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Transdermal and transbuccal drug delivery systems: Enhancement using iontophoretic and chemical approaches

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ABSTRACT

We investigated the enhancement effect of chemical enhancers and iontophoresis on the *in vitro* transdermal and transbuccal delivery of lidocaine HCl (LHCl), nicotine hydrogen tartrate (NHT), and diltiazem HCl (DHCl) using porcine skin and buccal tissues. Dodecyl 2-(*N*,*N*-dimethylamino) propionate (DDAIP), dodecyl-2-(*N*,*N*-dimethylamino) propionate hydrochloride (DDAIP HCl), N-(4-bromobenzoyl)-*S*,*S*-dimethyliminosulfurane (Br-iminosulfurane), and azone (laurocapram) were used as chemical enhancers. The study results showed that the application of iontophoresis at either 0.1 mA or 0.3 mA significantly enhanced transdermal and transmucosal delivery of LHCl, NHT and DHCl. It was also demonstrated that iontophoresis had a more pronounced enhancement effect on transdermal delivery than on transbuccal delivery of LHCl, NHT and DHCl. In addition, DDAIP HCl was found to be the most effective enhancer for transbuccal delivery of LHCl and NHT.

1. Introduction

Human skin provides a significant surface area as well as various locations for drug absorption. Its uppermost layer – stratum corneum (SC) is the major barrier preventing compounds from inward and outward diffusion through skin. The SC is mainly composed of keratinized cells (corneocytes) embedded in a lamellar lipid-rich interstitium. These lipid arrangements are crucial for establishing the barrier properties of SC and maintain cohesion between corneocytes. These lipids contain a mixture of roughly 27% cholesterol, 10% cholesteryl esters, 41% ceramides and 9% fatty acids (Suhonen et al., 1999) These are the main lipid components of SC. Polar lipids: glucosylceramides and cholesterol sulfate are the minor lipid components of SC. The existence of lipid bilayer of SC greatly reduces skin permeability (Bouwstra et al., 2000; Brod, 1991; Coderch et al., 2003; Wertz and van den Bergh, 1998).

Buccal mucosa refers to the mucous membrane tissues located on the inside of the cheek area. It contains non-keratinized stratified squamous epithelium, basement membrane, lamina propria and the submucosa (Gandhi, 1988). The epithelium has about 40–50 cell layers with a thickness of about 200–400 μm (Smart, 1993). The buccal mucosa was believed to have about 4–4000 times higher permeability than the skin (Galey et al., 1976; Squier et al., 1976). The intercellular lipids within the epithelium form a highly organized gel phase membrane structure to provide the major physical barrier for the buccal mucosa (Garza et al., 1998; Squier et al., 1991). The superficial barrier region within buccal epithelium are enriched with phospholipids, cholesterol and glycolipids (Garza et al., 1998; Wertz, 1983). Ceramides, cholesterol and saturated fatty acids are the major components of the intercellular lamellae of buccal epithelium. Its lipid mixture is similar to that of the intercellular lamellae of SC (Wertz, 1983). Human and pig skin and buccal mucosa have similar lipid contents, permeability barrier properties and membrane morphology (Collins et al., 1981; Lesch and Squier, 1989; Wertz and Squier, 1991; Wertz, 1996). Lesch and Squier (1989) demonstrated that human skin and buccal tissues had similar permeability constants for tritium-labeled water. This further validated pig as an effective permeation study model. Porcine skin and mucosa were much easier to be harvested than human skin and mucosa. Therefore, porcine skin and buccal mucosa were used to replace human skin and buccal mucosa in this study.

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Transdermal drug delivery is a method of delivering drug systematically by applying a drug formulation on top of intact skin. The use of plasters and poultices hundreds of years ago was the first application of this route of drug administration (Scheindlin, 2004). Transdermal delivery has some advantages over other conventional routes of drug delivery such as oral, parenteral or pulmonary. Transdermal systems are non-invasive, self-controllable and can provide sustained plasma concentration profiles for long periods of time. The systems can also greatly improve patient compliance through avoiding first-past metabolism, improved bioavailability and dosing schedule, and reduction of systemic side effects. In the past three decades, transdermal drug delivery has advanced to the stage where transdermal systems are becoming a feasible way of delivering clinically effective drugs (Segal, 1991). However, there are also some challenges facing this route of drug delivery such as local irritation, erythema, itching, and local edema at the site of application (Naik et al., 2000; Prausnitz et al., 2004; Scheindlin, 2004; Thomas and Finnin, 2004). So far only a limited number of small molecules (Da <500) can be delivered transdermally (Naik et al., 2000). Similar to skin, the buccal route is also an attractive alternative for non invasive systemic delivery of drugs. Compared to oral drug delivery, transbuccal drug delivery provides great advantages such as a much milder environment for drug absorption, elimination of first-past metabolism, rapid drug uptake, and improved patient compliance. However, there are some disadvantages associated with this route of drug delivery such as its small surface area for drug application and dilution of drug concentrations due to saliva secretion.

Various physical and chemical enhancement methods have been investigated to a great extent for enhancing transdermal and transbuccal delivery of hydrophilic and hydrophobic drugs in the past 30 years. Typical physical enhancement methods are iontophoresis, electroporation, sonophoresis, microneedles, etc. (Naik et al., 2000). Chemical enhancement methods refer to the application of various chemical enhancers such as organic solvents surfactants, etc. (Suhonen et al., 1999). The goal of this study was to develop enhanced transdermal and transbuccal delivery systems for hydrophilic drugs: LHCl, NHT and DHCl using iontophoresis, chemical enhancers and a combination of both enhancement techniques.

LHCl has a primary indication of anesthetic used as an antiarrhythmic drug. There are several pharmaceutical dosage forms of LHCl on the market. Lidocaine hydrochloride injection, USP is a sterile solution administered intravenously by either direct injection or continuous infusion (http://www.drugs.com/pro/lidocaine.html). LIDODERM® (lidocaine patch 5%) was approved for relieving the pain of post-herpetic neuralgia (http://www.lidoderm.com/). Iontophoresis and chemical enhancers have been utilized to enhance both topical and transdermal delivery of lidocaine. Sintov and Brandys-Sitton (2006) used a short-term (10 min) iontophoresis (1.13 mA/cm^2) plus a special microemulsion formulation to enhance transdermal delivery of lidocaine. Their *in vitro* and *in vivo* studies showed that this unique combination delivery system not only shortened lag times but significantly increased flux when compared to control – an aqueous lidocaine solution only. Lee et al., 2006 developed *in vitro* transdermal delivery systems for lidocaine using different chemical enhancers via pig and human skin. Their results demonstrated that the binary chemical enhancer system: isopropyl myristate (IPM) plus *n*-methyl pyrrolidone (NMP) enhanced flux of lidocaine by 4-fold and 25-fold over NMP alone and over IPM alone ($p < 0.001$), respectively. In 2004, Vyteris, NJ gained FDA's approval of its LidoSite™ System (www.vyteris.com/Our_Products/Lidosite.php) which delivers lidocaine and epinephrine simultaneously using iontophoresis to achieve dermal analgesia effect.

Nicotine is a well known drug for the treatment of nicotine withdrawal following cessation of smoking. Smoking cessation products include different dosage forms of nicotine such as transdermal patches, chewing gum, sublingual tablets, nasal spray and oral inhalers (Cheng et al., 2002). Nicorette patch (3.6 cm × 4.9 cm) (http://www.nicorette.co.uk/stop-smoking/products/patch.aspx) is a typical transdermal system that delivers 10–15 mg of nicotine per day. Chewing gum, sublingual tablets and (oral mucosal) inhalers can deliver nicotine into blood stream as fast as within 30 min (Cheng et al., 2002). These nicotine products were proven to be effective in aiding smoking cessation. However, none of these nicotine products is able to gain rapid uptake of nicotine at a blood level with similar pharmacological effect of cigarette smoking. Thus, there is a significant need for improved nicotine replacement formulations or delivery systems. Iontophoresis and chemical enhancer pretreatment combination approaches are well fitted in this case to meet the need. Conaghey et al. (1998) studied an enhanced *in vitro* nicotine gel delivery system at a nicotine concentration range of 7.8–39.5 mg/ml using iontophoresis (0.5 mA/cm^2) via human skin tissues. The nicotine release rate was found to show a plateau when the nicotine concentration reached to 20 mg/ml. Nolan et al. (2007), reported that the combined treatments of oleic acid and iontophoresis provided synergistic enhancement effect on the *in vitro* transport of nicotine across murine skin, and oleic acid even increased the post-iontophoretic nicotine permeation (Nolan et al., 2007).

DHCl is a calcium ion influx inhibitor used for the treatment of hypertension. Nolan and Corish (Nolan et al., 2007) investigated the combined enhancement effect of iontophoretic and chemical enhancers on transdermal delivery of DHCl. Their data suggested that the use of iontophoresis increased skin permeability, thus the diffusion of DHCl into skin; the incorporation of oleic acid into the drug formulation enhanced the iontophoretic transport of DHCl by 2.6-fold ($P \leq 0.02$), indicating that oleic acid enhanced the engagement of the drug in the conductive process of iontophoresis application.

In this work, the effects of iontophoresis, chemical enhancers and their combined treatments on transdermal and transbuccal delivery of LHCl, NHT and DHCl were investigated. Chemical enhancers used were DDAIP and DDAIP HCl, and Br-iminosulfurane. DDAIP, DDAIP HCl and Br-iminosulfurane at ≤5% were previously proved to be low toxic and biodegradable (Pfister et al., 2006; Song et al., 2005). They were also reported to enhance transdermal or transbuccal delivery of alprostadil, ketoprofen, ondansetron, miconazole, indomethacin, clonidine and hydrocortisone (Büyüktimkin et al., 1993; Fujii et al., 2002; Hu et al., 2011; Song et al., 2005). A popular enhancer – 1-dodecylazacycloheptan-2-one (azone, laurocapram) was used as a control. However, the enhancement effects of these enhancers on transdermal and transbuccal drug delivery have not been compared. Also, no comparison was made between transdermal and transbuccal drug delivery using iontophoresis or the combined treatment of chemical enhancers and iontophoresis.

2. Materials and methods

2.1. Materials

NexMed (U.S.A.), Inc. (San Diego, CA, USA) supplied DDAIP and DDAIP HCl. Azone and Br-iminosulfurane were made in New Jersey Center for Biomaterials, Rutgers-The State University of New Jersey (Piscataway, NJ, USA). Silver wire, citric acid, LHCl, propylene glycol (PG) and NHT were obtained from Sigma–Aldrich (Saint Louis, MO, USA). Polymed, Inc. (Houston, TX, USA) supplied DHCl dihydrate. MP Biomedicals (LLC, Solon, OH, USA) supplied phosphate buffer saline tablets. Barton's Farms and Biologicals (Great Meadows, NJ, USA) was the original source of porcine buccal tissue. Porcine skin

Table 1
Lidocaine HCl, nicotine hydrogen tartrate and diltiazem HCl gel formulations.

Ingredients	Formulations (%, w/w)		
	2.5% lidocaine HCl gel	2% nicotine hydrogen tartrate gel	2% diltiazem HCl gel
Lidocaine HCl	2.5		
Nicotine hydrogen tartrate		2.0	
Diltiazem HCl			2.0
Cellulose gum	2.0	2.0	1.0
Water	95.5	96.0	97.0
pH	6.0	4.0	6.0
Viscosity (cps)	9000	9200	800

tissue was harvested from University of Medicine and Dentistry, Newark, NJ, USA. TIC Gums (Belcamp, MD, USA) supplied cellulose gum (CMC 200 SF, Food and Pharmaceutical Grade with molecular weight of about 25,000 and viscosity of 20,000–30,000 cps at 2% (w/w) in water).

2.2. Lidocaine HCl, nicotine hydrogen tartrate and diltiazem HCl gel formulations

Cellulose gum was dispersed in water first, then right amount of LHCl or NHT or DHCl was added and mixed well using lightning mixer until uniform to obtain LHCl, NHT and DHCl gel formulations, respectively (Table 1).

2.3. Skin and buccal tissue preparation

Porcine skin with a thickness of about 500–600 μm obtained from young Yorkshire pigs (3–4 months old; 25–30 kg) was prepared using Padgett® Model B Electric Dermatome (Integra LifeSciences, Plainsboro, NJ). The dermatomed skin was then cut into a size of 1.0 cm^2 and stored at −80 °C no more than 3 months prior to use. In the beginning of a permeation experiment, at room temperature the skin was defrosted first and then soaked in phosphate buffer saline (PBS) solution for 1 h.

Buccal mucosa samples were harvested from pig's cheek area and placed below −30 °C. The tissues samples were defrosted at room temperature first before use. Then a scalpel blade and a surgical scissor were used to remove the underlying connective tissue and trim the buccal mucosa to about 300–400 μm in thickness. Before each experiment the buccal tissues were submerged in PBS (pH = 7.5) for 1 h.

2.4. Preparation of anodal and cathodal electrodes

Anodal electrodes (Ag) were prepared using pure silver (Ag) wire (0.5 mm in diameter). Cathodal electrodes (AgCl) were made by connecting AgCl powder coated Ag wires and pure Ag wires

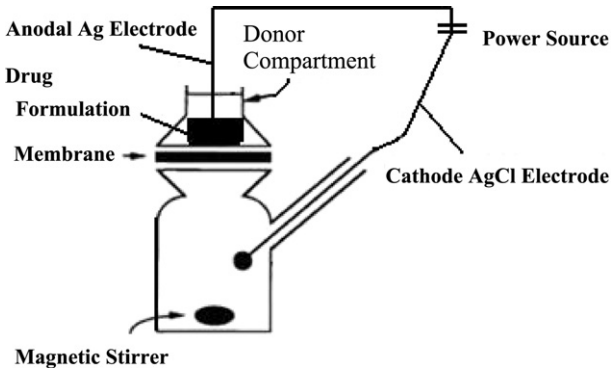

Fig. 1. Iontophoretic experimental design for *in vitro* transdermal and transbuccal drug delivery.

partially dipped in 0.1 N HCl solution to a power source of 3 mA for 12 h.

2.5. Enhancer solution preparation

5% (w/v) DDAIP, 5% Br-iminosulfurane and 2% (w/v) azone enhancer solutions were prepared using PG as the vehicle. 5% (w/w) DDAIP HCl in PG and water solutions were prepared using water and PG as separate vehicles.

2.6. In vitro transdermal and transbuccal permeation study

In vitro transdermal and transbuccal drug permeation experiments were conducted using Franz diffusion cells (PermeGear, PA, USA) using porcine skin and buccal tissues. The following experiments were performed: passive (control) permeation, permeation with 1.0 h enhancer pretreatment, permeation with 8.0 h iontophoresis (0.1 or 0.3 mA) treatment, and permeation with 1.0 h enhancer pretreatment plus 8.0 h iontophoresis (0.3 mA) treatment. At 37 °C, the duration for all experiments was 8 h.

For the passive *in vitro* permeation study, PBS (pH = 7.5) solution was added into Franz cell receptor compartment and stirred at 600 rpm. The skin or buccal tissue was sandwiched between donor and receptor compartments with the side of epidermal or connective tissue attached to the receptor compartment. The available diffusion area was 0.64 cm^2. 0.3 ml of each tested gel formulation was added into the donor compartment at the start of each experiment. At each time points (0.0, 0.5, 1.0, 3.0, 5.0, or 8.0 h), 300 μl samples were taken from the receptor compartment for HPLC sample analysis and then quickly filled with an exact amount of 300 μl PBS (pH = 7.5) (Diaz del Consuelo et al., 2005; Jacobsen, 2001).

For permeation study with enhancer pretreatment, the skin or buccal tissue was treated first for 1 h by adding 30 μl of

Table 2
HPLC methods for analysis of lidocaine HCl, nicotine hydrogen tartrate and diltiazem HCl.

Drug	HPLC column	HPLC conditions	Mobile Phase
Lidocaine HCl	Waters column Nova-Pak C18 column 4 μm 3.9 mm × 300 mm	Flow rate: 1.5 ml/min Column temperature: 25 °C UV wavelength: 254 nm Injection volume: 15 μl	35 ml glacial acetic acid (99%) 930 ml deionized water; adjusted pH = 3.4 using 1 N NaOH solution; 4 volume of the above solution plus 1 volume of acetonitrile
Nicotine hydrogen tartrate	Phenomenex column 150 × 4.6 mm C18 (2) 100 A Luna 5 μm	Flow rate: 1.4 ml/min Column temperature: 25 °C UV wavelength: 256 nm	5 Phosphate buffer saline (PBS) tablets; 1000 ml water; 7.5 mL triethylamine adjusted pH = 6.8 using glacial acetic acid (99%); 500 ml methanol
Diltiazem HCl	Phenomenex column 150 × 4.6 mm C18 (2) 100 A Luna 5 μm Phenyl-hexyl	Flow rate: 1.0 ml/min Column temperature 25 °C UV wavelength: 310 nm Injection volume: 20 μl	Glacial acetic acid aqueous solution (pH = 3.0): methanol = 1:4; triethylamine to adjust pH to 6.8.

Table 3
Effect of 8 h iontophoresis treatment on transdermal and transbuccal delivery of lidocaine HCl.[a]

Treatment (mA)	Transdermal		Transbuccal	
	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)
Control	7.4 ± 5.8	59.7 ± 43.4	44.7 ± 9.6	345.6 ± 74.3
0.1	61.7 ± 20.8[b]	494.0 ± 152.0[b]	137.1 ± 13.1[b,d]	1085.2 ± 92.1[b,d]
0.3	375.6 ± 69.44[c]	2879.2 ± 531.1[c]	241.7 ± 60.5[c]	1910.2 ± 454.7[c]

Control – untreated passive; 0.1 mA ≈ 0.16 mA/cm²; 0.3 mA ≈ 0.47 mA/cm². Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Statistically significantly higher than control ($p < 0.05$).
[c] Statistically significantly higher than 0.1 mA and the control ($p < 0.05$).
[d] Transbuccal delivery is significantly higher than transdermal delivery at 0.1 mA ($p < 0.05$).

Table 4
Effect of 8 h iontophoresis treatment on transdermal and transbuccal delivery of nicotine hydrogen tartrate.[a]

Treatment (mA)	Transdermal		Transbuccal	
	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)
Control	1.3 ± 1.9	9.9 ± 14.6	0.9 ± 0.4	6.9 ± 2.6
0.1	56.1 ± 11.4[b,c]	433.2 ± 85.4[b,c]	17.6 ± 6.9[b]	141.5 ± 58.6[b]
0.3	138.4 ± 72.3[d]	1326.6 ± 186.2[d]	81.7 ± 35.9[d]	629.5 ± 276.8[d]

Control – untreated passive; 0.1 mA ≈ 0.16 mA/cm²; 0.3 mA ≈ 0.47 mA/cm². Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Statistically significantly higher than control ($p < 0.05$).
[c] Transdermal delivery is significantly higher than transbuccal delivery at 0.1 mA ($p < 0.05$).
[d] Statistically significantly higher than 0.1 mA and the control ($p < 0.05$).

Table 5
Effect of 8 h iontophoresis treatment on transdermal and transbuccal delivery of diltiazem HCl.[a]

Treatment (mA)	Transdermal		Transbuccal	
	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)	Flux ($\mu g/cm^2$ h)	Q_8 ($\mu g/cm^2$)
Control	0.4 ± 0.3	3.0 ± 2.6	32.6 ± 9.5	258.3 ± 73.6
0.1	18.9 ± 10.4[b]	154.1 ± ±83.5[b]	54.5 ± 2.6[d]	430.0 ± 18.7[b,d]
0.3	100.3 ± 33.7[c]	796.8 ± 276.6[c]	80.7 ± 18.0[b]	650.9 ± 139.1[b]

Control – untreated passive; 0.1 mA ≈ 0.16 mA/cm²; 0.3 mA ≈ 0.47 mA/cm². Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Statistically significantly higher than control ($p < 0.05$).
[c] Statistically significantly higher than 0.1 mA and the control ($p < 0.05$).
[d] Transbuccal delivery is significantly higher than transdermal delivery at 0.1 mA ($p < 0.05$).

chemical enhancer solution on the top of skin or buccal tissue in the donor compartment before the addition of a tested gel formulation. Then the same procedures described above for passive permeation experiment were followed.

For iontophoretic experiment, 0.1 and 0.3 mA for 8 h of treatment was provided by Phoresor II Auto (Model PM 850). The anodal electrode (Ag) was submerged in the gel formulation in the donor compartment, but stayed about 2 mm above the skin or buccal tissue. The cathode electrode (AgCl) was placed into the receptor compartment (Fig. 1). The anodal and cathode electrodes were connected to the positive and negative terminators of Phoresor II Auto power source to conduct iontophoresis treatment on skin or buccal tissue. Iontophoresis was terminated after 8 h application. The same sampling method and time points were used as described above for passive permeation experiment.

2.7. HPLC analysis of LHCl, NHT and DHCl

An Agilent HP 1100 HPLC system with a VWD detector and Agilent ChemStation for LC were used to analyze LHCl, NHT, and DHCl concentrations (Table 2) in the receptor compartment at different time points.

2.8. Data analysis for permeation study

Steady state flux at time t ($J\,\mu g\,cm^2$) was represented by the slope of the linear section of the plot of cumulative drug amount permeated *vs.* time. Q_8 ($\mu g\,cm^2$) was defined as the cumulative drug amount permeated into the receptor compartment at 8 h from the drug formulation in the donor compartment. The enhancement ratio (*ER*) for flux was obtained from the following formula:

$$ER = \frac{\text{flux for treated skin or buccal tissue with enhancer or iontophoresis or their combination}}{\text{flux for treated and untreated skin or buccal tissue}}$$

Results were demonstrated as mean ± standard deviation (S.D.) (n) where n was the number of experiment replicates. The unpaired Student's t-test was used to analyze the difference between fluxes for treated tissue and untreated (control) tissue. ANOVA was used to compare fluxes among different treated tissues, and a difference with $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of iontophoretic treatment on transdermal and tranbuccal delivery of LHCl, NHT and DHCl

Anodal iontophoretic (0.1 mA or 0.3 mA) treatment was conducted on porcine skin and buccal tissue for 8 h. Tables 3–5 and Figs. 2–4 show the results of the flux, cumulative amount of drug

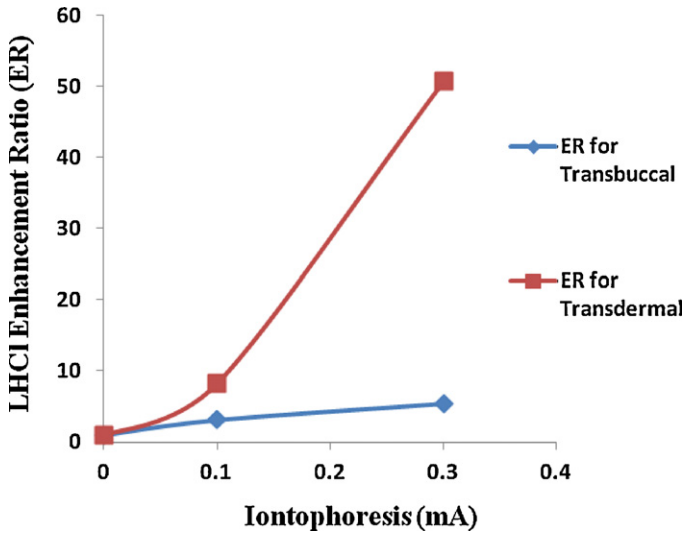

Fig. 2. Enhancement ratios of iontophoresis on transdermal and transbuccal delivery of lidocaine HCl at 8 h.

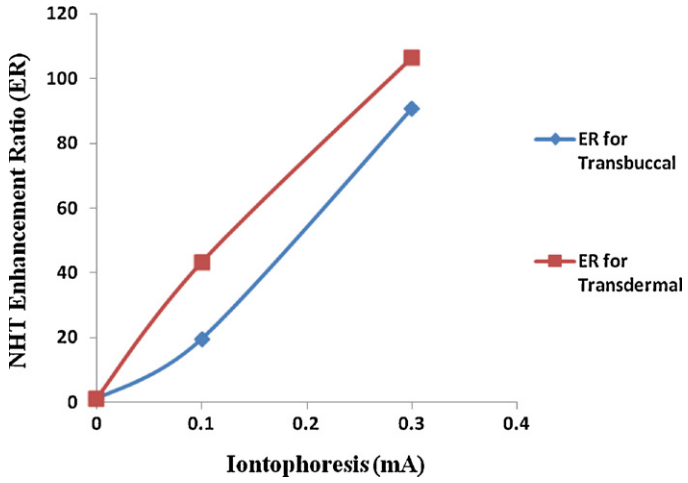

Fig. 3. Enhancement ratios of iontophoresis on transdermal and transbuccal delivery of nicotine hydrogen tartrate at 8 h.

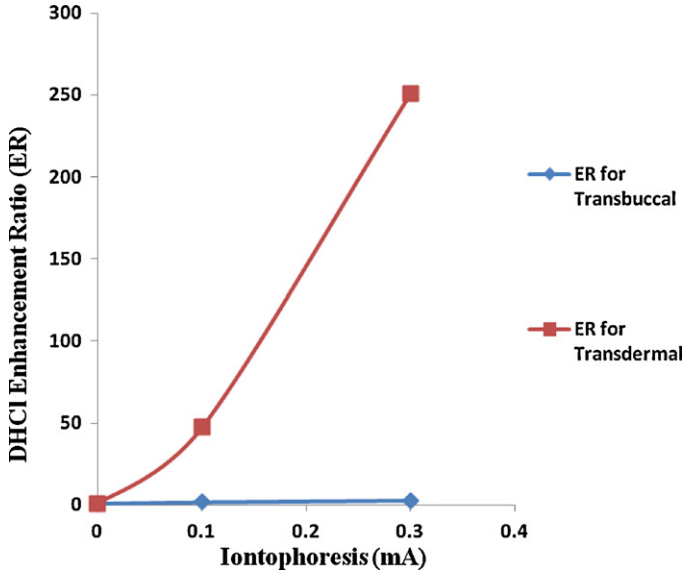

Fig. 4. Enhancement ratios of iontophoresis on transdermal and transbuccal delivery of diltiazem HCl at 8 h.

permeated and ER. The effect of iontophoresis (0.1 and 0.3 mA) on transdermal and transbuccal delivery of LHCl, NHT and DHCl was compared. During the same 8 h period of permeation study, LHCl and DHCl passively diffused through porcine buccal tissue much more effectively ($p < 0.05$) than through porcine skin which was in agreement with published literature (Rojanasakul et al., 1992). But, it was interesting to note that the difference between passive diffusion of transdermal and transbuccal delivery of NHT was not significant. When compared to control, iontophoresis at 0.1 mA and 0.3 mA significantly enhanced both transdermal and transbuccal delivery of LHCl, NHT and DHCl ($p < 0.05$). It was also observed that enhancement ratio (ER) from iontophoresis treatment (0.1 and 0.3 mA) on buccal tissue was consistently less than on skin tissue for the three tested drugs (Figs. 2–4). This may be due to the fact that the major barrier of skin – SC has pores in hair shaft and eccrine gland areas that exhibit less resistance to ionized molecules. Meanwhile, compared to SC of skin, the major barrier of buccal tissue – epithelium – contains no pores, small amounts of neutral lipids, but about 10 times more water and 8 times more polar lipids, mainly cholesterol sulfate and glucosylceramides (Wertz, 1983), which may compete for iontophoresis, thus reduce the effect of iontophoresis on transbuccal drug delivery. As a result, when iontophoresis is applied, ionized compounds such as LHCl, NHT and DHCl may be transferred through hair shafts and eccrine glands more easily of skin than epithelium of buccal tissue, i.e. the impact of iontophoresis on transdermal delivery of LHCl, NHT and DHCl was more significant than on transmucosal delivery.

Furthermore, for LHCl and DHCl, at 0.1 mA, flux and accumulative amount permeated at 8 h for transbuccal delivery were significantly higher than that of transdermal drug delivery ($p < 0.05$). At 0.3 mA, flux and accumulative amount permeated at 8 h for transdermal delivery were higher, but not significantly higher ($p > 0.05$) than that of transbuccal drug delivery. It may be explained as at 0.1 mA, the flux of these two drugs may be predominantly electroosmosis driven and the effect of electrorepulsion may not be significant during iontophoretic transdermal and transbuccal transport of LHCl and DHCl. When the current intensity of iontophoresis increased to 0.3 mA, the flux of these two drugs may be predominantly electrically driven (electrorepulsion) and the effect of electroosmosis became relatively less significant. However, for NHT, at both 0.1 mA and 0.3 mA, accumulative amount permeated at 8 h for transdermal delivery were significantly higher ($p < 0.05$) than that of transbuccal drug delivery, indicating that the flux of the drug may be predominantly electrically driven (electrorepulsion) and the effect of electroosmosis may not be significant during iontophoretic transbuccal transport of NHT.

3.2. *Effect of chemical enhancers on transdermal and transbuccal delivery of LHCl, NHT and DHCl*

Tables 6–8 and Figs. 5–7 demonstrated that enhancement effects of the various enhancer pretreatments (1 h) on transdermal and transbuccal delivery of LHCl, NHT and DHCl were different. When compared to control, azone had significantly higher ($p < 0.05$) enhancement effect on transbuccal than transdermal delivery of LHCl and DHCl, but had no enhancement effect on transdermal and transbuccal delivery of NHT. When compared to control, the hydrophobic enhancer Br-iminosulfurane significantly ($p < 0.05$) enhanced both transdermal and transbuccal delivery of LHCl, and the enhancement effect on transdermal was significantly higher ($p < 0.05$) than on transbuccal delivery of LHCl. It also had significantly higher ($p < 0.05$) enhancement effect on transbuccal than transdermal delivery of DHCl. It had no enhancement effect on either transdermal or transbuccal delivery of NHT. DDAIP had

Table 6
Enhancement effect of 1 h enhancer pretreatment on transdermal and transbuccal delivery of lidocaine HCl at 8 h.[a]

Treatment (mA)	Transdermal		Transbuccal	
Enhancer	Flux (μg/cm² h)	Q_8 (μg/cm²)	Flux (μg/cm² h)	Q_8 (μg/cm²)
Control	7.4 ± 5.8	59.7 ± 43.4	44.7 ± 9.6[b]	345.6 ± 74.3[b]
PG	8.4 ± 11.4	74.1 ± 97.9	39.1 ± 7.3[b]	299.4 ± 59.9[b]
2.5% Azone in PG	9.6 ± 2.8	87.3 ± 23.2	92.8 ± 62.5[b]	754.2 ± 543.7[b]
5.0% DDAIP in PG	15.0 ± 9.6	113.9 ± 73.8	91.6 ± 34.4[b,c]	716.5 ± 281.8[b,c]
5.0% DDAIP HCl in water	9.8 ± 7.1	79.9 ± 57.8	368.5 ± 111.5[b,c]	2902.0 ± 853.1[b,c]
5.0% DDAIP HCl in PG	7.0 ± 3.3	66.8 ± 31.7	217.7 ± 54.0[b,c]	1703.9 ± 419.2[b,c]
5.0% Br-iminosulfurane in PG	35.4 ± 8.8[c]	266.5 ± 69.4[c]	92.4 ± 26.9[b,c]	749.7 ± 216.8[b,c]

Control – untreated passive. Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Transbuccal delivery is significantly higher than transdermal delivery ($p < 0.05$).
[c] Statistically significantly higher than control ($p < 0.05$).

Table 7
Enhancement effect of 1 h enhancer pretreatment on transdermal and transbuccal delivery of nicotine hydrogen tartrate at 8 h.[a]

Treatment (mA)	Transdermal		Transbuccal	
Enhancer	Flux (μg/cm² h)	Q_8 (μg/cm²)	Flux (μg/cm² h)	Q_8 (μg/cm²)
Control	1.3 ± 1.9	9.9 ± 14.6	0.9 ± 0.4	6.9 ± 2.6
PG	1.5 ± 3.7	10.9 ± 26.8	1.0 ± 1.0	1.0 ± 1.0
2.5% Azone in PG	0.9 ± 1.4	7.7 ± 12.4	1.0 ± 1.0	1.0 ± 1.0
5.0% DDAIP in PG	1.3 ± 0.6	9.7 ± 4.9	70.3 ± 60.3[b,c]	579.8 ± 490.2[b,c]
5.0% DDAIP HCl in water	2.2 ± 5.3	11.3 ± 33.9	335.2 ± 104.5[b,c]	2768.0 ± 789.0[b,c]
5.0% DDAIP HCl in PG	0.6 ± 0.7	4.7 ± 5.8	171.1 ± 58.9[b,c]	1304.6 ± 415.4[b,c]
5.0% Br-iminosulfurane in PG	0.6 ± 1.4	4.4 ± 10.8	1.0 ± 1.0	9.6 ± 19.0

Control – untreated passive. Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Statistically significantly higher than control ($p < 0.05$).
[c] Transbuccal delivery is significantly higher than transdermal delivery ($p < 0.05$).

Table 8
Enhancement effect of 1 h enhancer pretreatment on transdermal and transbuccal delivery of diltiazem HCl at 8 h.[a]

Treatment (mA)	Transdermal		Transbuccal	
Enhancer	Flux (μg/cm² h)	Q_8 (μg/cm²)	Flux (μg/cm² h)	Q_8 (μg/cm²)
Control	0.4 ± 0.3	3.0 ± 2.6	32.6 ± 9.5[b]	258.3 ± 73.6[b]
PG	0.3 ± 0.2	2.7 ± 1.9	26.2 ± 5.8[b]	208.6 ± 46.4[b]
2.5% Azone in PG	0.8 ± 0.1	5.6 ± 1.3	83.8 ± 27.4[b]	662.6 ± 218.3[b]
5.0% DDAIP in PG	3.0 ± 1.2[c]	25.2 ± 10.4[c]	54.9 ± 11.2[b]	428.0 ± 83.9[b,c]
5.0% DDAIP HCl in water	0.1 ± 0.0	1.0 ± 0.4	58.9 ± 14.5[b]	485.1 ± 113.3[b,c]
5.0% DDAIP HCl in PG	0.3 ± 0.2	2.8 ± 1.6	37.2 ± 29.6[b]	299.7 ± 236.1[b]
5.0% Br-iminosulfurane in PG	0.3 ± 0.1	2.5 ± 0.7	66.2 ± 22.4[b]	532.2 ± 179.7[b,c]

Control – untreated passive. Q_8 – drug cumulative amount permeated within 8 h.
[a] Data are presented as means ± S.D. ($3 \le N \le 9$).
[b] Transbuccal delivery is significantly higher than transdermal delivery ($p < 0.05$).
[c] Statistically significantly higher than control ($p < 0.05$).

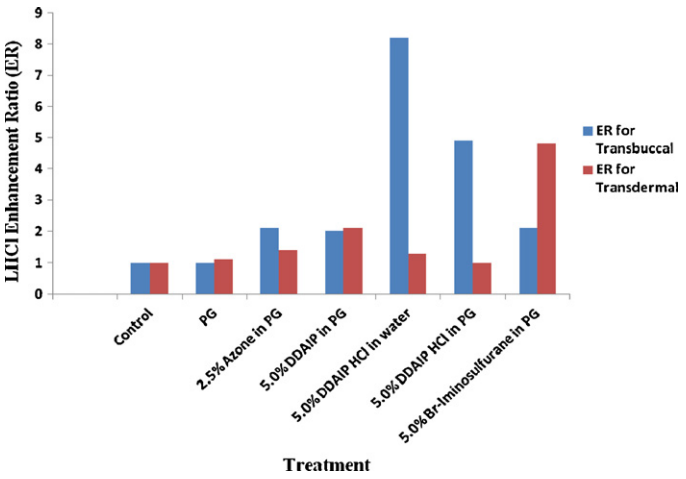

Fig. 5. Enhancement ratios of enhancers on transdermal and transbuccal delivery of lidocaine HCl at 8 h.

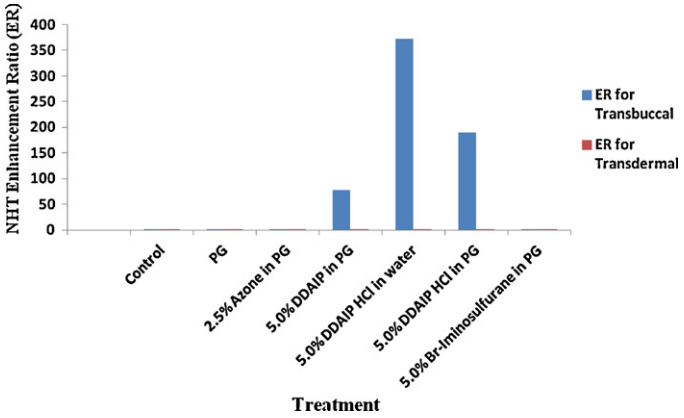

Fig. 6. Enhancement ratio of enhancer on transdermal and transbuccal delivery of nicotine hydrogen tartrate at 8 h.

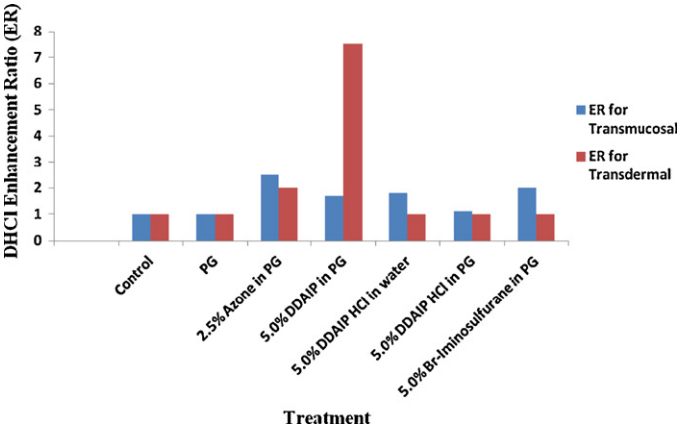

Fig. 7. Enhancement ratios of enhancer on transdermal and transbuccal delivery of diltiazem HCl at 8 h.

significantly higher ($p < 0.05$) enhancement effect on transdermal and transbuccal delivery of LHCl and DHCl, and had no enhancement effect on transdermal delivery of NHT. DDAIP HCl had no enhancement effect on transdermal delivery of LHCl, NHT and DHCl. However, DDAIP and DDAIP HCl had significantly higher ($p < 0.05$) enhancement effect on transbuccal than on transdermal delivery of LHCl, DHCl and NHT. The different chemical properties and different enhancement mechanisms of action of these enhancers may contribute to their different enhancement effects. Hydrophobic enhancer–azone is known to enhance intercellular drug permeation through skin by loosing up the lipid bilayer structure of stratum corneum (Barry, 1987). Hydrophobic enhancer Br-iminosulfurane was reported to enhance hydrophobic drug penetration through lipid enriched membranes (Song et al., 2005). DDAIP was recommended for enhancing drug transport through increasing lipid fluidity within the polar region of the lipid bilayer (Wolka et al., 2004). However, the non-keratinized buccal tissue is enriched with polar lipids which may have more interactions with hydrophilic compounds than with hydrophobic compounds (Squier et al., 1991). This may explain that hydrophilic enhancer DDAIP HCl was more effective in enhancing transbuccal delivery of

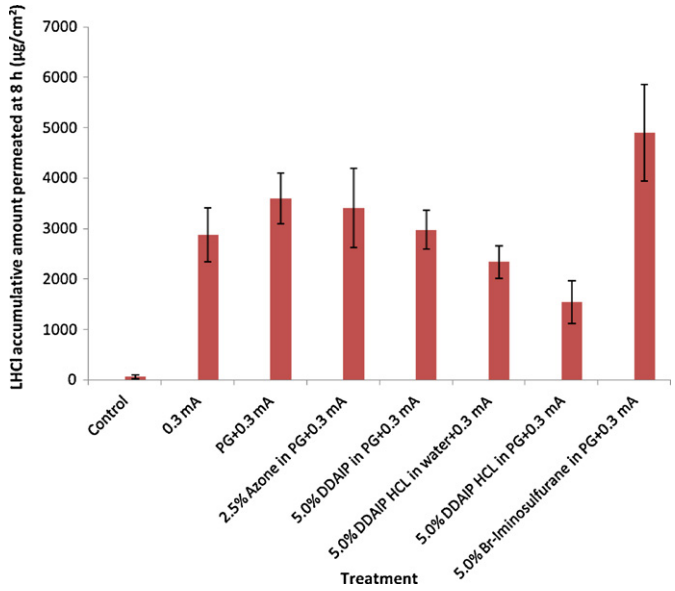

Fig. 8. Combined effect of iontophoresis and enhancer on transdermal delivery of lidocaine HCl. Data are presented as means ± S.D. ($3 \le N \le 9$).

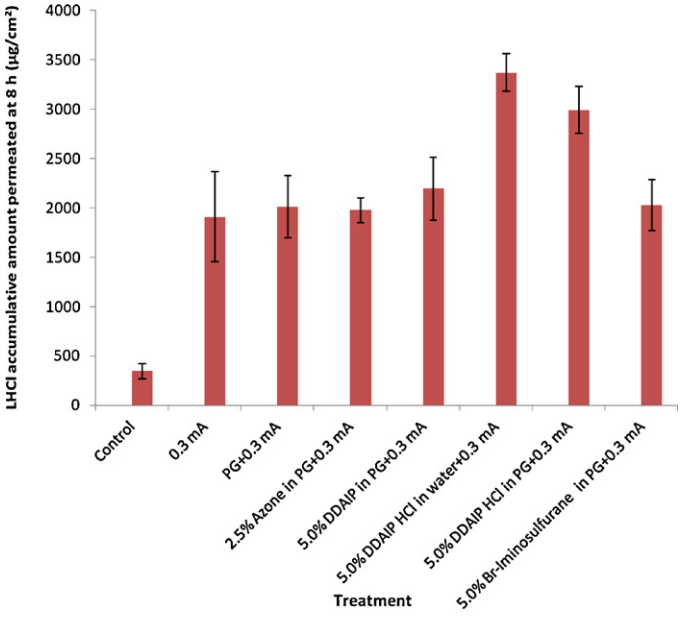

Fig. 9. Combined effect of iontophoresis and enhancer on transbuccal delivery of lidocaine HCl. Data are presented as means ± S.D. ($3 \le N \le 9$).

hydrophilic drugs than hydrophobic enhancers Br-iminosulfurane, azone and DDAIP.

3.3. Combined enhancement effect of chemical enhancer and iontophoresis on transdermal and transbuccal delivery of LHCl, NHT and DHCl

Figs. 8–16 show the results of the combined enhancement effect of iontophoresis (0.3 mA for 8 h) and enhancer pretreatment (1 h) on transdermal and transbuccal delivery of LHCl, NHT and DHCl. The results demonstrated that the combined treatments of iontophoresis (0.3 mA for 8 h) and enhancer: azone, Br-iminosulfurane, DDAIP and DDAIP HCl pretreatments (1 h) significantly enhanced both transdermal and transbuccal delivery of LHCl, NHT and DHCl ($p < 0.05$). It was also observed that the combined enhancement effect of enhancers (azone, Br-iminosulfurane, and DDAIP) and

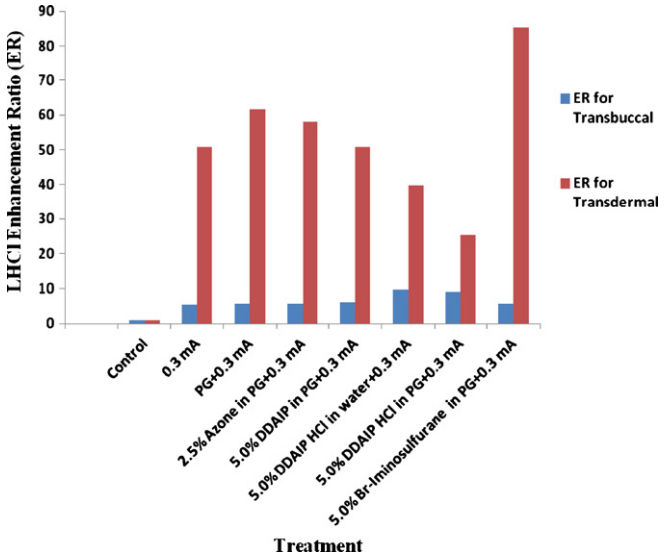

Fig. 10. Enhancement ratios of combined treatment of iontophoresis and enhancer on transdermal and transbuccal delivery of lidocaine HCl at 8 h.

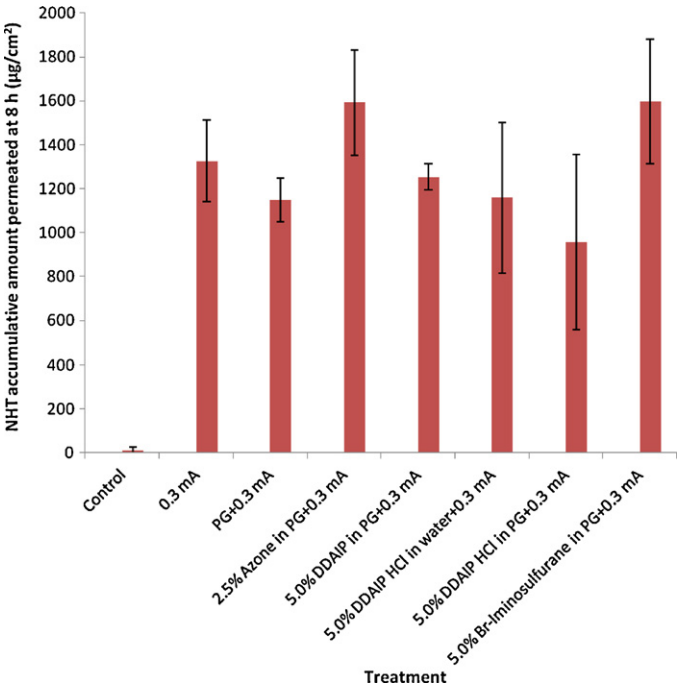

Fig. 11. Combined effect of iontophoresis and enhancer on transdermal delivery of nicotine hydrogen tartrate. Data are presented as means ± S.D. ($3 \leq N \leq 9$).

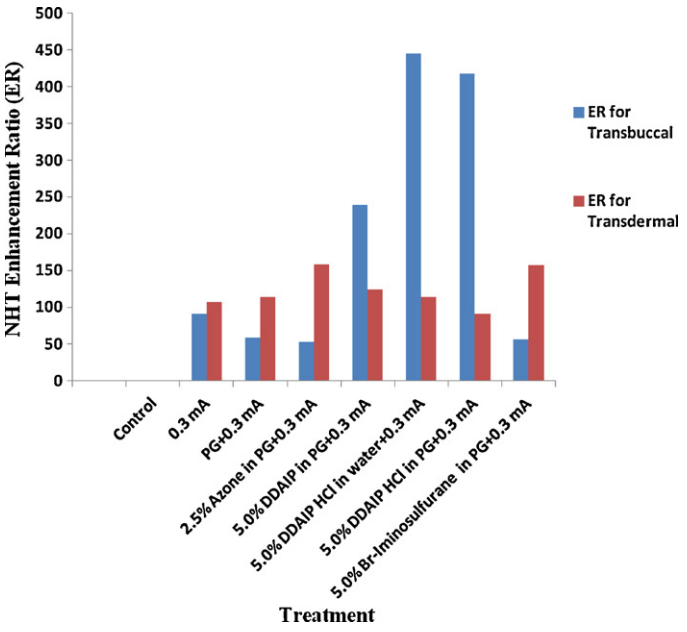

Fig. 13. Enhancement ratios of combined treatment of iontophoresis and enhancers on transdermal and transbuccal delivery of nicotine hydrogen tartrate at 8 h.

iontophoresis on transdermal delivery were significantly higher ($p < 0.05$) than on transbuccal delivery of LHCl, NHT and DHCl, indicating that iontophoresis was the major contributor of the combined enhancement effect. Synergistic enhancement effect was only observed on transdermal delivery of LHCl and NHT from the combined treatment of iontophoresis and Br-iminosulfurane. In the case of DDAIP HCl, the combined enhancement effect of DDAIP HCl and iontophoresis was significantly higher ($p < 0.05$) on transbuccal delivery than on transdermal delivery of LHCl, NHT and DHCl, indicating that DDAIP HCl was the major contributor to the combined enhancement effect. It was also found that the combined enhancement effect was less than the sum of enhancement effects of DDAIP HCl and iontophoresis. It can be explained as the hydrophilic enhance DDAIP HCl may be competing with hydrophilic drugs LHCl, NHT and DHCl for iontophoresis which was in agreement with the observation from our previous study on ondansetron HCl (Hu et al., 2011).

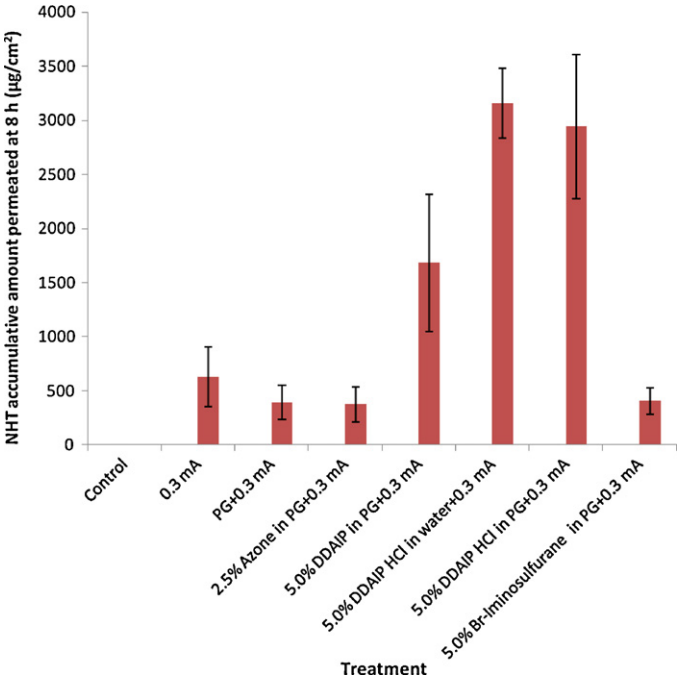

Fig. 12. Combined effect of iontophoresis and enhancer on transbuccal delivery of nicotine hydrogen tartrate. Data are presented as means ± S.D. ($3 \leq N \leq 9$).

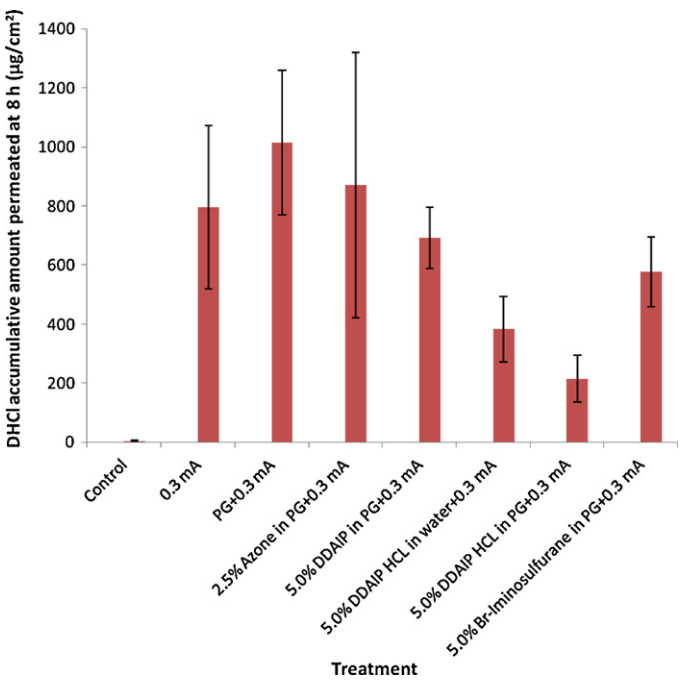

Fig. 14. Combined effect of iontophoresis and enhancer on transdermal delivery of diltiazem HCl. Data are presented as means ± S.D. ($3 \leq N \leq 9$).

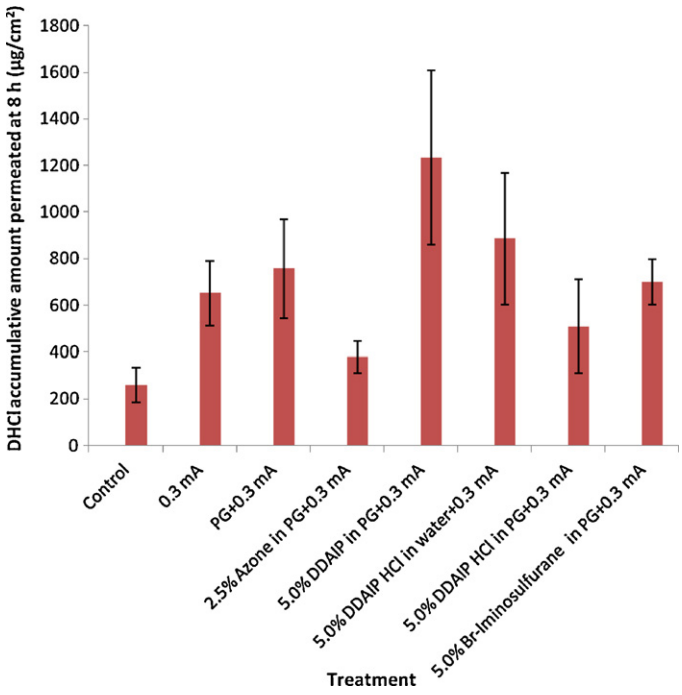

Fig. 15. Combined effect of iontophoresis and enhancer on transbuccal delivery of diltiazem HCl. Data are presented as means ± S.D. ($3 \leq N \leq 9$).

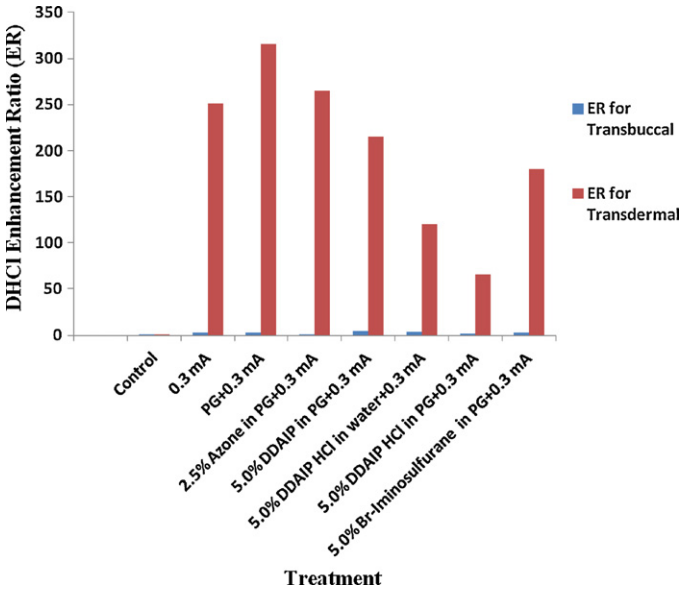

Fig. 16. Enhancement ratios of combined treatment of iontophoresis and enhancers on transdermal and transbuccal delivery of diltiazem HCl at 8 h.

4. Conclusions

Iontophoresis (0.3 mA) was effective in enhancing both transdermal and transbuccal drug delivery of hydrophilic drug LHCl, NHT and DHCl. At tested concentrations of these three drugs: 2.5% (w/w) LHCl, 2% (w/w) NHT, and 2% (w/w) DHCl, the enhancement effect of iontophoresis on transdermal tend to be much higher than on transbuccal drug delivery. The enhancement effect from chemical enhancer pretreatments was varied depending on the enhancers and drugs. Br-iminosulfurane had higher enhancement effect on transdermal than transbuccal delivery of LHCl. DDAIP significantly enhanced transdermal delivery of LHCl and DHCl. DDAIP HCl was significantly more effective in enhancing transbuccal than transdermal delivery of LHCl, NHT and DHCl. The combined treatment of iontophoresis and chemical enhancers provided limited synergistic effects on transdermal drug delivery and had no synergistic effect on transbuccal drug delivery.

From the perspective of cumulative total amount of drug delivered after 8 h (Q_8), as expected, transbuccal was more effective than transdermal delivery. For LHCl and NHT, although the major contributing factor for the enhancement was the chemical enhancer, the combination of iontophoresis and DDAIP HCl provided the best overall results. For DHCl, although the major contributing factor for the enhancement was iontophoresis, the combination of iontophoresis and DDAIP base provided the best overall results. However, more drugs and enhancers at different strengths will be tested to obtain a comprehensive understanding of the impact of iontophoresis and enhancers on enhancing transdermal and transbuccal drug delivery systems.

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