Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 297 (2005) 162–171
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

The effects of electrically assisted methods on transdermal delivery of nalbuphine benzoate and sebacoyl dinalbuphine ester from solutions and hydrogels

Jeng-Fen Huang [a], K.C. Sung [a], Oliver Yoa-Pu Hu [b], Jhi-Joung Wang [c], Yi-Hsin Lin [a], Jia-You Fang [d,*]

[a] *Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan Hsien, Taiwan*
[b] *School of Pharmacy, National Defense Medical Center, Taipei, Taiwan*
[c] *Department of Medical Research, Chi-Mei Medical Center, Tainan Hsien, Taiwan*
[d] *Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hua 1st Road, Kweishan, Taoyuan, Taiwan*

Received 4 October 2004; received in revised form 21 March 2005; accepted 25 March 2005
Available online 25 April 2005

Abstract

The aim of this study was to assess the effects of iontophoresis and electroporation on transdermal delivery of nalbuphine (NA) and its two novel prodrugs: nalbuphine benzoate (NAB) and sebacoyl dinalbuphine ester (SDN) from solutions as well as from hydrogels. Hydroxypropyl cellulose (HPC) and carboxymethyl cellulose (CMC) were used in hydrogel formulations to evaluate their feasibility for delivery of NA and its prodrugs. Application of iontophoresis or electroporation significantly enhanced the in vitro permeation of NA and its prodrugs. The enhancement effect was more pronounced after applying iontophoresis. The combination of two electrically assisted methods enhanced the delivery of NA; however, no such enhancement was observed for the permeation of NAB and SDN. Hydrogels containing low concentration HPC did not affect the passive as well as electrically assisted permeation of NA and its prodrugs. The increase of hydrogel concentration as well as molecular weight significantly decreased the electrically assisted permeation of NA, whereas the permeation of NAB and SDN remained unchanged. For the electrically assisted permeation from CMC-based hydrogels, the reduced permeation from higher percentage of CMC hydrogels may be attributed the viscosity effect as well as the ion competition effect. The above results demonstrated that lipophilicity and molecular size, as well as hydrogel compositions had significant effects on skin permeation of NA, NAB and SDN via passive diffusion or under the electric field.

Keywords: Nalbuphine; Prodrugs; Transdermal delivery; Iontophoresis; Electroporation; Hydrogels

1. Introduction

The delivery of drugs via skin pathway has been studied extensively in the pharmaceutical field. The

* Corresponding author. Tel.: +886 3 2118800x5521; fax: +886 3 2118236.
E-mail address: fajy@mail.cgu.edu.tw (J.-Y. Fang).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.03.015

advantages of transdermal delivery of drugs include its non-invasiveness, compliance, safety and effectiveness. Nevertheless, the barrier properties of skin, especially the stratum corneum, have limited its clinical applications. Consequently, two electrically assisted methods including iontophoresis and electroporation have been developed and demonstrated as effective means to enhance the transdermal delivery of drugs (Riviere and Heit, 1997; Banga et al., 1999).

Iontophoresis applies a small low voltage (typically 10 V or less) and constant current (typically 0.5 mA/cm^2 or less) to push a charged molecule into skin or other tissues. Electroporation involves the application of a high voltage (typically >100 V) pulse for a very short (μs–ms) duration to permeabilize the skin (Banga et al., 1999). Electrically assisted delivery by iontophoresis/electroporation provides the advantage of programming the delivery rate and extent in responding to the level of therapy desired. Previous studies have demonstrated that the electrically assisted methods may allow transdermal administration of narcotics with a rapid achievement of steady-state concentrations and desired delivery rates (Grond et al., 2000; Fang et al., 2002). These observations indicate that the transdermal delivery system modulated by iontophoresis/electroporation can be utilized to deliver various narcotic analgesics.

Nalbuphine (NA) is a narcotic analgesic used in the treatment of both acute and chronic pain. It is a potent analgesic with relatively low side effects (Cherny, 1996). Due to its short elimination half-life and low oral bioavailability (Lo et al., 1987), frequent injection is needed. In order to maintain the blood nalbuphine concentration to improve the patient compliance and therapeutic effectiveness in pain management, a series of nalbuphine prodrugs have been synthesized (Wang, 1992; Sung et al., 1998). The pharmacokinetic and biopharmaceutic characteristics of NA and its prodrugs suggest the practicality of transdermal route (Sung et al., 2000; Fang et al., 2001). Among those prodrugs synthesized, nalbuphine benzoate (NAB) and sebacoyl dinalbuphine ester (SDN) are relatively novel synthetic prodrugs of NA (Fig. 1) (Pao et al., 2000). Since the functional groups and molecular structures for the prodrugs are different, the drug lipophilicity, molecular size and thus, the skin permeation characteristics can be significantly different.

The major goal of this study was to assess the permeation characteristics of NA and the two prodrugs, NAB and SDN, under various driving forces as well as from various hydrogel formulations. The driving forces studied include passive diffusion, iontophoresis and electroporation. The combination of both iontophoresis and electroporation on permeation rates was also tested to explore more effective means for delivery of the prodrugs. The skin permeation of NA, NAB and SDN from hydrogel formulations containing hydroxypropyl cellulose (HPC) or carboxymethyl cellulose (CMC) was studied to evaluate their influence on permeation rates under the application of electrically assisted methods.

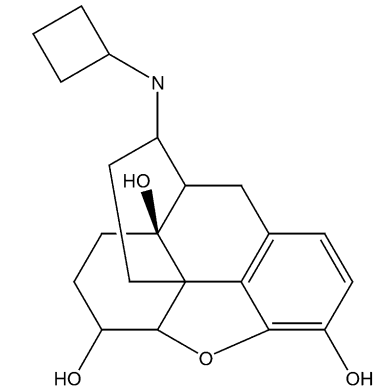
2. Materials and methods

2.1. Materials

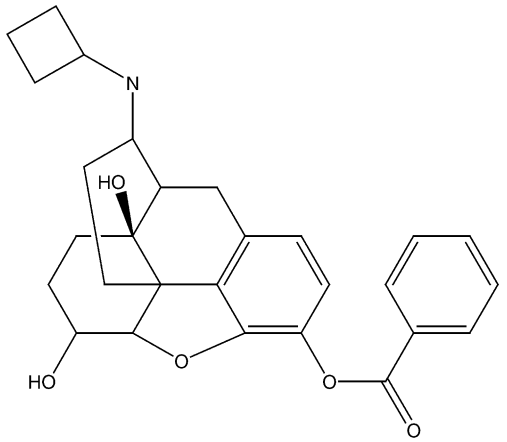
Hydroxypropyl cellulose (HPC) (150–400 cps and 1000–4000 cps determined 20 g/l at 20 °C) and carboxymethyl cellulose sodium salt (CMC) were purchased from Wako Chemical Industries (Japan). Nalbuphine (NA, MW = 357.46, melting point = 222–223 °C) was obtained from the Narcotic Bureau, Department of Health, Executive Yuan, Taiwan. Nalbuphine benzoate (NAB, MW = 461.56, melting point = 153–156 °C) and sebacoyl dinalbuphine ester (SDN, MW = 881.12, melting point = 130–131 °C) were synthesized by Yung-Shin Pharmaceutical Co. (Taiwan) by a method developed from Pharmaceutical Research Institute, National Defense Medical Center (Pao et al., 2000). All chemicals and solvents were analytical or HPLC grade and used as received.

2.2. Preparation of hydrogels

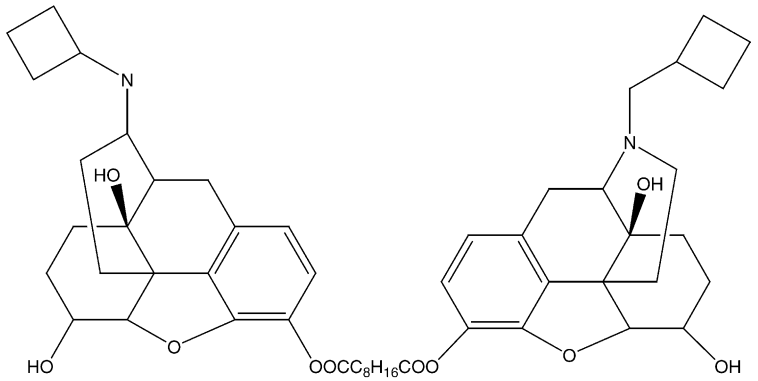
For the preparation of cellulose hydrogels, appropriate amounts of polymer were added into half of pH 4 citrate–phosphate buffer and the mixture was stirred continuously for 1 h. After 24 h, the residual half of pH 4 buffer and appropriate amounts of drug were added into the mixture with continuous stirring for 1 h. The final concentrations for NA, NAB and SDN were 1.5, 1.5 and 0.75 mM, respectively. The in vitro permeation experiments were performed 24 h after the preparation of hydrogels.



Nalbuphine (NA)



Nalbuphine benzoate (NAB)



Sebacoyl dinalbuphine ester (SDN)

Fig. 1. Chemical structures of nalbuphine (NA), nalbuphine benzoate (NAB) and sebacoyl dinalbuphine ester (SDN).

2.3. Viscosity determinations

Measurements of the viscosity were carried out on hydrogels before the performance of in vitro permeation experiments. The viscosity of hydrogel was determined in a cone and plate viscometer (Brookfield Co., Model DV-2, USA). Hydrogel of 0.5 g was placed in the sample cup of the viscometer and allowed to stand for 30 min to reach equilibrium temperature of 37 °C. For each measurement, the readings were recorded for 20 s; the stabilized values were then averaged and converted to the hydrogel viscosity.

2.4. In vitro permeation experiments

The in vitro permeation studies were performed using horizontal glass diffusion cells. The dorsal skin of excised female nude mouse (Balb-c/nu strain, 7 weeks old) was used as the model skin barrier. The receptor phase contained 8 ml of 0.06 M citrate–phosphate buffer (pH 7.4). For drug permeation from solutions, the donor compartment was filled with 8 ml of 0.06 M citrate–phosphate buffer (pH 4) containing 1.5 mM of NA or NAB, or 0.75 mM of SDN. For permeation studies from hydrogel vehicles, 8 g of hydrogel-containing drug was used as the donor vehicle. The available skin diffusion area was 0.785 cm^2. The cells were agitated by magnetic stirrers at 600 rpm. The samples (300 μl) were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh buffer solution. The samples were then analyzed by the HPLC methods (Sung et al., 1998; Pao et al., 2000).

2.5. Iontophoresis protocols

For the in vitro permeation experiments under iontophoresis, a pair of Ag/AgCl wires (0.5 mm in diameter) with an effective length of 15 mm were immersed in the buffer as electrodes, with the anode in donor site and the cathode in receptor site. The electrodes were each positioned 3 cm from the side of skin. The electrodes were connected to a current power supplier (Yokogawa Co., Model 7651, Japan). The current density was set at 0.3 mA/cm^2 by continuous mode for 3 h.

2.6. Electroporation protocols

Electroporation was performed using an exponential decay pulse generator (BTX Co., ECM 630 Electro Cell Manipulator®, USA). A pair of platinum electrodes (0.5 × 1.5 cm^2) were used and each located 3 cm from the skin membrane. The anode was positioned in the donor compartment and the cathode was in the receptor compartment. The electroporation protocol was 1 pulse per 30 s, applied for 10 min; the pulse voltage was 300 V and pulse duration was 200 ms. The voltages were expressed as applied values but not transdermal values. In the study of combining electroporation and iontophoresis, the iontophoresis was started after applying 10 min of skin electroporation.

2.7. Data analysis

In the permeation study, the total amount of NA permeated across the unit diffusion surface into receptor was calculated and plotted as a function of time. The permeation data were analyzed using the following equation:

$$J_{ss} = \frac{dQ}{dt \times A}$$

where J_{ss} is the flux at apparent steady state, Q the cumulative mass of drug transferred to the receptor compartment, and A the membrane surface area. Both Students' *t*-test and one-way ANOVA were utilized as appropriate to test the various treatment effects.

3. Results and discussion

3.1. Transdermal permeation of NA, NAB and SDN by passive diffusion

Prior to perform skin permeation experiments, the stability of both prodrugs in pH 4 buffer was evaluated. There were no detectable decomposition products observed within 6 h with or without applying current and voltage pulsing, indicating that the prodrugs were chemically stable within the experimental time (data not shown). Preliminary studies also show that within the limit of HPLC detection (1.2 ng/ml), no measurable amount of prodrugs was observed in the receptor cell during 6 h. This demonstrates that the prodrugs were completely hydrolyzed during the permeation process. Since the epidermis is a metabolically active tissue, the degradation of prodrugs during the permeation process can be attributed primarily to the biotransformation

and enzymatic degradation within the skin. The transformed NA from prodrugs may be largely accumulated in viable epidermis and then, the NA molecules were passively released from the epidermis into the receptor. The diffusion of NA from viable skin to receptor was fast according to the data of NA permeated across stratum corneum-stripped skin (Sung et al., 2003).

Fig. 2 shows the cumulative amount of NA ($\mu g/cm^2$) in the receptor compartment as a function of time for the passive permeation of NA and its prodrugs. The apparent steady-state fluxes ($\mu g/cm^2/h$) from the profiles are summarized in the figure legend. The amount of drug permeated via passive diffusion was low, with flux increased in the order of NAB > NA > SDN. The more lipophilic NAB may result in higher skin membrane–water partition and thus, higher NAB skin permeation. The first sampling time plot for in vitro permeation was 0.5 h. Only NA molecules were detected in this first sampling, indicating a fast biodegradation for NAB within the epidermis.

Previous report also showed that for any drug molecule with molecular weight higher than 500 Da may not be appropriate for transdermal delivery via passive diffusion (Finnin and Morgan, 1999). Although the SDN is more lipophilic than NA, its higher molecular weight (MW = 881.12) exceeded the cut-off point for passive permeation through skin. As a result, there was essentially no SDN permeation observed within the 6 h of experimental time. According to the study of the permeation of buprenorphine and its prodrugs (Stinchcomb et al., 1996), as one approaches the extremes of high lipophilicity the aqueous tissue resistance, such as viable epidermis/dermis, becomes the predominant resistance. It appears that the stratum corneum is not the sole significant contributor of resistance for the prodrugs. The other narcotic analgesics such as sufentanil, which has highly lipophilic nature, also showed a case of low permeation by the resistance of epidermis/dermis (Roy et al., 1994). Another possibility for the negligible permeation of SDN is the slower enzymatic biotransformation to form NA within the epidermis, thus retarding its passive diffusion.

3.2. Transdermal permeation of NA, NAB and SDN by electrically assisted methods

Fig. 2 shows the amount of drug permeated versus time for NA and its prodrugs under iontophoresis with a

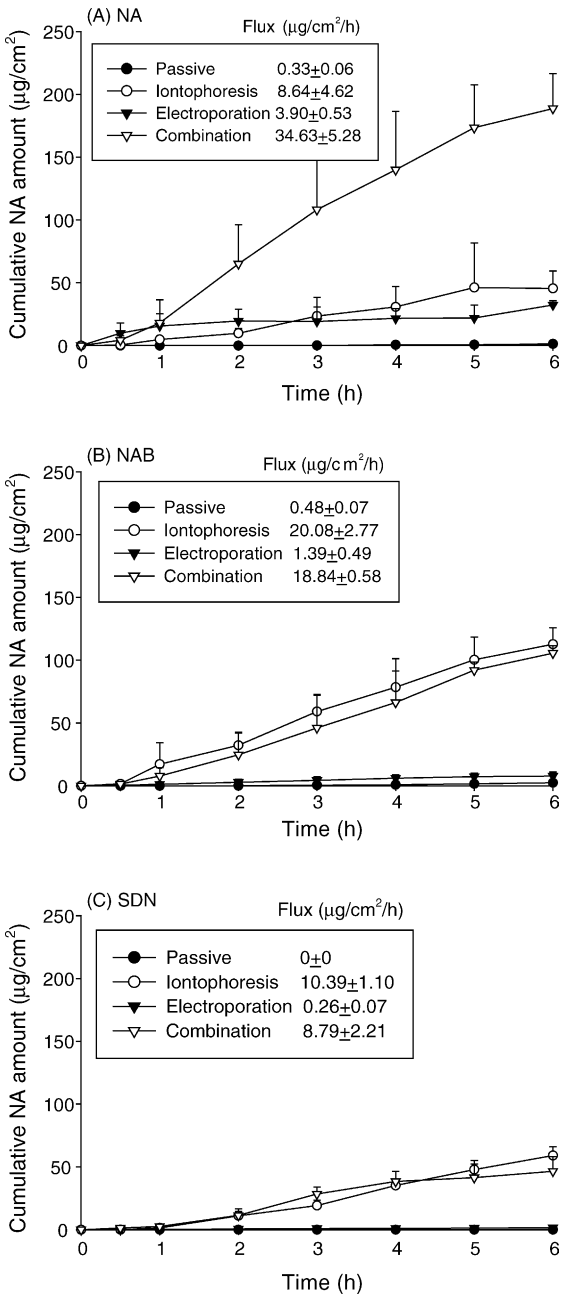


Fig. 2. Cumulative amount vs. time profiles for nalbuphine after application of nalbuphine (A), nalbuphine benzoate (B) and sebacoyl dinalbuphine ester (C) permeated across nude mouse skin from pH 4 buffer under various driving forces. All data represent the means of four experiments ± S.D.

constant current of 0.3 mA/cm^2 and 3 h of application. Iontophoresis markedly increased the transport of NA and its prodrugs, presumably due to the electrochemical potential gradient. The data also showed that the flux of SDN increased from 0 to 10.39 μg/cm^2/h after application of iontophoresis, suggesting the cut-off limit was increased by iontophoresis. The iontophoresis significantly enhanced the permeation of prodrugs rather than the parent drugs. The appendageal pathways are important for iontophoretic delivery of drugs (Tyle, 1986; Sung et al., 2000). Since the viable epidermis/dermis may be a predominant barrier for the lipophilic prodrugs to permeate across skin, the appendageal routes, which pass through epidermis/dermis, may offer a convenient pathway for NAB and SDN to permeate. Hence, the administration of iontophoresis extends this effect, resulting in the higher enhancement of prodrug permeation. The enhancement effects remained elevated even after cessation of electrical current (Fig. 2). This observation may be attributed to the formation of drug reservoir within the skin and the drug was slowly released from reservoir into the receptor even after 3 h of application.

Transdermal delivery of NA and its prodrugs was also investigated by application of high-voltage pulses at time zero. By comparing to the passive permeation, the application of electroporation significantly increased the skin permeation of all drugs tested (Fig. 2). The observation can be due to the creation of transient micropores in skin and permit transport of drugs across these pathways (Prausnitz et al., 1995; Jadoul et al., 1998; Fang et al., 2002). Nevertheless, fluxes enhanced by electroporation was less pronounced than those by iontophoresis (t-test, $p < 0.05$), especially for NAB and SDN. Electroporation predominantly acts on the stratum corneum to create micropores (Jadoul et al., 1998). Since the layers of epidermis/dermis was a rate-limiting barrier for prodrugs but not for NA itself, the subjection of the barrier property of stratum corneum by the pulsing may be not enough for NAB and SDN. Although electroporation could largely increase the accumulation of lipophilic prodrugs within the stratum corneum, the further transport across viable skin may be difficult.

The effects of combining iontophoresis and electroporation on skin permeation of NA, NAB and SDN were also examined in the present study. Since the enhancement mechanism for iontophoresis and electroporation are different, higher permeation would be expected for the application of short high-voltage pulses prior to applying iontophoresis. Fig. 2A demonstrates the permeation results by applying 20 pulses of 300 V at time zero and followed by applying 0.3 mA/cm^2 of iontophoresis. The application of electroporation pulses prior to iontophoresis consistently yielded four-fold higher flux for NA. The creation of a permeabilized state of the skin by high voltage pulses before iontophoresis is more likely the origin of the synergistic effect (Jadoul and Préat, 1997). Iontophoresis is believed to primarily transport drugs through pre-existing pathways (Cullander, 1992); it has also been shown that the application of high-voltage pulses may attribute to the creation of new pores or new pathways (Banga et al., 1999). As a result, the newly aqueous pores created by electroporation may allow hydrophilic NA to pass through under iontophoresis, thus causing the synergistic effect after combining iontophoresis and electroporation.

The application of electroporation prior to iontophoresis did not produce a synergistic effect for permeation of NAB as well as SDN (t-test, $p > 0.05$) (Fig. 2B and C). Since the application of electroporation has limited effect on the permeation of NAB and SDN, the aqueous pores created by electroporation thus could not offer an efficient pathway for NAB and SDN under iontophoresis. Hirvonen and Guy (1997) demonstrate that lipophilic cations, such as propranol, can decrease the electro-osmosis, thus decreasing the iontophoretic permeation of their own. The application of electroporation may accumulate larger numbers of lipophilic NAB and SDN within the stratum corneum. This may cause the reduction of electroosmotic flow, resulting in the negligible synergism by combining both electrically assisted methods.

3.3. Transdermal permeation of NA, NAB and SDN from hydrogels

The skin permeation of NA and its prodrugs from HPC as well as CMC-based hydrogels were investigated. Fig. 3 shows the permeation profiles of NA, NAB and SDN from HPC-based hydrogels. There is no significant difference (t-test, $p > 0.05$) between the flux from pH 4 buffer and from 2.5% HPC hydrogels for all three drugs studied, indicating that the cross-linkage structure formed by 2.5% HPC did not interact with NA

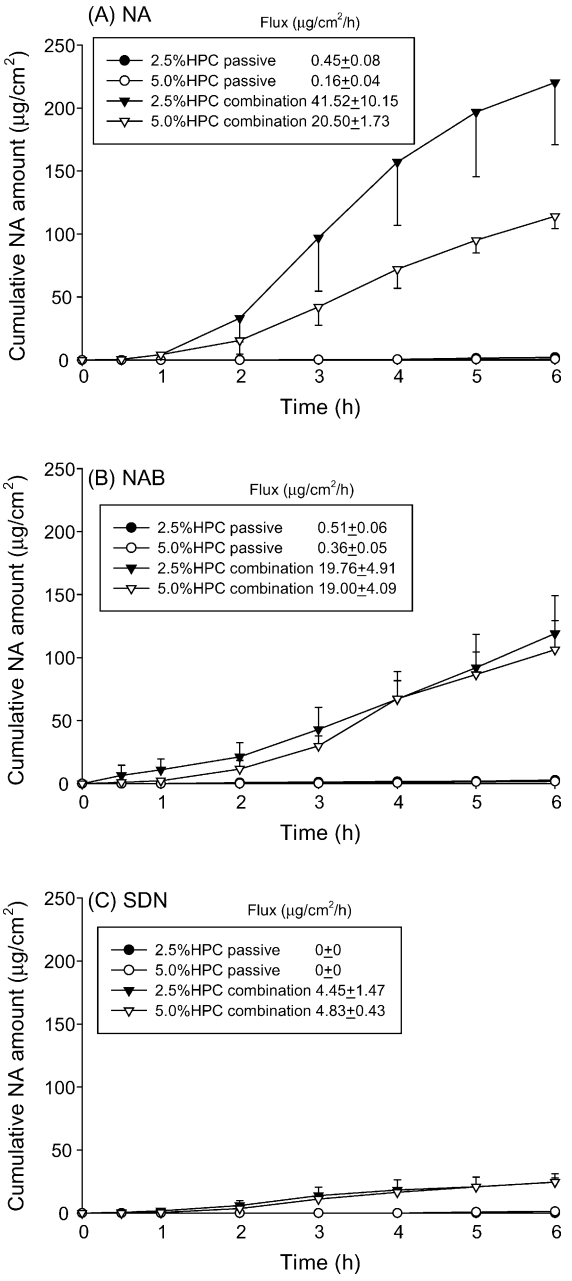


Fig. 3. Cumulative amount vs. time profiles for nalbuphine after application of nalbuphine (A), nalbuphine benzoate (B) and sebacoyl dinalbuphine ester (C) permeated across nude mouse skin from HPC hydrogels with different concentrations under various driving forces. All data represent the means of four experiments ± S.D.

Table 1
The viscosity (cps × 10^{-2}) of various hydrogel formulations

Polymer	Concentration (%)	Viscosity (cps × 10^{-2})
High-MW HPC[a]	2.5	7.20 ± 0.24
High-MW HPC	5.0	203.33 ± 24.05
Low-MW HPC	5.0	17.63 ± 0.60
CMC[b]	2.5	10.94 ± 0.26
CMC	5.0	308.80 ± 12.44

Each value represents the mean ± S.D. ($n = 3$).
[a] HPC: hydroxypropyl cellulose.
[b] CMC: carboxymethyl cellulose.

and its prodrugs. As the HPC concentration increased from 2.5 to 5.0% (Fig. 3A and B), the passive permeation of NA and NAB decreased (*t*-test, $p < 0.05$). The increased HPC concentration may result in higher entanglement density and thus, decrease the passive permeation rates (Fang et al., 1998). However, there was still no passive permeation of SDN through the skin. Table 1 shows the measured viscosity for those hydrogels. The viscosity increases drastically with the increase of HPC concentration. Accordingly, the results clearly demonstrate that the hydrogel concentration can have significant impact on the hydrogel viscosity and thus, the passive permeation rate.

By comparing the passive permeation, the application of both electrically assisted methods significantly increased the permeation of NA and its prodrugs from 2.5% HPC hydrogels (Fig. 3). Fig. 3 also demonstrates that the flux of NA decreased as the polymer concentration increased from 2.5 to 5%, whereas the flux of NAB and SDN remained unchanged. The results suggest that the permeation through hydrogel and skin were both important rate processes for NA, whereas the permeation through skin is the predominant rate process for NAB and SDN under the electric field.

The permeation of NA and its prodrugs from 5% HPC with different HPC molecular weight was also investigated (Table 2). For all the permeants studied, the permeation rates from low-molecular-weight HPC were similar to those from pH 4 buffer under either passive diffusion or electrically assisted methods, which can be due to the low viscosity for the low molecular weight of HPC hydrogel. Table 2 also shows that, irrespective the driving forces, the permeation rates of NA through skin were higher from the low-molecular-weight HPC, whereas the permeation rates of NAB and SDN were less sensitive to the changes in HPC

Table 2
The flux (μg/cm^2/h) of nalbuphine and its prodrugs from hydrogels with 5% low-MW HPC (150–400 cps determined 20 g/l at 20 °C)

Condition	Compound	Flux (μg/cm^2/h)
Passive diffusion	Nalbuphine	0.32 ± 0.07
	Nalbuphine benzoate	0.35 ± 0.06
	Dinalbuphine	0
Iontophoresis + electroporation	Nalbuphine	30.55 ± 3.56
	Nalbuphine benzoate	24.25 ± 5.66
	Dinalbuphine	5.17 ± 1.32

Each value represents the mean ± S.D. ($n = 4$).

molecular weight. The results again indicate that the hydrogel-controlled mechanism is an important permeation process for NA (Ho et al., 1994); on the other hand, the permeation of NAB and SDN through skin was the rate-determined process for both permeants.

CMC is an ionic water-soluble cellulose derivative used in various pharmaceutical dosage forms. Fig. 4 shows the transdermal permeation of NA, NAB and SDN under passive permeation and electrically assisted methods from CMC hydrogels. The gel with 2.5% CMC did not influence the passive permeation (*t*-test, $p > 0.05$) of NA and NAB as compared to the vehicle of pH4 buffer (Fig. 4A and B). Similarly, the increase of CMC concentration to 5.0% reduced the passive permeation rates of NA and NAB (*t*-test, $p < 0.05$). Nevertheless, there was no cumulative SDN observed via passive diffusion.

The transport of NA and its prodrugs was largely decreased by the presence of CMC after application of iontophoresis combined with electroporation (Figs. 2 and 4). Although the fluxes were not significantly different for the prodrugs from 2.5 and 5% CMC hydrogels, the trend of decrease in permeation rates from hydrogel with higher polymer percentage can still be observed. The results may be attributed to the viscosity effect of CMC as well as the ion competition effect. Table 1 demonstrates a significantly higher viscosity for 5% CMC hydrogel as compared to that of 2.5% CMC hydrogel, suggesting that the viscosity effect may partially attribute to the slow permeation rates from higher percentage of CMC hydrogel. Furthermore, since CMC is an ionized polymer with sodium (Na^+) as the counter-ion, the low permeation from CMC hydrogels may also partially be due to the competition of ionic drugs and Na^+ for the applied cur-

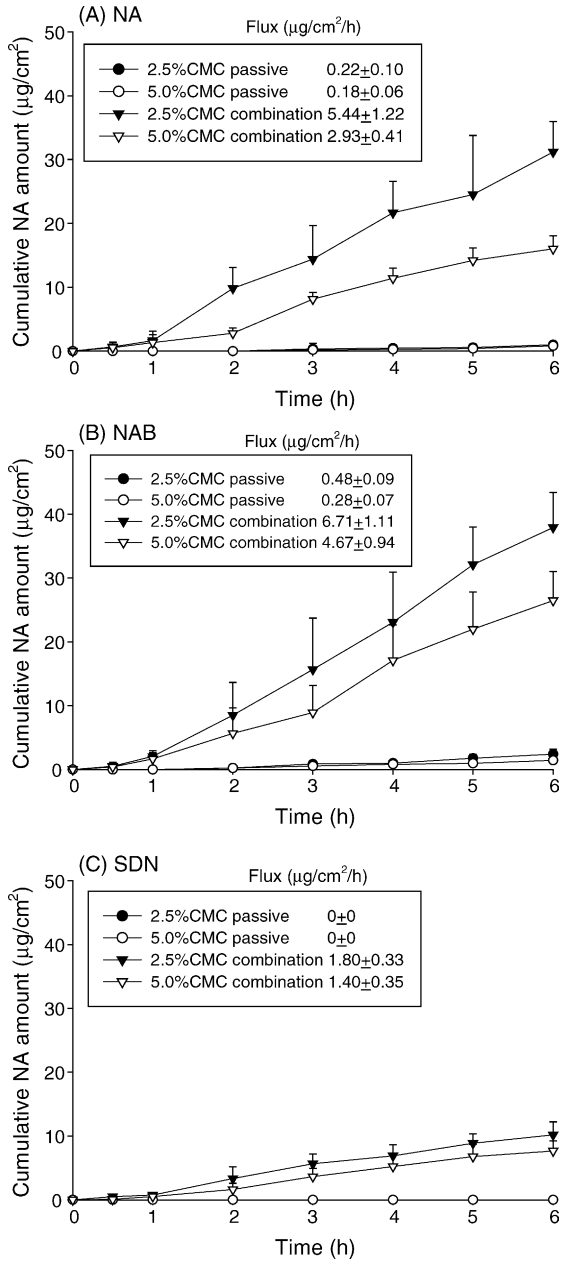


Fig. 4. Cumulative amount vs. time profiles for nalbuphine after application of nalbuphine (A), nalbuphine benzoate (B) and sebacoyl dinalbuphine ester (C) permeated across nude mouse skin from CMC hydrogels with different concentrations under various driving forces. All data represent the means of four experiments ± S.D.

rent. A part of current would be carried by Na^+ with relatively high mobilities, so that the residual fraction of applied current was carried by NA and its prodrugs. The two inferences may account for the slower permeation rates for NA and its prodrugs from higher percentage of CMC hydrogels under electric field.

4. Conclusions

The transdermal permeation of NA, NAB and SDN under various driving forces from pH 4 buffer as well as from hydrogels were assessed in this study. The passive permeation studies demonstrated that NAB had higher permeation rates than the parent drug (NA). The SDN prodrug containing double molecules of NA had essentially no passive permeation, which can be due to its large molecular size. Both iontophoresis and electroporation enhanced the skin permeation of NA and its prodrugs. The enhancement effects by iontophoresis were more pronounced as compared to that by electroporation. The combination of iontophoresis and electroporation further increase the flux of NA; however, no such enhancement effect was observed for NAB and SDN. Hydrogels containing low concentration HPC did not affect the passive as well as electrically assisted permeation of NA and its prodrugs. The increase of polymer concentration as well as molecular weight significantly decreased the electrically assisted permeation of NA, whereas the permeation of NAB and SDN remained unchanged. For the CMC-based hydrogels, both the viscosity effect as well as the competitive ion effect by the counterions of CMC (Na^+) may account for the reduced drug permeation under electric field from higher percentage of CMC hydrogels. The present study demonstrated the feasibility of skin permeation of NA, NAB and SDN from solutions and hydrogels by passive diffusion and electrically assisted methods.

References

Banga, A.K., Bose, S., Ghosh, T.K., 1999. Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. 179, 1–19.

Cherny, N.I., 1996. Opoid analgesics: comparative features and prescribing guidelines. Drugs 51, 713–737.

Cullander, C., 1992. What are the pathways of iontophoretic current flow through mammalian skin? Adv. Drug Deliv. Rev. 9, 119–135.

Fang, J.Y., Huang, Y.B., Lin, H.H., Tsai, Y.H., 1998. Transdermal iontophoresis of sodium nonivamide acetate IV. Effect of polymer formulation. Int. J. Pharm. 173, 127–140.

Fang, J.Y., Hwang, T.L., Huang, Y.B., Tsai, Y.H., 2002. Transdermal iontophoresis of sodium nonivamide acetate V. Combined effect of physical enhancement methods. Int. J. Pharm. 235, 95–105.

Fang, J.Y., Sung, K.C., Hu, O.Y.P., Chen, H.Y., 2001. Transdermal delivery of nalbuphine and nalbuphine pivalate from hydrogels by passive diffusion and iontophoresis. Arzneim-Forsch./Drug Res. 51, 408–413.

Finnin, B.C., Morgan, T.M., 1999. Transdermal penetration enhancers: application, limitation, and potential. J. Pharm. Sci. 88, 955–958.

Grond, S., Radbruch, L., Lehmann, K.A., 2000. Clinical pharmacokinetics of transdermal opioids. Clin. Pharmacokinet. 38, 59–89.

Hirvonen, J., Guy, R.H., 1997. Iontophoretic delivery across the skin: electroosmosis and its modulation by drug substances. Pharm. Res. 14, 1258–1263.

Ho, H.O., Huang, F.C., Sokoloski, T.D., Sheu, M.T., 1994. The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. J. Pharm. Pharmacol. 46, 636–642.

Jadoul, A., Lecouturier, N., Mesens, J., Caers, W., Préat, V., 1998. Transdermal alniditan delivery by skin electroporation. J. Control. Release 54, 265–272.

Jadoul, A., Préat, V., 1997. Electrically enhanced transdermal delivery of domperidone. Int. J. Pharm. 154, 229–234.

Lo, M.W., Schary, W.L., Whitney Jr., C.C., 1987. The disposition and bioavailability of intravenous and oral nalbuphine in healthy volunteers. J. Clin. Pharmacol. 27, 866–873.

Pao, L.H., Hsiong, C.H., Hu, O.Y.P., Ho, S.T., 2000. High-performance liquid chromatographic method for the simultaneous determination of nalbuphine and its prodrugs, sebacoyl dinalbuphine ester, in dog plasma and application to pharmacokinetic studies in dogs. J. Chromatogr. B 746, 241–247.

Prausnitz, M.R., Edelman, E.R., Gimm, J.A., Langer, R., Weaver, J.C., 1995. Transdermal delivery of heparin by skin electroporation. Biotechnology 13, 1205–1209.

Riviere, J.E., Heit, M.C., 1997. Electrically-assisted transdermal drug delivery. Pharm. Res. 14, 687–697.

Roy, S.D., Hou, S.Y.E., Witham, S.L., Flynn, G.L., 1994. Transdermal delivery of narcotic analgesics: comparative metabolism and permeability of human cadaver skin and hairless mouse skin. J. Pharm. Sci. 83, 1723–1728.

Stinchcomb, A., Paliwal, A., Dua, R., Imoto, H., Woodard, R.W., Flynn, G.L., 1996. Permeation of buprenorphine and its ester prodrugs through skin. Pharm. Res. 13, 1519–1523.

Sung, K.C., Fang, J.Y., Hu, O.Y.P., 2000. Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis. J. Control. Release 67, 1–8.

Sung, K.C., Fang, J.Y., Wang, J.J., Hu, O.Y.P., 2003. Transdermal delivery of nalbuphine and its prodrugs by electroporation. Eur. J. Pharm. Sci. 18, 63–70.

Sung, K.C., Han, R.Y., Hu, O.Y.P., Hsu, L.R., 1998. Controlled release of nalbuphine prodrugs from biodegradable polymer matrices: influence of prodrug hydrophilicity and polymer composition. Int. J. Pharm. 172, 17–25.

Tyle, P., 1986. Iontophoretic devices for drug delivery. Pharm. Res. 3, 318–326.

Wang, J.J., 1992. Pharmacodynamic and pharmacokinetic studies with long acting novel nalbuphine prodrugs. Ph.D. Thesis, National Defense Medical Center, Taiwan.