

# Potential Mucoadhesive Dosage Form of Lidocaine Hydrochloride: II. In Vitro and In Vivo Evaluation

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**ABSTRACT** The aim of this study was to develop a controlled release buccal mucoadhesive delivery system for systemic delivery of lidocaine hydrochloride as a model drug. In vitro release and buccal permeation as well as in vivo permeation of LDHCL patches were evaluated. The drug release and the permeability of the drug through porcine buccal mucosa were evaluated using Franz diffusion cell. In vivo evaluation of patches was carried out on rabbits as an animal model.

Patches were designed in two fashions, bi-layer (BLP; LDHCL, carbopol, glycerin, penetration enhancer, and Tween 20 as the first layer; and EVA as the second layer) and triple layer (TLP; LDHCL, carbopol and glycerin as the first layer; carbopol, glycerin, penetration enhancer and pluronic F-127 as the middle layer; and EVA as the third layer) patches, respectively.

Presence of oleic acid as PE in the formulation significantly enhanced the in vitro permeability of LDHCL ( $p < 0.05$ ), while propylene glycol monolaurate as PE suppressed it ( $p < 0.05$ ). The in vivo evaluation in rabbits showed that TLP had significantly higher  $C_{max}$  and  $AUC_{0-8}$  ( $p < 0.05$ ) than BLP. Furthermore, TLP showed a well-controlled drug plasma concentration over 6 hr which was significantly longer than BLP ( $p < 0.05$ ). Patches were well adhered to buccal mucosa of the rabbits over the 8-hr study period. It was postulated that the hypothetical release mechanism of the drug and oleic acid from TLP was controlled by their diffusion through the swollen polymer network and micelled gel.

**KEYWORDS** Lidocaine hydrochloride, buccal patch, in vitro release, permeability, carbopol

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## INTRODUCTION

Transmucosal drug delivery has unique characteristics not easily obtained with other routes, like sustained release of the drug, and rapid decline in the serum concentration of the drug when the transmucosal patch is removed. Transmucosal delivery appears to have low inter-subject variability, particularly in comparison with oral controlled release formulation, and a significantly faster initiation and decline of delivery than do transdermal patches

(Swarbrick, 1996; Veuillez et al., 2001). The buccal mucosa was investigated as a potential site for local drug delivery several decades ago (Burgalassi et al., 1996; Khanna et al., 1997), but the interest on systemic transmucosal drug administration is growing fast nowadays (Shojaei et al., 2001; Bredenberg et al., 2003). Buccal route offers a series of advantages compared with other routes. The permeability of the buccal mucosa is higher than that of skin and hence, a lower loading dose in a transbuccal device could provide the same therapeutic effect as transdermal patch. The buccal mucosa is more resistant to tissue damage or irritation because of its rapid cell turn over and daily exposure to xenobiotics, such as food (Xiang et al., 2002). It is highly vascularized (Veuillez et al., 2001) providing high blood levels and rapid onset of action. In addition to its good accessibility, robustness of the epithelium, facile removal of the dosage form in case of need, possibility of elimination of the administered dosage form from the buccal area by natural clearance mechanisms. Moreover, it has relatively low enzymatic activity, and in the case of systemic delivery, this route offers the advantage of partly circumventing drug degradation in the gastrointestinal tract and of avoiding the hepatic first pass metabolism (Shojaei, 1998; Hao & Heng, 2003). It is useful for patients suffering from nausea or vomiting, or in state of unconsciousness (Swarbrick, 1996; Veuillez et al., 2001). Various bioadhesive mucosal dosage forms have been developed as tablets, gels, disks, patches, and films (Michael Rathbone, 1996). A variety of drug substances have been administered by the buccal route as calcitonin, testosterone, metoprolol tartarate, burenorphine, and oxycodone.

The major challenge for such delivery is the retention of the delivery system in the oral cavity for the desired duration. In addition, it should release the drug in a controlled and predictable manner to elicit the required therapeutic response (Shin et al., 2000). The use of excellent mucoadhesive polymers such as carbopol gives a great opportunity to meet these challenges (Lee et al., 2000; Singla et al., 2000). Carbopol 971P NF polymer is a carboxyvinyl hydrophilic polymer. This type of polymer is an excellent candidate for bioadhesion.

Buccal penetration enhancers might also be needed in order to increase the flux of the drugs through the mucosa (Gandhi & Robinson, 1992; Turunen et al., 1994; Senel et al., 2000). Buccal penetration enhancers

are capable of decreasing penetration barrier of the buccal mucosa by increasing cell membrane fluidity, extracting the structural intercellular and/or intracellular lipids, altering cellular proteins, or altering mucus structure and rheology (Veuillez et al., 2001; Hao & Heng, 2003).

Lidocaine hydrochloride [2-diethylamineoacetate-2',6'-xylidide] is a white odorless crystalline powder, with a slightly bitter taste. Lidocaine (lignocaine) is the most important amide local anesthetic. It is also used as an antiarrhythmic agent given only by intravenous route. Lidocaine provides a rapid onset of action (15–30 min). However, it undergoes extensive first-pass hepatic metabolism, where only 3% of orally administered lidocaine appear in plasma.

The aim of the present study was to develop a buccal mucoadhesive patches containing the drug lidocaine hydrochloride and oleic acid or propylene glycol monolaurate as a penetration enhancers, and to evaluate the feasibility of transbuccal delivery of drug through in vitro, and in vivo release, and permeation studies. The purpose was attained by developing a mucoadhesive patches containing oleic acid or propylene glycol as a penetration enhancers by casting process.

## MATERIALS AND METHODS

### Materials

- Pharmaceutical grade lidocaine base (QC No. 98L1G.007), lidocaine hydrochloride (QC No. 01970121), carbopol 971P (Lot CC29MAJ126), and carbopol 934 (QC No. 01001333) were kindly donated by Arab Pharmaceutical Manufacture Company, (Salt, Jordan). Bupivacaine 0.5% (Marcaine) by Astrazeneca, Sweden was kindly donated by King Abdullah Hospital, Irbid, Jordan.
- Polyethylene glycol 400 was supplied by Montplet & Estebansa (Barcelona-Spain). Acetonitrile HPLC grade, dichlormethane HPLC grade stabilized with ethanol, glycerol 99.5% reagent grade, *n*-octyl alcohol extra pure, triethyl amine acetate, sodium hydroxide, and ethanol absolute-analytical grade were supplied by Sharlau, (Barcelona-Spain).
- Ethylene vinyl acetate copolymer 40 wt. % vinyl acetate stabilized, and hexane HPLC grade (95% *n*-hexane) were supplied by Acros, New Jersey.

All chemicals were used as supplied and water used in all experiments was HPLC grade supplied by Across, New Jersey.

## Methods

### **Preparation of the Mucoadhesive Patches**

A total of 12 mL from 3 g% w/v polyethylene co-vinyl acetate (EVA) in dichloromethane was cast into plexi-glass petri dishes with a diameter of 5.0 cm (surface area 19.63 cm<sup>2</sup>) and drying at room temperature. The EVA showed low water permeability and excellent flexibility (Guo & Cooklock, 1996) comparing to others backing films previously used (Lopez et al., 1998; Jay et al., 2002; Cui & Mumper, 2002). Then the plasticized drug/polymer aqueous solution was poured onto the dried backing layer [casting weight = 10 g; total carbopol 971P solids content = 0.15 g; glycerin content based on polymer = 30% (w/w); lidocaine hydrochloride = 2 mg/cm<sup>2</sup>] and placed in the dry hot air oven at 32°C until a constant weight of the patch was obtained. The dried bilaminated films were peeled from the glass dish after drying, cut into circular shape of smaller size (diameter = 2 cm; surface area = 3.14 cm<sup>2</sup>), and stored at 20 ± 1°C in a desiccator containing saturated solution of sodium dichromate (Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) which provided an atmosphere of 52% relative humidity, for at least 2 days before testing. The thickness of each patch was measured using a micrometer at five locations (center and four corners), and the mean thickness was calculated. Samples with air bubbles, nicks or tears or having mean thickness variations of greater than 5% were excluded from analysis.

### **Lidocaine Hydrochloride Analysis in Aqueous Samples**

A Lachrome, Merck high-performance liquid chromatography (HPLC); Hitachi-Japan, equipped with Lichrospher 60 RB-select B C18 column, 15 cm length and 4.6 mm in diameter, pore size 5 µm was used for lidocaine hydrochloride quantification. The column effluent was monitored by photodiode array detector L-7455 at 220 nm. Samples were applied to the system through an autoinjector L-7200 (volume of injection = 10 µl), and processed with a computer integrating system. The mobile phase was acetonitrile-water (25:75 v/v) and triethylamine (0.8 mL/L), with pH

adjusted to 3.4 using phosphoric acid. The flow rate was 1 mL/min.

### **In Vitro Release-Dissolution Studies**

The in vitro drug release studies were conducted using Franz diffusion cells at 37 ± 1°C with a receptor compartment carrying 15.2 mL of saline phosphate buffer (SPB) of pH 7.0. The patches were placed on a wire mesh mounted between the two compartments of the diffusion cell, in such a way that the backing layer was facing the donor compartment and the adhesive film facing the receiver compartment and fastened with an O-ring. Samples of 300 µL were taken periodically through the sampling port from the receiver cell at predetermined time intervals (10, 20, 30, 45, 60, 90, 120, 180, 300, and 480 min), and replaced with an equal volume of fresh receptor solution. Samples were analyzed for drug content using HPLC. All release experiments were repeated in triplicates.

To investigate the unidirectional drug release, permeability of lidocaine hydrochloride through the backing layer was studied using the Franz cell. The medicated mucoadhesive films backed with EVA was placed between the two compartments of the diffusion cell. One sample (1 mL) was taken from the donor compartment after 24 hr of starting the experiment to be analyzed for drug content using HPLC.

### **In Vitro Permeation Studies**

For permeability studies, oleic acid or propylene glycol monolaurate were added as penetration enhancers. Six different types of patches were tested, without (standard) and with (A–E) either oleic acid or propylene glycol monolaurate, in addition to patch F, which has Tween 20, as shown in Table 1 and Fig. 1.

The difference in casting process was due to the fact that oleic acid is a hydrophobic fatty acid that requires the addition of a surface-active agent in order to be incorporated into the aqueous hydrophilic polymer solution thus Tween 20 as well as pluronic F-127 were used in a concentration of 1% w/v. Adding pluronic F-127 to medicated plasticized-carbopol solution resulted in a heterogeneous dispersion. In order to get homogenous cast dispersion, the casting process was performed in three steps to form triple layer.

**TABLE 1** Composition of Mucoadhesive Patches Loaded With Lidocaine Hydrochloride

Type of patches	First layer	Second layer	Third layer
Standard patch	EVA	0.150 g Carbopol 971P 0.045 g Glycerol 5.670 mg LDHCL	–
Patch A	EVA	0.075 g Carbopol 971P 0.023 g Glycerol 0.025 g Pluronic F-127 0.05 g Oleic acid	0.150 g Carbopol 971P 0.045 g Glycerol 5.670 mg LDHCL
Patch B	EVA	0.150 g Carbopol 971P 0.045 g Glycerol 0.050 g Tween 20 0.100 g Oleic acid 5.670 mg LDHCL	–
Patch C	EVA	0.075 g Carbopol 971P 0.023 g Glycerol 0.025 g Tween 20 0.050 g Oleic acid	0.150 g Carbopol 971P 0.045 g Glycerol 5.670 mg LDHCL
Patch D	EVA	0.075 g Carbopol 971P 0.023 g Glycerol 0.025 g Pluronic F-127 0.050 g Propylene glycol monolaurate	0.150 g Carbopol 971P 0.045 g Glycerol 5.670 mg LDHCL
Patch E	EVA	0.150 g Carbopol 971P 0.045 g Glycerol 0.050 g Tween 20 0.100 g Propylene glycol monolaurate 5.670 mg LDHCL	–
Patch F	EVA	0.150 g Carbopol 971P 0.045 g Glycerol 0.050 g Tween 20 5.670 mg LDHCL	–

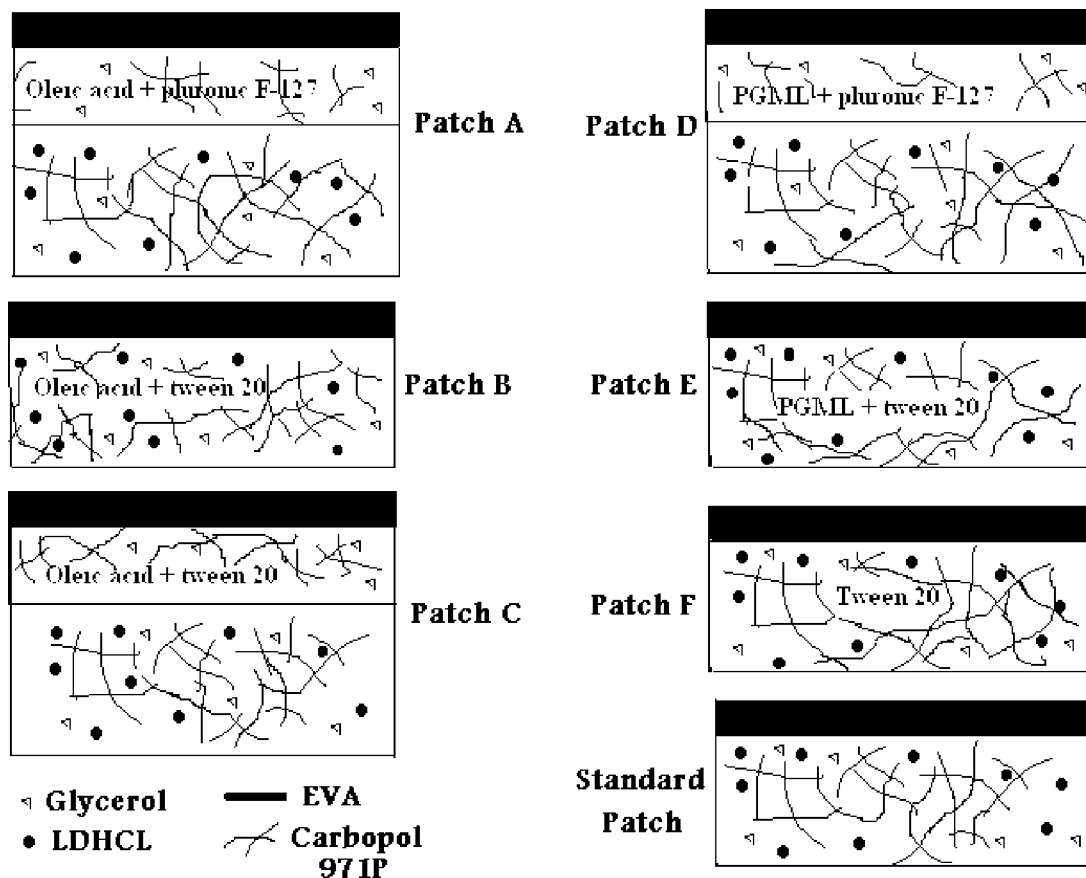
Freshly excised buccal tissue obtained from pigs weighing between 35 and 40 kg was kindly provided by a local slaughterhouse in Amman-Jordan. The tissue was taken immediately after the animal was slaughtered and stored in normal saline at 4°C to be used within 2 hr (Shojaei et al., 1998). The buccal mucosa was dermatomed with surgical scissors at a thickness of approximately 400 µm.

In vitro permeation studies were conducted at 37 ± 1°C using the Franz cells with a diffusional area of 2.84 cm<sup>2</sup>. Porcine buccal membranes were mounted between donor and receiver compartments of the diffusion cell with the epithelial side facing the donor compartment. Medicated patches were placed on the top of the porcine buccal mucosa fastened with an O-ring, in such a way that the backing layer was facing the donor chamber and the adhesive film facing the porcine epithelial mucosa. SPB of pH 7 was used as a receptor medium. Samples of 300 µL were taken from

the receptor side at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 6.5, and 8.0 hr, and replaced with the same volume of SPB. Samples were analyzed for drug content using HPLC. Permeation experiments were conducted in not less than triplicates.

For comparison studies, the permeability of a controlled solution composed of 5.67 mg of lidocaine hydrochloride in 2 mL of SPB was added to the donor cell, instead of mounting the patch between the two compartments of Franz cell.

The cumulative amount of drug permeating (Q) through a unit surface area of mucosal tissue (µg/cm<sup>2</sup>) was plotted versus time, and the flux (J) was calculated from the slope of the linear (steady-state) part of the line obtained (Deneer et al., 2002). The slope was calculated using linear regression analysis of the data. Lag time was obtained by extrapolating the steady-state lines to the time axis. The efficacy of the different enhancers was determined by comparing the flux of



**FIGURE 1** Schematic Representation of the Composition of the Prepared Multilayered Medicated Patches.

lidocaine hydrochloride (LDHCL) in the presence and absence of enhancer. It was defined as the enhancement factor (EF) which was calculated using the following equation (Stott et al., 1998):

$$EF = \frac{J_{\text{of LDHCL in the presence of enhancer}}}{J_{\text{of LDHCL in the absence of enhancer}}}$$

### ***In Vivo Permeability Study***

Patches A and B (had highest penetration rate and insignificant lag time) were selected for use in the subsequent in vivo studies. They are a circle of 1 cm in diameter containing oleic acid as enhancer and lidocaine hydrochloride and applied directly to the buccal pouch of the rabbits. Eight Poland rabbits weighing between 2.700 and 3.200 kg were slightly sedated by ether. The rabbits were divided into two groups, composed of four rabbits each. Group one has

patch A, and group two has patch B (one rabbit died to a reason unrelated to the experiment). A blood sample of 0.5 mL was collected 5 min before and then at 15, 30, 60, 120, 240, 360, and 480 min following the application of mucoadhesive buccal patches. All blood samples were collected into 1.5 mL Eppendorf tubes containing heparin sodium (100 U/mL) and were centrifuged at 3000 rpm for 10 min to separate the plasma. The retrieved plasma was transferred into a clean Eppendorf and stored at  $-20^{\circ}\text{C}$  until the time of analysis.

### ***Drug Analysis in Plasma***

Analysis of lidocaine in biological samples can be done using GC-MS, enzyme-multiplied immunoassay technique (Heusler, 1985), HPLC (Yahagi et al., 1999; Neal & Poklis, 1996; Dollo et al., 2001) and fluorescence detection (Kang et al., 1999).

Klein et al. (1994) method was adopted with slight modifications, it was simple, sensitive, and only 200  $\mu\text{L}$  plasma was needed for analysis. Assay procedure

was as follows: 200  $\mu\text{L}$  of plasma was pipetted with micro-syringe into a 15 mL conical centrifuge tube. 100  $\mu\text{L}$  of internal standard (bupivacaine 0.1 mg/mL) and 100  $\mu\text{L}$  of 2 M sodium hydroxide were added and vortex-mixed for 2 min. Then 5 mL of *n*-hexane was added and vortex-mixed for 2 min, centrifuged at 2000 rpm for 10 min. A total of 4 mL of the upper organic layer was transferred to a clean conical centrifuge tube, and 250  $\mu\text{L}$  of 0.0125 M sulfuric acid was added. Once again the mixture was vortex-mixed for 2 min, centrifuged at 2000 rpm for 10 min. The upper organic *n*-hexane layer was discarded, and 100  $\mu\text{L}$  aliquot from the acid phase was pipetted into a clean HPLC vial.

A Lachrome, Merck high-performance liquid chromatography (HPLC) equipped with Lichrospher 60 RB-select B column, 15 cm length and 4.6 mm in diameter, pore size 5  $\mu\text{m}$  was used for lidocaine hydrochloride quantification. The column effluent was monitored by photodiode array detector at 210 nm. Samples were applied to the system through an auto-injector (volume of injection = 50  $\mu\text{L}$ ), and processed with a computer integrating system. The mobile phase was acetonitrile-water (15:85 v/v) and triethylamine (0.8 mL/L), with pH adjusted to 3.4 using phosphoric acid. The flow rate was 1 mL/min.

### Data Analysis

The values of  $C_{\text{max}}$  and  $t_{\text{max}}$  were estimated directly from plasma lidocaine hydrochloride versus time profiles following buccal administration. Area under the plasma lidocaine hydrochloride concentration (AUC) versus time curve was calculated using trapezoidal rule (Shargel & Yu, 1993).

## RESULTS AND DISCUSSION

Mucoadhesive film was preferred over mucoadhesive tablet to be developed in terms of flexibility and comfort (Periolo et al., 2004). It can circumvent the relatively short residence time of oral gels on the mucosa, which is easily washed away and removed by saliva. Buccal film is able to protect the wound surface, thus reduce pain and also could treat oral diseases more effectively (Peh & Wong, 1999). Solvent evaporation method was applied rather than direct compression method. Okamoto et al. (2002) showed that solvent evaporation method was more effective at supplying lidocaine to the oral mucosa than those

prepared by direct compression of physical mixture and direct compression of the spray-dried powder.

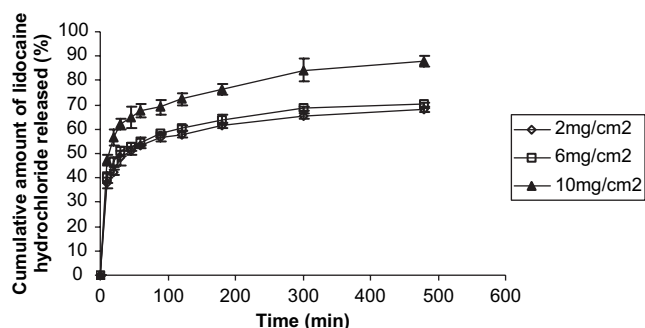
Different concentrations of carbopol 971 were prepared (4.0, 3.0, 2.0, 1.5, 1.0 % w/v) in order to screen for the optimum concentration with suitable viscosity and flowability. It was evident that high concentration of carbopol 971P gel (>1.5% w/v) prevented homogeneous mixing and good casting of the gel which was viscous.

The best condition for a fast drying, well-spread mucoadhesive layer over the backed layer was to dry the films at 32°C in hot air oven. Cast gels dried overnight at 40, and 50°C in dry hot oven resulted in fast drying with shrunk films (not evenly spread over the backing layer). Cast gels left to dry overnight in a controlled room temperature at 20°C took a long time (5 days) to get dried. Drying in a vacuum oven resulted in an uneven film thickness. Attempts to store patches in a desiccator containing silica at 20  $\pm$  1°C resulted in the separation of the mucoadhesive film and EVA. Therefore, patches were stored at 20  $\pm$  1°C and 55% RH.

### In Vitro Release

The HPLC analytical method was developed for the analysis of lidocaine hydrochloride. This method was validated through the evaluation of its performance expressed as linearity, precision, specificity and sensitivity. Calibration curve was done each time a new analysis was started. Linearity was demonstrated by the correlation coefficients ( $r^2 = 0.9999$ ) for a concentration 0.02–50 mg%. The minimum detectable level of the method was 0.01 mg% and the minimum quantitative concentration was 0.02 mg%. The selectivity of the method was approved by applying a placebo sample containing the receiving solution (saline phosphate buffer pH =7) except the drug. No peaks were observed at the retention time of the drug (2.6 min), the polymer and plasticizer did not interfere in the assay.

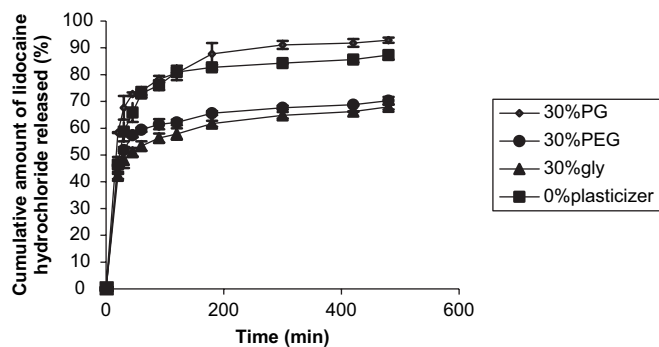
In vitro release profile showed a burst effect of the drug during the first hour, followed by a more sustained pattern. The release was completed after 24 hr. As the amount of carbopol cast and consequently the thickness increased, the release of the drug was decreased, because of increasing hindrance in diffusivity of the drug. Fig. 2 shows the in vitro release profiles for patches with various loading amounts of lidocaine hydrochloride. A greater cumulative amount of drug



**FIGURE 2** Effect of Using Different Initial Loading Amounts of Lidocaine Hydrochloride on its In Vitro Release in SPB of pH 7 at 37°C From Plasticized Carbopol 971P Bi-layered Patches ( $n = 3$ ).

released was observed from patches with higher loading of lidocaine hydrochloride. Release of lidocaine hydrochloride from patches loaded with 10 mg/cm<sup>2</sup> was significantly higher than those loaded with 2 mg/cm<sup>2</sup> ( $p = 0.008$ ) and 6 mg/cm<sup>2</sup> ( $p = 0.020$ ). While the difference in the drug cumulative amount of drug released from patches loaded with 6 and 2 mg/cm<sup>2</sup>, respectively was insignificant ( $p = 0.590$ ).

Fig. 3 shows the effect of different plasticizers addition on the release of lidocaine hydrochloride. The average cumulative amount of lidocaine hydrochloride released from nonplasticized bilayered patches loaded with 2 mg drug/cm<sup>2</sup>, after 120 min was  $80.97 \pm 0.32\%$ . This was similar to the amount of drug released from propylene glycol plasticized patches, where the cumulative amount released was  $80.62 \pm 2.70\%$  ( $p = 0.870$ ). In contrast, the cumulative amounts of lidocaine hydrochloride released from plasticized patches containing glycerol or PEG 400 were significantly lower, representing  $57.85 \pm 2.12\%$  ( $p = 0.033$ ),



**FIGURE 3** Effect of Adding 30% (w/w of Polymer Content) From Different Plasticizers to Medicated Carbopol 971P Film (2 mg/cm<sup>2</sup> Lidocaine Hydrochloride) on the In Vitro Release of Lidocaine Hydrochloride in SPB of pH 7 at 37°C ( $n = 3$ ).

and  $62.18 \pm 1.04\%$  ( $p = 0.010$ ), respectively. Previous studies showed when beta-CyD polymer was added to hydroxypropylcellulose (HPC) or polyvinylalcohol (PVA) film dosage forms, the release of lidocaine into artificial saliva (pH 5.7) was reduced by 40% of the control (Arakawa et al., 2005). Propylene glycol had no effect on the release profile of lidocaine hydrochloride, where the release of the drug was as high as the release from the nonplasticized patches. This behavior could be according to water uptake by the films, where higher hydration of the film was accompanied with faster drug release. Glycerol is a small hydrophilic compound with three hydroxyl groups, capable of hydrogen bond formation with the polymer that decreases the capability of water uptake.

The physical configurational contribution of polyethylene glycol 400 increased the potential for hydrogen bond formation because the lone pair of electrons of oxygen in the repeat unit (CH<sub>2</sub>CH<sub>2</sub>O) of polyethylene glycol served as hydrogen bond acceptors. However, films plasticized with polyethylene glycol had lower hydrogen bond formation than the one plasticized with glycerol. That could be attributed to its high molecular weight and chain length. Lower hydrogen bond formation between the polymer and the plasticizer means higher water uptake that leads to higher drug release.

Presence of a hydrophobic methyl group and only two hydroxyl groups in propylene glycol compared to the three hydroxyl groups in glycerol, and the repeated unit (CH<sub>2</sub>CH<sub>2</sub>O) of polyethylene glycol 400, could contribute to a significant lower hydrogen bond formation, and higher water uptake and consequently higher drug release.

In addition, the release of drug was not affected when carbopol 934 was added in different ratios, analogues to Qvist et al. (2002) studies, which reported that acrylic adhesives showed no difference on the release rate of the permeation enhancers from adhesive transdermal patches.

## In Vitro Permeation

The concentration of lidocaine hydrochloride in the donor compartment cell after 24 hr from starting the release of the drug at 37°C was very low and considered to be negligible (<1 mg%) assuring the unidirectional release of the drug.

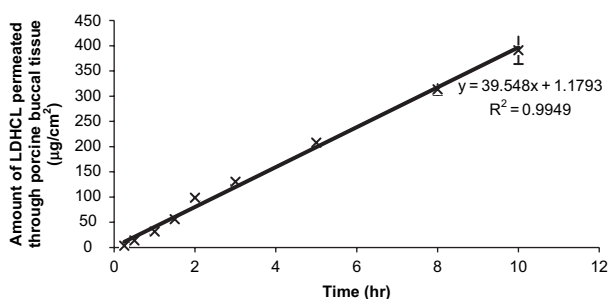
In vitro release experiment did not represent the real situation of the mucoadhesive patches, where a

biological barrier had to be present in order to simulate the *in vivo* release. Porcine buccal mucosa was found to be a good model for *in vitro* transbuccal permeation experiments (Guo & Cooklock, 1996; Senel et al., 2000; Xiang et al., 2002; Deneer et al., 2002). Porcine and human epithelia are similar in several important parameters including the anatomy and metabolism, in addition to permeability, barrier lipid composition, histology, and ultrastructure organization. A porcine mucosal tissue thickness of 400  $\mu\text{m}$  was chosen instead of the entire epithelium to avoid the high variability of permeability in the presence of connective tissue as reported by Xiang et al. (2002). The integrity of the tissue before and after starting the experiment was assured using the light microscope with a magnification of 40 $\times$ .

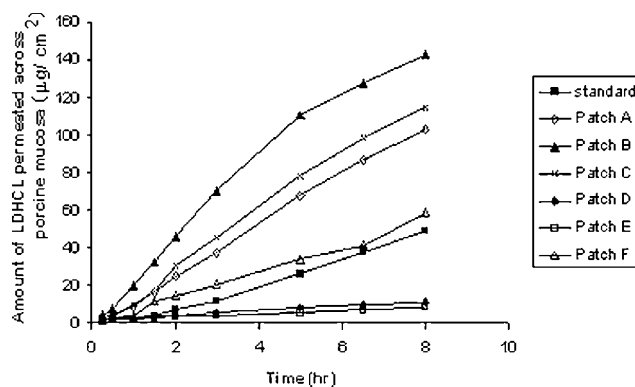
All patches significantly ( $p < 0.001$ ) prolonged the permeation of lidocaine hydrochloride through porcine buccal tissue as compared with a control solution. The overall permeation from a control solution was linear in respect to time ( $r^2 = 0.9949$ , permeability coefficient = 0.106 cm/hr), as shown in Fig. 4.

Fig. 5 shows the typical penetration rates of lidocaine hydrochloride, where the same permeation profile was obtained for all types of patches, with a progressive decrease in the lag time, which was the least when 1% w/v oleic acid was used. The lag times before the appearance of lidocaine hydrochloride in the receptor cell for the tested patches were  $< 2$  hr.

Table 2 represents the values of the steady-state fluxes and the enhancement factor for the patches studied. The patches containing oleic acid (A, B, and C) had significantly ( $p < 0.05$ ) higher values than standard ones, while the values for the patches containing propylene glycol monolaurate (D and E) were significantly ( $p < 0.05$ ) lower. It is worth noting that the



**FIGURE 4** In Vitro Permeability of Lidocaine Hydrochloride Across Porcine Buccal Tissue From a Control Solution of 2.84 mg Drug per mL in SBP of pH 7 at 37°C.



**FIGURE 5** Effect of Penetration Enhancers on the In Vitro Permeability of Lidocaine Hydrochloride Through Porcine Buccal Tissue in SPB at 37°C. (Total Drug Content = 5.67 mg, Area of the Patch = 2.84 cm<sup>2</sup>, Patch A, B, & C: Oleic Acid Effect; Patch D & E: Propylene Glycol Monolaurate Effect; Patch F: Tween 20 Effect)

**TABLE 2** Parameters for the *in vitro* permeability of lidocaine hydrochloride through porcine buccal tissue in SPB of pH 7 at 37°C (n = 3)

Type of patch	Steady state flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Enhancement Factor
Standard	7.04 $\pm$ 0.19	Reference = 1
Type A	13.33 $\pm$ 0.35	1.89
Type B	20.74 $\pm$ 0.12	2.95
Type C	15.23 $\pm$ 0.68	2.16
Type D	1.24 $\pm$ 0.04	0.18
Type E	0.77 $\pm$ 0.06	0.11
Type F	7.07 $\pm$ 0.33	1.00

fluxes of drug increased in the presence of oleic acid and decreased in the presence of propylene glycol monolaurate. Previous studies showed that oleic acid has been shown to disrupt strongly the polar head group and the hydrophobic region of the membrane lipids in a concentration dependent and time dependent manner in the deep bi-layer region (Turunen et al., 1994) and effective in promoting the absorption of lidocaine hydrochloride through porcine oral mucosa (Ganem-Quintanar et al., 1998). Unsaturated acids are usually more disruptive than their saturated counterparts having the same carbon number (Veuillez et al., 2001).

While propylene glycol monolaurate is a nonionic surfactant with HLB value 4.5, previous studies showed that the maximal effect for nonionic surfactants on buccal absorption was for nonionic surfactants with HLB range between 8 and 14 (Aungst & Rogers, 1989). Similar suppression was observed with



thiocolchicoside permeation across buccal tissue when certain penetration enhancers were added (Artusi et al., 2003).

Considering patches B and C, patch B showed significantly ( $p = 0.0005$ ) higher flux than patch C. With no doubt, the differences in the values are attributed to the way of casting process that led to deeper packing of oleic acid and Tween 20 in type (C), and drug had to diffuse through the swollen carbopol network toward the mucosa. Deep packing of oleic acid (as a middle layer) increased its diffusion length. Longer diffusion length led to late availability of the penetration enhancer, and consequently, gave lower drug permeation. Moreover, the amount of carbopol in type (C) was 0.225 mg, comparing to 0.15 mg in type (B), leading to larger thickness of diffusion layer, that retard the diffusion of the drug and the penetration enhancer.

It is clear that pluronic F-127 played a crucial role over Tween 20 when compared to patches A–C. The steady-state flux of patch C was higher than that of patch A. Pluronic F-127 and Tween 20 probably formed micelles and controlled the release of oleic acid that consequently controlled the permeability of lidocaine hydrochloride across the mucosa. However, at physiological temperatures, pluronic F-127 could play an additional effect in retarding the permeability of the drug. Because it is a thermogelling block polymer, it converted the low viscous solution into high viscous one (Kabanov et al., 2002, 2003). This viscosity might retard the diffusion of both the drug and oleic acid through the patch.

Double layered (patch B) showed a significant ( $p = 0.0001$ ) higher flux than the triple layered (patch A), also because of the way of casting, amount of carbopol and presence of pluronic.

The steady-state fluxes for type D patches were higher than those of patches E, but this difference did not reach significance level ( $p = 0.1038$ ).

Oleic acid was the major compound responsible for penetration enhancement, and not Tween 20. The steady-state flux of patch F was almost the same as the standard patch, with no significant difference. Moreover, the steady-state flux of the drug for patches B and C were significantly higher than that for patch F ( $p < 0.05$ ).

Preliminary stability study of Lidocaine hydrochloride patch was studied. Patches stored at 20°C and 55% RH for 3 months did not show any physical

changes. General appearance was the same, where transparent, elastic, and clear patches were observed, with good binding between EVA film and carbopol film. Lidocaine hydrochloride permeation through porcine buccal tissue was evaluated, where no significant difference in the release profile and flux value was noticed.

## In Vivo Permeability

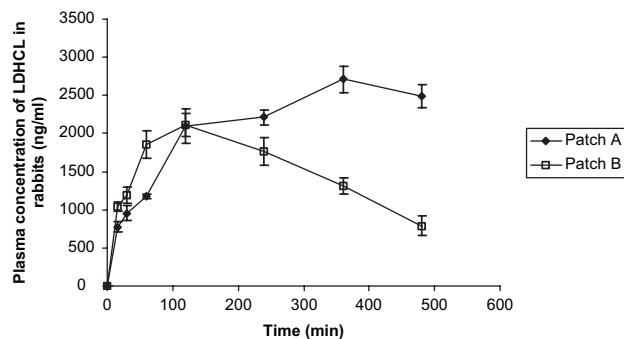
The chromatogram obtained from a blank rabbit plasma and standard sample after buccal administration of lidocaine hydrochloride patches shows sharp peaks with complete baseline separation between peaks. The retention times of lidocaine hydrochloride and bupivacaine were 6.5 and 17.5 min, respectively. The standard curves in rabbit plasma were linear in the range 60–2000 ng/ml ( $r^2 = 0.9819$ ).

On the basis of preliminary in vivo studies, a dose of 2 mg/kg of lidocaine hydrochloride was selected (Katzung, 1998).

Fig. 6 represents the mean plasma lidocaine hydrochloride levels after buccal administration of type A and B patches. Table 3 represents the pharmacokinetic parameters for the drug in the rabbits after administration of each type.

All rabbits were observed to have remained intact with the mucoadhesive layer and EVA backing layer still bonded to each other. There was no discernible redness or irritation around the treated buccal tissue after 8 hr.

Type (A) exhibited good controlled and delayed release pattern of lidocaine hydrochloride. The concentration of the drug released was maintained well



**FIGURE 6** Mean Plasma Lidocaine Hydrochloride Levels After Buccal Administration of Type (A & B) Patches on the Buccal Pouches of the Rabbits (Area of the Patch = 0.79 cm<sup>2</sup>, Total Drug Loading = 4.71 mg).

**TABLE 3** Plasma concentration and pharmacokinetic parameters of lidocaine hydrochlorides in the rabbits after buccal administration of type A (n=4) and type B (n=3) patches. (Total drug content = 6 mg/cm<sup>2</sup>, area of the patch = 0.78 cm<sup>2</sup>, values are presented as mean ± standard deviations)

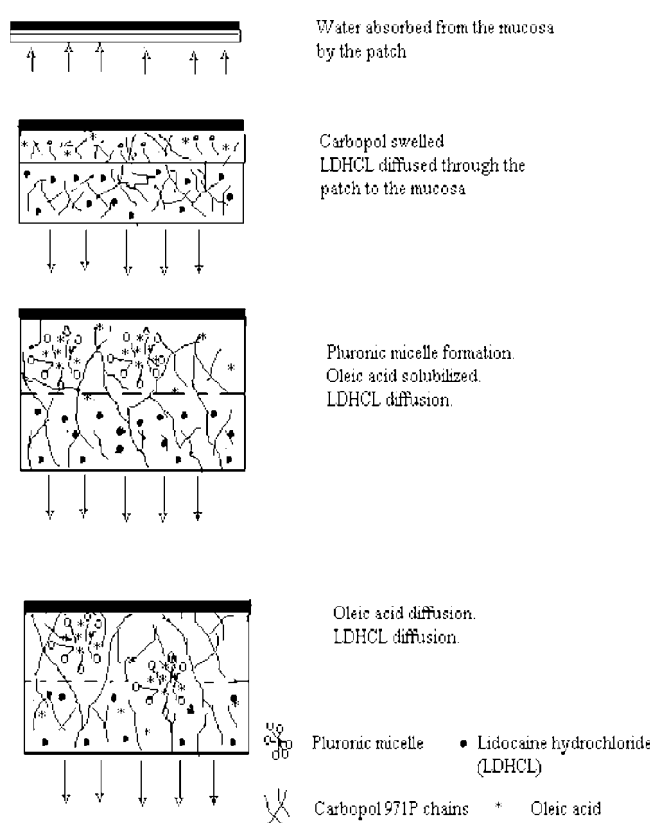
Time (min)	Patch A (ng/ml)	Patch B (ng/ml)	Test of significance (P)
15	776.44 ± 67.32	1037.71 ± 64.22	0.004402
30	954.64 ± 96.56	1189.9 ± 108.24	0.039270
60	1180.88 ± 32.02	1857.17 ± 185.15	0.022070
120	2095.18 ± 227.38	2111.60 ± 151.58	0.913300
240	2212.85 ± 96.29	1765.57 ± 181.56	0.039560
360	2709.70 ± 167.8	1315.17 ± 109.36	0.000100
480	2489.87 ± 153.77	791.34 ± 129.17	0.000153
<b>C<sub>max</sub> (ng/ml)</b>	2709.70 ± 167.80	2111.6 ± 151.58	0.005103
<b>T<sub>max</sub> (hrs)</b>	6	2	–
<b>AUC<sub>0-8</sub> (µg.min/ml)</b>	1024.53 ± 31.21	733.12 ± 20.11	0.000429
<b>AUC<sub>0-1</sub> (µg.min/ml)</b>	149.12 ± 9.46	189.25 ± 15.37	0.026000

above its therapeutic level (2 µg/mL; Katzung, 1998) over a period of 6 hr. Concentration of drug released was in the range of 776.44–2709.70 ng/mL, the extent of absorption of lidocaine hydrochloride through the buccal pouch in rabbits ( $n = 4$ ) was 1024.50 ± 31.21 µg.min/mL. The plasma level concentrations of the drug for the first hour after administering patch B were significantly higher than that after administering type A. The AUC<sub>0-1</sub> for type B was significantly higher than that of type A, while the AUC<sub>0-8</sub> for type A was significantly higher than type B. This higher initial plasma concentration for type B patch might be attributed to the higher initial availability of oleic acid at the site of penetration as previously described (smaller thickness of diffusion layer, longer diffusion length, and absence of gel depot of pluronic F-127 micelles).

The sustained release of the in vitro permeability for type (B) was not attained by the in vivo studies that might be attributed to side diffusion of the drug that leads to loss of the drug by enzymatic degradation (first pass effect) through the gastrointestinal tract. This side diffusion was not noticeable in type (A), again because of the presence of a condensed network depot.

This result showed that it is feasible to control the release of drug substances from hydrogel by controlling its diffusion through the swollen polymer network and micelled gel. Furthermore, micelles controlled the release of oleic acid, leading to a concentration gradient of oleic acid across the patch that prolonged its action on mucosa and consequently controlled the permeability of the drug for longer time.

In general, as shown in Fig. 7, partitioning of oleic acid from pluronic micelles, and diffusion of the drug



**FIGURE 7** Theoretical Mechanism of Drug Release From Type A Mucoadhesive Patches.

and oleic acid through the swollen polymer controlled the release and the permeability of the drug across the buccal mucosa. Analogues to the mechanism of lidocaine release from both of the hydroxypropyl cellulose (HPC) and hydroxypropyl methyl cellulose (HPMC) films that assumed predominantly diffusion of the drug through the polymer matrices (Repka et al., 2005).

## CONCLUSION

In vitro permeability study through porcine tissue showed that diffusion of LDHCL from patches could be affected by adding a penetration enhancer like oleic acid or propylene glycol monolaurate. Presence of oleic acid in the formulation significantly enhanced the permeability of LDHCL ( $p < 0.05$ ), while propylene glycol monolaurate suppressed it ( $p < 0.05$ ). Steady-state flux of the drug from BLP was found to be significantly ( $p < 0.05$ ) higher than that of TLP. Oleic acid was the main factor for drug permeability enhancement and significantly more efficient than Tween 20 ( $p < 0.05$ ), particularly in BLP because it came in a high concentration in a direct contact with the buccal mucosal membrane.

In vivo evaluation on rabbits showed that patch A exhibited good mucoadhesion with good controlled release pattern. It was composed of TLP (LDHCL, carbopol and glycerin as the first layer; carbopol, glycerin, oleic acid, and pluronic F-127 as the middle layer; and EVA as the third layer). It had significantly higher  $C_{max}$  and  $AUC_{0-8}$  ( $p < 0.05$ ) than BLP (LDHCL, carbopol, glycerin, oleic acid and Tween 20 as the first layer; and EVA as the second layer). Patches were well adhered to buccal mucosa of the rabbits over the eight hours study period.

These results were noteworthy for several reasons. First, lidocaine hydrochloride was maintained in the plasma for at least 6 hours and has a plasma concentration above the therapeutic level ( $2 \mu\text{g/mL}$ ; Katzung, 1998), therefore it can be effective for overnight therapy. It can be given over a longer period of time thus increasing efficacy compliance and better clinical usefulness. Second, the overall reported bioavailability ( $AUC_{0-6}$ ) after using double phased mucoadhesive suppositories of lidocaine (50 mg) in rabbits was around  $1.5 \mu\text{g}\cdot\text{hr/mL}$  (Yahagi et al., 1999), and after applying 1 g of local anesthetic cream (EMLA) on pigs, that contains 25 mg lidocaine was  $126.39 \text{ ng}\cdot\text{hr/mL}$  (Klein et al., 1994).

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