself and consequently affecting its thermodynamic activity within the donor solution from which it originates [74].

The selection of Transcutol for integration into binary systems was primarily guided by its established ability to enhance the flux of pharmaceuticals in buccal delivery models, as well as to facilitate an investigation into the influence exerted by water on its hygroscopic properties, which are critical for understanding its behavior in various formulations. The synergistic interaction between Transcutol and water in these binary systems had a significant impact on the permeation of the drug domperidone through biological membranes, particularly at specific concentrations of Transcutol, which were determined to be 40% and 60% (v/v). This underscores the importance of precise formulation in drug delivery applications. The combination of Transcutol and water was served to alleviate the hygroscopic tendencies that were observed when Transcutol was utilized in isolation. These tendencies had previously resulted in water backflux and a subsequent reduction in the thermodynamic properties of domperidone present in the donor compartment, ultimately affecting the overall efficacy of the drug delivery system. Additionally, the varying concentrations of Transcutol were shown to have a profound effect on the flux of the drug across both biological membranes, a phenomenon attributed to the synergistic action of water, which conferred a lyotropic effect on the mucosal lipids while simultaneously enhancing the solubilization of Transcutol itself, thereby optimizing the drug's bioavailability [55].

The binary system, which is composed of a mixture containing 20% Transcutol and the remaining proportion of water in a volume/volume ratio, exhibited the minimal impact on the permeation of domperidone, a phenomenon that can presumably be ascribed to the limitations imposed on the solubility of domperidone when it is subjected to this particular mixture. Numerous scholarly articles and empirical studies available in the existing literature suggest that Transcutol functions effectively as a penetration enhancer when utilized at concentrations that approximate 50%, thereby enhancing the overall permeation of various pharmaceutical compounds [75, 76]. The similar behavior of different solvent systems on domperidone permeation through both models suggests that there is a similarity on lipid barrier structure in both membranes. Squier *et al*. studied the lipid content and water permeability of porcine skin and oral mucosa and he concluded that water permeability across buccal mucosa is about 10 times more than the skin [6]. Ganem *et al*. gave a comprehensive review about the lipid composition in the oral mucosa and its effect on the permeability barrier of the mucosa [7]. From these studies, it can be seen that buccal mucosa contains more phospholipids than skin,

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which is mainly in the form of phosphatidyl ethanolamine. In case of ceramides, buccal mucosa is very deficient in free ceramides if compared to the skin while it is rich in the more hydrophilic glycosylceramide due to the deficiency in the glycosidase enzymes in the buccal epithelium and this may result in the higher water permeability of this region [70]. For EpiOral™ model, lipid analysis showed that this model is rich in polar phospholipids with small amounts of glucisphingolipids and ceramide 2 [77].

5. CONCLUSIONS

A Solvent uptake experiment can be used as a valuable tool to study the effect of different solvents on the barrier membrane. The huge uptake of water and the high uptake of the polar cosolvent suggest the relative hydrophilic nature of the buccal mucosal lipids. The permeation of domperidone through the buccal mucosa of porcine and tissue culture origins showed the same rank of solvent system. More experiments with more model drugs and solvent systems are required for full establishment of correlation between the two buccal models.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1.] Rathbone, M.J., B.K. Drummond, and I.G. Tucker, The oral cavity as a site for systemic drug delivery. Advanced Drug Delivery Reviews, 1994. 13(1-2): p. 1-22.
[2.] Goodman, C.H. and C.A. Squier, Blood-Flow in the Oral-Mucosa of Normal and Atherosclerotic Rhesus-Monkeys. Journal of Oral Pathology & Medicine, 1988. 17(1): p. 34-38.
[3.] Squier, C., The permeability of oral mucosa. Critical Reviews in Oral Biology & Medicine, 1991. 2(1): p. 13-32.
[4.] Galey, W.R., H. Lonsdale, and S. Nacht, The in vitro permeability of skin and buccal mucosa to selected drugs and tritiated water. Journal of Investigative Dermatology, 1976. 67(6): p. 713-717.
[5.] Squier, C.A. and P.W. Wertz, Structure and function of the oral mucosa and *implications for drug delivery.* Drugs and the Pharmaceutical Sciences, 1996. **74**: p. 1-26.
[6.] Squier, C.A., P. Cox, and P.W. Wertz, *Lipid-Content and Water Permeability of Skin and Oral-Mucosa.* Journal of Investigative Dermatology, 1991. **96**(1): p. 123-126.
[7.] Ganem-Quintanar, A., F. Falson-Rieg, and P. Buri, *Contribution of lipid components to the permeability barrier of oral mucosa.* European journal of pharmaceutics and biopharmaceutics, 1997. **44**(2): p. 107-120.
[8.] Ceschel, G., et al., *In vitro permeation through porcine buccal mucosa of Salvia desoleana Atzei & Picci essential oil from topical formulations.* International journal of pharmaceutics, 2000. **195**(1-2): p. 171-177.
[9.] Ganem-Quintanar, A., *et al*., *Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers.* International journal of pharmaceutics, 1998. **173**(1-2): p. 203-210.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[10.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *Enhanced buccal mucosal retention and reduced buccal permeability of estradiol in the presence of padimate O and Azone®: A mechanistic study.* Journal of pharmaceutical sciences, 2005. 94(4): p. 873-882.
[11.] Lesch, C.A., et al., *The Permeability of Human Oral-Mucosa and Skin to Water.* Journal of Dental Research, 1989. 68(9): p. 1345-1349.
[12.] Nielsen, H.M. and M.R. Rassing, *TR146 Cells Grown on Filters as a Model of Human Buccal Epithelium: IV. Permeability of Water, Mannitol, Testosterone and Beta-adrenoceptor Antagonists. Comparison to Human, Monkey and Porcine Buccal Mucosa.* International Journal of Pharmaceutics, 2000. 194(2): p. 155-167.
[13.] Artusi, M., et al., *Buccal Delivery of Thiocolchicoside: In Vitro and In Vivo Permeation Studies.* International Journal of Pharmaceutics, 2003. 250(1): p. 203-213.
[14.] Chen, L.L.H., D.J. Chetty, and Y.W. Chien, *A Mechanistic Analysis to Characterize Oramucosal Permeation Properties.* International Journal of Pharmaceutics, 1999. 184(1): p. 63-72.
[15.] Devries, M.E., et al., *Localization of The Permeability Barrier Inside Porcine Buccal Mucosa - A Combined Invitro Study of Drug Permeability, Electerical-Resistance and Tissue Morphology.* International Journal of Pharmaceutics, 1991. 76(1-2): p. 25-35.
[16.] Deneer, V.H.M., et al., *Buccal Transport of Flecainide and Sotalol: Effect of a Bile Salt and Ionization State.* International Journal of Pharmaceutics, 2002. 241(1): p. 127-134.
[17.] Hansen, L.B., L.L. Christrup, and H. Bundgaard, *Enhanced Delivery of Ketobimidone Through Porcine Buccal Mucosa Invitro Via More Lipophilic esters Prodrugs.* International Journal of Pharmaceutics, 1992. 88(1-3): p. 237-242.
[18.] Hoogstraate, A.J., et al., *Effects of Bile Salts on Transport Rates and Routes of FITC-labelled Compounds Across Porcine Buccal Epithelium In Vitro.* Journal of Controlled Release, 1996. 40(3): p. 211-221.
[19.] Hoogstraate, A.J., et al., *Diffusion Rates and Transport Pathways of Fluorescein Isothyocyanate (FITC)-Labeled Model Compounds Through Buccal Epithelium.* Pharmaceutical Research, 1994. 11(1): p. 83-89.
[20.] Jasti, B.R., et al., *Permeability of Antisense Oligonucleotide Through Porcine Buccal Mucosa.* International Journal of Pharmaceutics, 2000. 208(1-2): p. 35-39.
[21.] Le Brun, P.P.H., et al., *In Vitro Penetration of Some [beta]-Adrenoreceptor Blocking Drugs Through Porcine Buccal Mucosa.* International Journal of Pharmaceutics, 1989. 49(2): p. 141-145.
[22.] Nair, M.K., et al., *Biomembrane Permeation of Nicotine: Mechanistic Studies with Porcine Mucosae and Skin.* Journal of Pharmaceutical Sciences, 1997. 86(2): p. 257-262.
[23.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *The effect of various in vitro conditions on the permeability characteristics of the buccal mucosa.* Journal of pharmaceutical sciences, 2003. 92(12): p. 2399-2410.
[24.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *Assessment of the effects of sodium dodecyl sulfate on the buccal permeability of caffeine and estradiol.* Journal of pharmaceutical sciences, 2004. 93(2): p. 431-440.
[25.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *Modification of buccal drug delivery following pretreatment with skin penetration enhancers.* Journal of pharmaceutical sciences, 2004. 93(8): p. 2054-63.
[26.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *Enhanced buccal mucosal retention and reduced buccal permeability of estradiol in the presence of padimate O and Azone (R): A mechanistic study.* Journal of pharmaceutical sciences, 2005. 94(4): p. 873-882.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[27.] Nicolazzo, J.A., B.L. Reed, and B.C. Finnin, *Enhancing the buccal mucosal uptake and retention of triamcinolone acetonide.* Journal of Controlled Release, 2005. 105(3): p. 240-248.
[28.] Senel, S., et al., *In Vitro Studies on Enhancing Effect of Sodium Glycocholate on Transbuccal Permeation of Morphine Hydrochloride.* Journal of Controlled Release, 1998. 51(2-3): p. 107-113.
[29.] Senel, S., et al., *Enhancing effect of chitosan on peptide drug delivery across buccal mucosa.* Biomaterials, 2000. 21(20): p. 2067-2071.
[30.] Shin, S.C. and J.Y. Kim, *Enhanced Permeation of Triamcinolone Acetonide Through The Buccal Mucosa.* European Journal of Pharmaceutics and Biopharmaceutics, 2000. 50(2): p. 217-220.
[31.] Shojaei, A.H., B. Berner, and X.L. Li, *Transbuccal Delivery of Acyclovir: I. In Vitro Determination of Routes of Buccal Transport.* Pharmaceutical Research, 1998. 15(8): p. 1182-1188.
[32.] Shojaei, A.H., et al., *Transbuccal Permeation of a Nucleoside Analog, Dideoxycytidine: Effects of Menthol as a Permeation Enhancer.* International Journal of Pharmaceutics, 1999. 192(2): p. 139-146.
[33.] Squier, C.A., et al., *Oral mucosal permeability and stability of transforming growth factor beta-3 in vitro.* Pharmaceutical Research, 1999. 16(10): p. 1557-1563.
[34.] Veuillez, F., et al., *Comparison of The Ex-Vivo Oral Mucosal Permeation of Tryptophan-Leucine (Trp-Leu) and Its Myristoyl Derivative.* International Journal of Pharmaceutics, 1998. 170(1): p. 85-91.
[35.] Veuillez, F., et al., *Permeation of a Myristoylated Dipeptide Across The Buccal Mucosa: Topological Distribution and Evaluation of Tissue Integrity.* International Journal of Pharmaceutics, 2002. 231(1): p. 1-9.
[36.] Xiang, J., X.L. Fang, and X.L. Li, *Transbuccal Delivery of 2 ',3 '-Dideoxycytidine: In Vitro Permeation Study and Histological Investigation.* International Journal of Pharmaceutics, 2002. 231(1): p. 57-66.
[37.] Hoogstraate, A.J. and H.E. Bodde, *Methods for Assessing the Buccal Mucosa as a Route of Drug-Delivery.* Advanced Drug Delivery Reviews, 1993. 12(1-2): p. 99-125.
[38.] Tavakolisaberi, M.R. and K.L. Audus, *Cultured Buccal Epithelium - An Invitro Model Derived from The Hamster Pouch for Studying Drug Transport and Metabolism.* Pharmaceutical Research, 1989. 6(2): p. 160-166.
[39.] Jacobsen, J., M. Pedersen, and M.R. Rassing, *TR146 Cells as a Model for Human Buccal Epithelium .2. Optimisation and Use of a Cellular Sensitivity MTS/PMS Assay.* International Journal of Pharmaceutics, 1996. 141(1-2): p. 217-225.
[40.] Jacobsen, J., et al., *TR146 Cells Grown on Filters as a Model for Human Buccal Epithelium .1. Morphology, Growth, Barrier Properties, and Permeability.* International Journal of Pharmaceutics, 1995. 125(2): p. 165-184.
[41.] Nielsen, H.M. and M.R. Rassing, *TR146 Cells Grown on Filters as a Model of Human Buccal Epithelium: III. Permeability Enhancement by Different pH Values, Different Osmolality Values, and Bile Salts.* International Journal of Pharmaceutics, 1999. 185(2): p. 215-225.
[42.] Nielsen, H.M. and M.R. Rassing, *TR146 Cells Grown on Filters as a Model of Human Buccal Epithelium: V. Enzyme Activity of The TR146 Cell Culture Model, Human Buccal Epithelium and Porcine Buccal Epithelium, and Permeability of Leu-enkephalin.* International Journal of Pharmaceutics, 2000. 200(2): p. 261-270.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[43.] Rupniak, H., et al., *Characteristics of Four New Human Cell Lines Derived From Squamous Cell Carcinomas of The Head and Neck.* Journal of the National Cancer Institute, 1985. 75(4): p. 621.
[44.] Jacobsen, J., et al., *Filter-grown TR146 Cells as an In Vitro Model of Human Buccal Epithelial Permeability.* European Journal of Oral Sciences, 1999. 107(2): p. 138-146.
[45.] Nielsen, H.M. and M.R. Rassing, *Nicotine permeability across the buccal TR146 cell culture model and porcine buccal mucosa in vitro: effect of pH and concentration.* European Journal of Pharmaceutical Sciences, 2002. 16(3): p. 151-157.
[46.] Nielsen, H.M., et al., *TR146 Cells Grown on Filters as a Model of Human Buccal Epithelium: Permeability of Fluorescein Isothiocyanate-labelled Dextrans in The Presence of Sodium Glycocholate.* Journal of Controlled Release, 1999. 60(2-3): p. 223-233.
[47.] Selvaratnam, L., et al., *Permeability Barrier Properties of Oral Keratinocyte Cultures: a Model of Intact Human Oral Mucosa.* Oral Diseases, 2001. 7(4): p. 252-258.
[48.] Walle, T., et al., *Benzo [a] pyrene-induced oral carcinogenesis and chemoprevention: studies in bioengineered human tissue.* Drug metabolism and disposition, 2006. 34(3): p. 346-350.
[49.] Delves, S.J., et al., *Development of an EpiOral (TM) in vitro human tissue model for oral irritancy testing.* Toxicology, 2008. 253(1-3): p. 12-13.
[50.] Koschier, F., V. Kostrubsky, and M.A. Gallo, *In vitro effects of ethanol and mouthrinse on permeability in an oral buccal mucosal tissue construct.* Food and Chemical Toxicology, 2011.
[51.] Hu, L., et al., *Enhanced in vitro transbuccal drug delivery of ondansetron HCl.* International journal of pharmaceutics, 2010.
[52.] Rai, V., H.S. Tan, and B. Michniak-Kohn, *Effect of surfactants and pH on naltrexone (NTX) permeation across buccal mucosa.* International journal of pharmaceutics, 2011.
[53.] Thakur, R.A., B.B. Michniak, and V.M. Meidan, *Transdermal and buccal delivery of methylxanthines through human tissue in vitro.* Drug development and industrial pharmacy, 2007. 33(5): p. 513-521.
[54.] Krevelen, D.W. and P. Hoftyzer, *Properties of polymers, their estimation and correlation with chemical structure*. 1976: Elsevier Scientific Pub. Co.
[55.] Small, D.M. and D.J. Hanahan, *The Physical Chemistry of Lipids: From Alkanes to Phospholipids*. 1986: Plenum Press New York.
[56.] Alonso, A., N.C. Meirelles, and M. Tabak, *Effect of hydration upon the fluidity of intercellular membranes of stratum corneum: an EPR study.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 1995. 1237(1): p. 6-15.
[57.] Knutson, K., et al., *Macro-and molecular physical-chemical considerations in understanding drug transport in the stratum corneum.* Journal of Controlled Release, 1985. 2: p. 67-87.
[58.] Golden, G.M., J.E. McKie, and R.O. Potts, *Role of stratum corneum lipid fluidity in transdermal drug flux.* Journal of pharmaceutical sciences, 1987. 76(1): p. 25-28.
[59.] Francoeur, M.L., G.M. Golden, and R.O. Potts, *Oleic-Acid - Its Effects on Stratum-Corneum in Relation to (Trans)Dermal Drug Delivery.* Pharmaceutical Research, 1990. 7(6): p. 621-627.
[60.] Golden, G.M., et al., *Lipid Thermotropic Transitions in Human Stratum-Corneum.* Journal of Investigative Dermatology, 1986. 86(3): p. 255-259.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[61.] Golden, G.M., et al., *Stratum corneum lipid phase transitions and water barrier properties.* Biochemistry, 1987. 26(8): p. 2382-2388.
[62.] Eliasz, A., D. Chapman, and D. Ewing, *Phospholipid phase transitions. Effects of n-alcohols, n-monocarboxylic acids, phenylalkyl alcohols and quatenary ammonium compounds.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 1976. 448(2): p. 220-233.
[63.] French, E., C. Pouton, and G. Steele, *Fluidisation of distearoylphosphatidylcholine bilayers by nonionic surfactants of single ethoxy chain length.* J. Pharm. Pharmacol, 1988. 40: p. 38P.
[64.] Hadgraft, J., K.A. Walters, and P.K. Wotton, *Facilitated transport of sodium salicylate across an artificial lipid membrane by Azone.* J Pharm Pharmacol, 1985. 37(10): p. 725-7.
[65.] Rowe, E.S., *Lipid chain length and temperature dependence of ethanol-phosphatidylcholine interactions.* Biochemistry, 1983. 22(14): p. 3299-3305.
[66.] Rowe, E.S., *Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines.* Biochimica et Biophysica Acta (BBA)-Biomembranes, 1985. 813(2): p. 321-330.
[67.] Wertz, P.W., A. Hoogstraate, and C. Squier, *Biochemical basis of the permeability barrier in skin and oral mucosa.* DRUGS AND THE PHARMACEUTICAL SCIENCES, 1996. 74: p. 27-49.
[68.] Wertz, P.W., D.C. Swartzendruber, and C.A. Squier, *Regional variation in the structure and permeability of oral mucosa and skin.* Advanced Drug Delivery Reviews, 1993. 12(1-2): p. 1-12.
[69.] Wertz, P.W., et al., *Lipids of epidermis and keratinized and non-keratinized oral epithelia.* Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1986. 83(3): p. 529-531.
[70.] Chang, F., P.W. Wertz, and C.A. Squier, *Comparison of glycosidase activities in epidermis, palatal epithelium and buccal epithelium.* Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1991. 100(1): p. 137-139.
[71.] Turunen, T.M., et al., *Effect of some penetration enhancers on epithelial membrane lipid domains: evidence from fluorescence spectroscopy studies.* Pharmaceutical Research, 1994. 11(2): p. 288-294.
[72.] Dias, M., Hadgraft, J. & Lane, M. E.. *Influence of membrane-solvent-solute interactions on solute permeation in skin.* International journal of pharmaceutics, 2007. 340(1-2): p. 65-70.
[73.] Ritschel, W., et al., *Development of an intracutaneous depot for drugs.* Skin Pharmacology and Physiology, 1991. 4(4): p. 235-245.
[74.] Ganem-Quintanar, A., et al., *Evaluation of the transepidermal permeation of diethylene glycol monoethyl ether and skin water loss.* International journal of pharmaceutics, 1997. 147(2): p. 165-171.
[75.] Mura, P., et al., *Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations.* European journal of pharmaceutical sciences, 2000. 9(4): p. 365-372.
[76.] Godwin, D.A., N.H. Kim, and L.A. Felton, *Influence of Transcutol® CG on the skin accumulation and transdermal permeation of ultraviolet absorbers.* European journal of pharmaceutics and biopharmaceutics, 2002. 53(1): p. 23-27.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[77.] Mattek, C. *EpiOral(TM) and EpiGingival(TM) data sheet*. Available from: http://www.mattek.com/pages/products/epioral.

Online ISSN: 3009-7800
Print ISSN: 3009-6324