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Evaluation of transdermal iontophoresis of enoxacin from polymer formulations: in vitro skin permeation and in vivo microdialysis using Wistar rat as an animal model

Jia-You Fang [a], Li-Ren Hsu [b], Yaw-Bin Huang [c], Yi-Hung Tsai [c,*]

[a] *Graduate Institute of Pharmaceutical Sciences, Taipei Medical College, Taipei, Taiwan ROC*
[b] *School of Pharmacy, Chia Nan College of Pharmacy and Science, Tainan Hsien, Taiwan ROC*
[c] *School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan ROC*

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Abstract

Polymers were used in vehicles to form hydrogel matrices in this study to evaluate the in vitro permeation and in vivo microdialysis of enoxacin. The highest transdermal delivery determined by area under flux-time curve (AUC) and intracutaneous enoxacin concentration were observed in methylcellulose (MC) and polyvinylpyrrolidone (PVP) hydrogels, respectively. To avoid the pH shift in vehicles during iontophoresis, buffer species were added to formulations to increase the buffer capacity. As expected, the permeability of enoxacin of anodal iontophoresis was larger than that of cathodal iontophoresis. Combination of benzalkonium chloride, a cationic surfactant as an enhancer, and iontophoresis exerted an enhancing effect for anionic enoxacin at pH 10.0. However, no effect or a negative effect was detected for cationic enoxacin in deionized water or pH 5.0 buffer, due to the shielding of the negative charge in the skin. The skin residue of enoxacin was slightly increased after the incorporation of Azone in PVP hydrogel. The result of in vivo microdialysis was in accordance with that of in vitro study. The effect of Azone on the intracutaneous enoxacin was more significant for in vivo microdialysis than in the in vitro study indicating the clinical feasibility of Azone for iontophoretic delivery. Microdialysis can be considered as a useful technique to investigate the pharmacokinetics of transdermal iontophoresis in vivo.

Keywords: Eenoxacin; Microdialysis; Polymer; Penetration enhancer; Transdermal iontophoresis; Wistar rats

1. Introduction

Most of the fluoroquinolones are available as oral and intravenous preparations. Anorexia, nau-

* Corresponding author.

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sea, vomiting, diarrhoea and metallic taste are occasionally reported after oral administration of fluoroquinolones. Furthermore, the interactions between fluoroquinolones and antacids containing magnesium and aluminum result in the inhibition of oral bioavailability (Sorgel et al., 1989). So transdermal drug delivery may be suitable for fluoroquinolones to reduce these adverse effects. In earlier research, enoxacin had the highest penetration capability through rat skin among six quinolone agents (Dzau, 1991). Accordingly enoxacin is used as the model drug for percutaneous absorption in this paper. The biological half-life of enoxacin is 3–6 h, and its therapeutic plasma concentration is 1–4 mg/ml for systemic use (Schulz and Schmoldt, 1997). Enoxacin also acts on topical skin and soft tissue infections (Henwood and Monk, 1988), so the experimental data on transdermal penetration and residues in the skin reservoir were assessed in this study.

Iontophoresis is a process by which the transport of ions into or through skin is increased by the application of an external electrical field across the skin (Sims et al., 1991). Since enoxacin is ionized as either cation or anion at appropriate pH, it is suitable for iontophoretic delivery (Fang et al., 1998). When the transdermal iontophoretic delivery system is administered clinically, the patch or semisolid dosage form may be more applicable than solution. However, most research on transdermal iontophoresis has focused on the discussion of solution vehicles. In order to hold promise for further in vivo application, the polymer is added to the vehicle to form a hydrogel matrix in this study of in vitro and in vivo transdermal iontophoresis. Polymers have been used extensively in the development of systems to provide controlled delivery of drugs (Langer, 1990). There are advantages for polymer formulation such as no risk of 'dumping' of a large part of the dose and the manufacturing process is notably straightforward (Vazquez et al., 1992). Furthermore, there is always a great volume of water employed in gel formulations which exhibits a high electrical conductivity (Fang et al., 1996).

Although there have been some successful polymer formulations for drugs developed and commercially available, one cannot simply substitute one drug for another in a given system. Each drug requires a dosage form specifically designed to fit its properties. Thus the formulation of enoxacin is adjusted for optimal penetration capacity and physicochemical characteristics using various types of hydrophilic polymers. In addition to the in vitro permeation study, the viscosity of the formulation is also determined since it is the most widely utilized method for the characterization of polymers (Mitchell et al., 1993). For good patient compliance the transdermal device such as a patch should be as small and as thin as possible. One of the ways is to incorporate a chemical enhancer (Hadgraft, 1996). Accordingly two enhancers, benzalkonium chloride and Azone are incorporated in polymer hydrogels.

In vivo transdermal iontophoresis in the rat is carried out here using microdialysis as a sampling technique since the microdialysis system has been found to be useful for assessing transdermal absorption (Matsuyama et al., 1994). Microdialysis is a non-traumatic method which allows continuous direct measurements of substances in the interstitial space of a tissue or organ (Lonnroth and Smith, 1990). In this study, a tubular dialysis membrane is introduced intradermally, the tube is perfused with Ringer's solution that equilibrates with the fluid outside the tube by diffusion in both directions. The most important features of microdialysis are that it samples the extracellular fluid; it samples continuously for hours; and it purifies the sample and simplifies chemical analysis by excluding large molecules such as proteins from perfusate (Ungerstedt, 1991). The demerit of this technique is that the sampling amount may be not enough for analyzing due to the slow perfusion rate.

2. Materials and methods

2.1. Materials

Enoxacin was supplied by Sigma (St. Louis MO, USA). Carbopol 940® was obtained from B.F. Goodrich (USA). Polyvinylpyrrolidone (PVP), with different molecular weights: M_w 40000, M_w 360000 and M_w 1200000, polyvinyl alcohol (PVA)

($n = 1750$), methyl cellulose (MC) with different viscosity: 20–30, 80–120 and 350–550 cps, carboxymethyl cellulose sodium (CMC) ($n = 500$) were obtained from Tokyo Kasei (Japan). Hydroxypropyl methylcelulose (Metolose®) (4000 cps) was purchased from Shin-Etsu (Japan). All other chemicals and solvents were of analytical grade.

2.2. Preparation of hydrogels

A 5% (w/w) polymer in purified water or buffer was prepared. Enoxacin was participated in the base to give a concentration of 0.2%. The Carbopol 940® hydrogel was prepared according to the previous study (Fang et al., 1996). Buffers of pH 5.0 and 7.5 were citrate-phosphate systems (McIlvaine buffer). A borax-sodium hydroxide system was used to prepare pH 10.0 buffer, the buffer capacity was 0.06 M. The pH of the polymer vehicle was adjusted with either 1 M NaOH or 1 M HCl. In the study of combination of iontophoresis and enhancer effect, 5% benzalkonium chloride or Azone was added to the hydrogel matrix. In vitro and in vivo experiments were performed after preparation of the hydrogels (Martin et al., 1983).

2.3. Instruments and in vitro permeation procedures

The in vitro permeation procedures of iontophoresis were determined using the horizontal glass diffusion cells. The abdominal skin of excised Wistar rat was used as the model membrane, soaked in the receptor buffer solution for 45 min prior to being placed in the cells. The receptor phase contained 8 ml of 0.06 M, pH 7.4 McIlvaine buffer was used. The available diffusion surface area was 0.785 cm^2. A pair of platinum wires having an effective length of 15 mm (99.99% purity, 0.5 mm in diameter) used as electrodes was immersed in the cell with the anode in donor and cathode in receptor when using for pH 5.0 and pH 7.5 hydrogels. The electrode polarity was reversed for anion iontophoresis when applying for pH 10.0 hydrogel. The electrodes were connected to a current power supplier (Yokogawa, Model 7651, Japan). Current density of 0.3 mA/cm^2 was applied to stimulate the penetration of enoxacin.

The donor and receptor compartments were maintained at 37°C and agitated by magnetic stirrer at 600 rpm. The 200 ml samples were withdrawn from receptor at regular intervals and immediately replaced by an equal volume of fresh receptor solution. The samples were assayed by HPLC as described previously (Fang et al., 1997). After the termination of the in vitro permeation study, a test was performed to determine the residual enoxacin in skin. The rat skin was washed twenty times using the cotton cloth immersed in 0.1 N HCl. The treated skin area was cut by the scissors in small pieces and weighed, then positioned in a glass homogenizer containing 6 ml of 0.1 N HCl, mixed for 2 min by the electric stirrer at appropriate rpm. The resulting homogenized solution was centrifuged for 15 min at 4000 rpm. The supernatant was filtered through 0.45 mm artificial membrane filter (Whatman, USA) prior to analysis by HPLC.

2.4. Viscosity measurements

Measurements of the viscosity were carried out on hydrogels before and after the performance of in vitro permeation study. Tests were maintained at 37°C in a cone and plate viscometer (Brookfield, Model DV-2, USA). Readings were recorded 30 s after the measurement was made, when the level had stabilized.

2.5. In vivo microdialysis

The microdialysis system basically consisted of a CMA/102 microinjection pump (Carnegie Medicin, Sweden), which delivers sterile Ringer's solution at a flow rate of 1 ml/min as perfusate to the probe, and a microfraction collector (CMA/142). The microdialysis probes used in this study were custom made, with a membrane length of 4 mm. Female Wistar rats were anesthetized by intraperitoneal injection of 6% sodium pentobarbital (0.1 ml/100 g weight). For intradermal microdialysis, modified from the method of Matsuyama et al. (1994), the abdominal fur of rats was shaved. As shown in Scheme 1, the skin

was then incised over the dermis, followed by intradermal insertion of an introducer assembled by inserting a stainless needle into the tubing. After placing the tubing under skin, the needle was withdrawn, followed by insertion of probe and the tubing removed. After probe implantation, a hemispherical glass reservoir with the available diffusion area of 3.80 cm^2 was placed above the tip of the probe on skin. A 8 g polymer formulation was added into the reservoir. The anode was inserted in the hydrogel, while the cathode was inserted into the incised region of rat skin. Both electrodes had an effective length of 15 mm. The probe was connected to the microdialysis system after introduction and positioning in skin.

2.6. In vivo recovery of enoxacin across the microdialysis probe

A probe was inserted into dermis as indicated above. Ringer's solution containing enoxacin (50 mg/ml) was passed through the probe using an infusion pump. The dialysate samples were collected every 1 h for 7 h in total. The recovery ratio of enoxacin was calculated from the following equation:

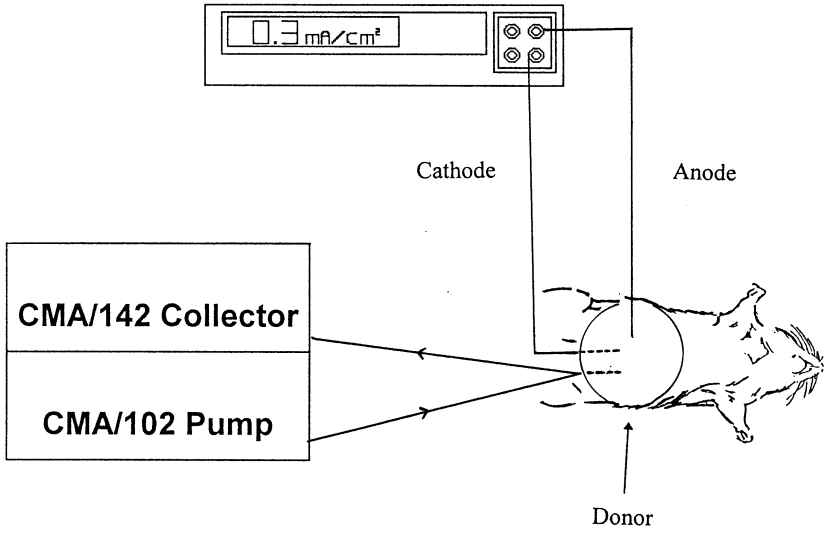
$$= 1 - \text{Effluent dialysate amount/Influent dialysate amount}$$

This equation can be derived assuming that drug recovery from the tissue to perfusate is the same as drug loss from perfusate to tissue, across the probe membrane (retrodialysis method) (Elmquist and Sawchuk, 1997).

3. Results

3.1. Effect of various polymer formulations

Various enoxacin formulations of polymer in deionized water were performed for 0.3 mA/cm^2 iontophoretic delivery for 6 h. Fig. 1 shows the flux (dQ/dt)-time profile of six polymer formulations. The flux at a given time point is calculated from the amount of enoxacin permeated per cm^2 of skin from one time point to the next. The polymers utilized in this study are frequently used to provide controlled release of drugs from matrices. To provide a summary measure to evaluate experimental data, the area under the curve (AUC) was calculated from 0 to 6 h after application. According to the pH values of hydrogels, the



Scheme 1. Scheme of the apparatus used for the in vivo microdialysis of transdermal iontophoretic delivery.

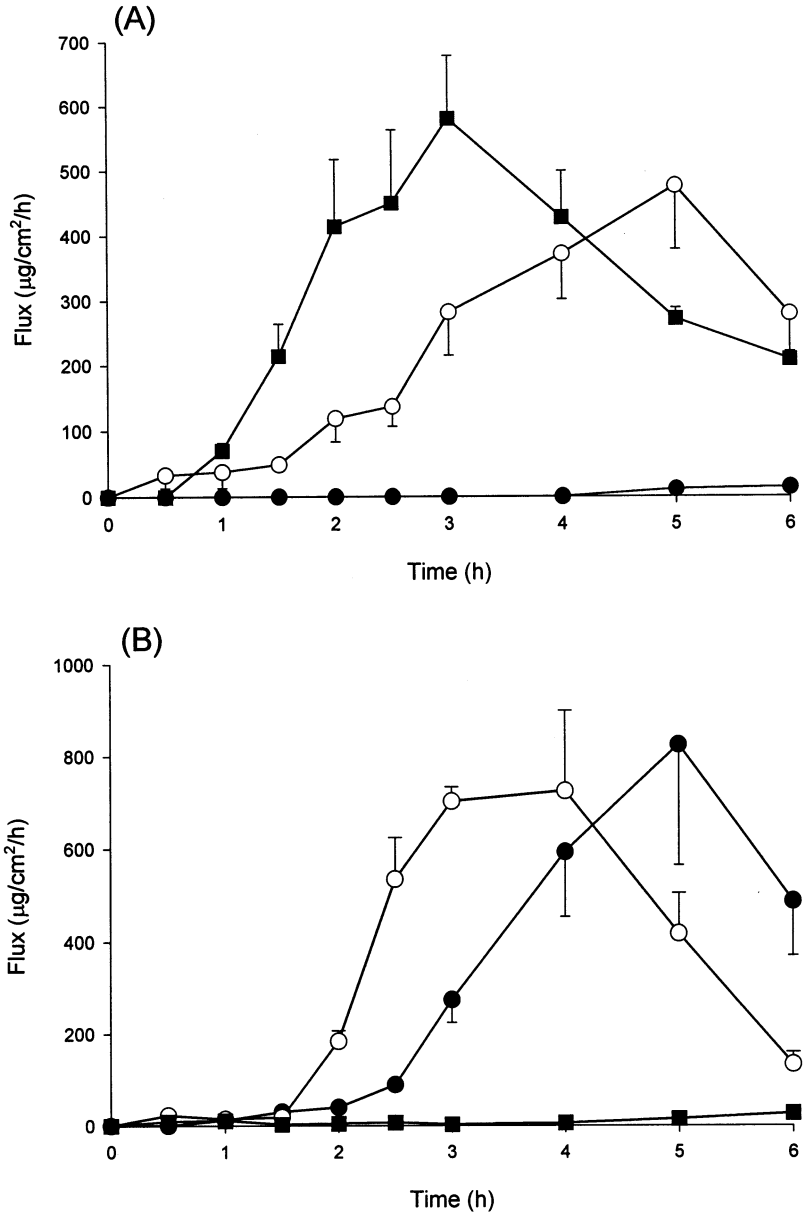


Fig. 1. Plot of iontophoretic flux versus time for enoxacin with various polymers in deionized water at 0.3 mA/cm^2: (A) (●) Carbopol 940®, (○) PVA, (■) PVP, (B) (●) MC, (○) HPMC, (■) CMC. All data represent the means of three experiments ± S.D.

anodal iontophoresis was carried out in all formulation except for Carbopol 940® gel since the isoelectric point of enoxacin is pH 7.5. As shown in Table 1, the AUC and intracutaneous enoxacin concentration are found to be influenced by the kind of polymers greatly. The AUC of enoxacin from the gels prepared with MC or HPMC were similar and showed the highest permeation capacity among the six polymers. However, the highest enoxacin levels retained in skin were detected in PVP and HPMC. Both anionic polymers of Carbopol 940® (polyacrylic acid) and CMC showed

negligible permeation amount and skin residues of enoxacin.

The pH of the donor gel was found to decrease after anodal iontophoresis and to increase after cathodal iontophoresis. This is a common phenomenon in platinum electrodes which the electrochemical decomposition of water with the production of H^+ ions at the anode and OH^- ions at the cathode. In general, the permeation capacity of enoxacin in polymers was directly related to the difference between the pH values of hydrogels before and after in vitro permeation study as shown in Table 1. Viscosity is the most widely utilized method for the characterization of hydrophilic polymers. In particular the most viscous hydrogel is found in HPMC as shown in Table 1. In light of the viscosity change after permeation, the viscosity was increased except for the anionic polymer of CMC for anodal iontophoresis and Carbopol 940® for cathodal iontophoresis. As depicted in Table 1, the highest AUC was observed with the MC formulation. On the other hand, the highest intracutaneous enoxacin amount was observed with the PVP formulation. For this reason, both hydrogels were utilized in the following experiments.

3.2. Effect of various vehicular pH values

As shown in Table 1, the pH of hydrogels shifts due to the lack of buffer capacity for deionized water in formulations. So it is important to optimize the pH value and ionic strength of buffer species used in the formulation to maintain good buffer capacity and stable pH but should not reach an extent such that the current is mostly carried by buffer species instead of the drug. The buffer pH values selected in this study were pH 5.0, 7.5 and 10.0 due to the charge variety of enoxacin of cationic, neutral and anionic molecules, respectively. As depicted in Fig. 2, the permeability of enoxacin increased in the order pH $10.0 < 7.5 < 5.0$. This trend was the same as that of enoxacin iontophoresis in aqueous solution (Fang et al., 1998). Moreover, Fig. 2 demonstrates that a steady-state flux may be achieved for anodal iontophoresis at pH 5.0. The time taken to reach the steady-state flux for enoxacin was approximately 1.5 h. As shown in Table 2, variations in pH and buffer species do not seem to significantly affect the viscosity of polymer. Besides, the viscosity after iontophoresis slightly increased for all formulations although not all formulations showed a significant difference (t-test, $p < 0.05$).

3.3. Effect of various polymer molecular weights

The permeability of enoxacin from gel formulations of PVP and MC with various molecular weights was determined and the result is shown in Table 3. As expected, the viscosity of polymer increased with increase of molecular weight, but both the AUC and enoxacin in skin were similar for the various molecular weights. As a result, the viscosity of the gel matrices shows only a minor

Table 1
Initial and final pH value and viscosity, AUC, enoxacin retained in skin of various polymer formulations after transdermal iontophoresis of enoxacin

Polymer	pH value of donor		Viscosity ($cps \times 10^3$)		AUC ($\mu g/cm^3$)	Enoxacin in skin (mg/g)
	Initial	Final	Initial	Final		
PVA	7.10	3.87	9.08 ± 0.21	9.76 ± 0.61	1383.39 ± 289.18	12.64 ± 4.95
PVP	6.53	3.69	6.03 ± 0.33	6.28 ± 0.43	1811.69 ± 85.93	23.90 ± 6.37
Carbopol 940	7.88	8.08	17.0 ± 1.16	12.3 ± 0.98	17.72 ± 5.60	0.05 ± 0.03
MC	6.95	3.05	7.24 ± 1.21	8.88 ± 2.11	2364.83 ± 572.96	15.95 ± 7.09
HPMC	7.40	3.46	73.6 ± 3.95	80.8 ± 4.96	2121.39 ± 420.88	22.97 ± 4.09
CMC	7.24	6.19	17.2 ± 1.01	14.2 ± 0.51	52.27 ± 13.63	0.12 ± 0.04

Each value represents the mean $\pm$ S.D. ($n = 3$) except pH value of donor.

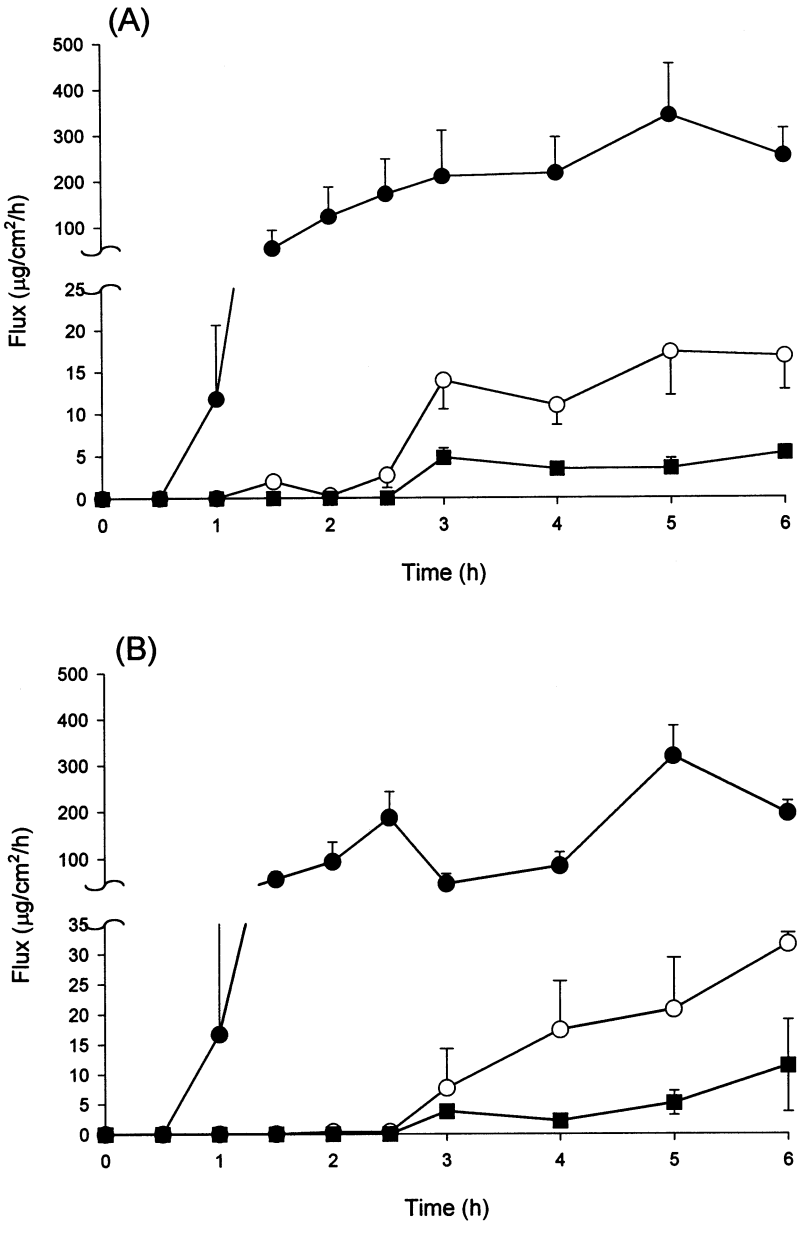


Fig. 2. Plot of iontophoretic flux versus time for enoxacin with various polymers in buffer at 0.3 mA/cm^2: (A) PVP, (B) MC; (●) pH 5.0 buffer, (○) pH 7.5 buffer, (■) pH 10.0 buffer. All data represent the means of three experiments ± S.D.

influence on enoxacin penetration. In general, higher molecular weight materials lead to more sustained release, but more careful analysis of the data shows that the release is only affected in the initial period (Doelker, 1987).

3.4. The incorporation of penetration enhancers

Benzalkonium chloride was chosen in this study since it can greatly enhance the percutaneous absorption of enoxacin (Fang et al., 1998). Be-

sides, Azone is known to achieve a synergistic effect with iontophoresis (Ganga et al., 1996; Kalia and Guy, 1997). The hydrogel without enhancers in formulation served as control group. As shown in Fig. 3, benzalkonium chloride and Azone have no effect or a negative effect on the penetration capacity of enoxacin in anodal iontophoresis. However, the cathodal iontophoresis at pH 10.0 showed an enhancement when incorporated with both enhancers. The presence of Azone in PVP hydrogel significantly (t-test, $p < 0.05$) promotes the retention of enoxacin in full-thickness skin in a pH 10.0 hydrogel, but this phenomenon is not observed for MC.

3.5. In vivo microdialysis

Recently, in vivo microdialysis has found important applications in the field of pharmacokinetics, especially in the area of drug distribution and metabolism. Firstly, knowledge of the fractional recovery of the drug is a prerequisite for calculating tissue extracellular concentrations of the drug (Elmquist and Sawchuk, 1997). The relative recovery-time profile of intradermal enoxacin is shown in Fig. 4. This data indicated that the enoxacin concentrations in the dialyzed tissue might be about 3- to 6-fold higher than those detected in the dialysate. Three hydrogel formulations of PVP were atudied in vivo. The result is shown in Fig. 5. The AUC increased in the order of PVP in pH 5.0 buffer < PVP in deionized water < PVP with Azone in deionized water.

4. Discussion

Platinum electrodes have been known to cause pH drift in vehicle as current density applied as observed in Table 1. The flux of enoxacin decreases at the end-stage of application of polymers as shown in Fig. 1. With a charged membrane, the ion flow may be diffusion limited, the membrane conductivity reaching a limiting value at high concentrations of ion when the pores become saturated. Subsequently a maximum flux is reached during enoxacin iontophoresis, after which there is a gradual reduction in the flux.

The PVP formulation offered high permeability and skin residues of enoxacin. Nalidixic acid interacts only weakly with PVP in aqueous solution (Sekikawa et al., 1978). According to this inference, enoxacin may also interacts weakly with PVP results. Since enoxacin is supersaturated at 2 mg/g in hydrogels, precipitation (crystallisation) was observed during experiments. PVP is an excellent crystal growth retardant for drugs. It slows the transformation of the drug from its high energy state to the stable crystallic form. The adsorption of PVP on to the hydrophobic surface of crystals has been (Megrab et al., 1995).

The permeability of cathodal iontophoresis of Carbopol 940® for enoxacin was low which may be due to the anionic characteristic of Carbopol 940® carrying a part of current. Another explanation is that enoxacin was not completely ionized in pH 7.88, the pH value of Carbopol 940® formulation, so that only a small fraction of current

Table 2
Initial and final pH value and viscosity, AUC, enoxacin retained in skin of PVP and MC polymer formulations in various buffer vehicles after transdermal iontophoresis of enoxacin

Polymer	pH value of donor		Viscosity ($cps \times 10^3$)		AUC ($\mu g/cm^3$)	Enoxacin in skin (mg/g)
	Initial	Final	Initial	Final		
PVP	5.0	3.65	8.28 ± 0.54	9.52 ± 0.52	973.45 ± 320.69	5.25 ± 1.97
	7.5	6.95	5.88 ± 0.32	7.32 ± 0.47	50.19 ± 12.16	0.26 ± 0.09
	10.0	10.33	6.84 ± 0.81	7.48 ± 1.15	13.11 ± 2.10	1.17 ± 0.07
MC	5.0	4.08	8.52 ± 1.21	9.28 ± 1.22	707.04 ± 96.95	2.78 ± 0.95
	7.5	7.21	6.36 ± 0.97	9.04 ± 0.34	59.87 ± 11.23	0.24 ± 0.07
	10.0	10.21	7.44 ± 0.64	10.9 ± 0.97	16.90 ± 11.47	1.17 ± 0.16

Each value represents the mean $\pm$ S.D. ($n = 3$) except pH value of donor.

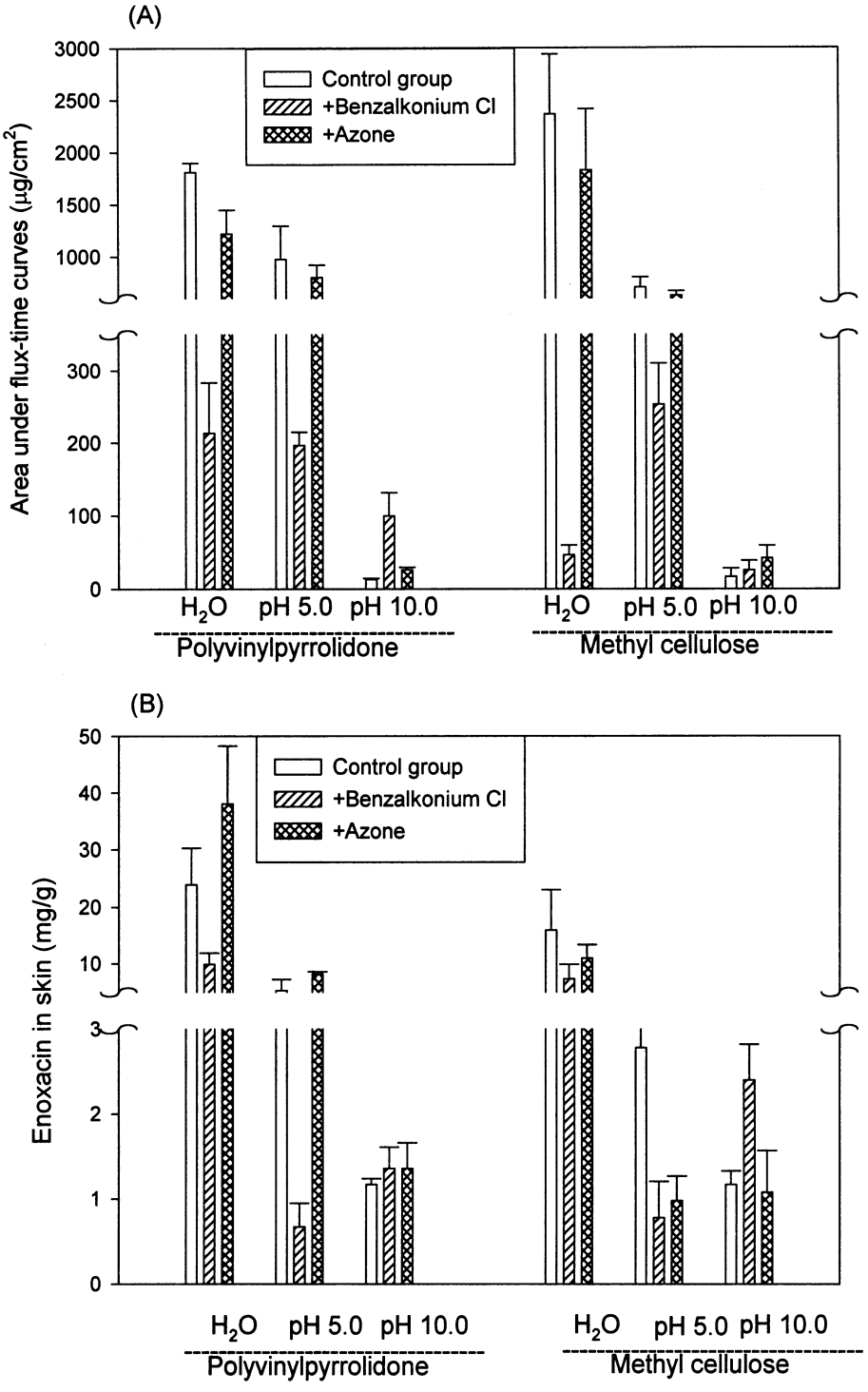


Fig. 3. Area under flux-time curves (A) and drug retained in skin (B) of enoxacin from PVP and MC hydrogels combined with benzalkonium chloride and Azone at 0.3 mA/cm^2. All data represent the means of three experiments $\pm$ S.D.

Table 3
Initial viscosity, AUC, enoxacin retained in skin of PVP and MC polymer formulations of various types in pH 5.0 buffer vehicle after transdermal iontophoresis of enoxacin

Polymer	Type	Viscosity (cps $\times 10^3$)	AUC (μg/cm^2)	Enoxacin in skin (mg/g)
PVP	40 000 M_w	6.32 ± 0.36	1125.14 ± 231.08	3.99 ± 0.70
	360 000 M_w	8.28 ± 0.54	973.45 ± 320.69	5.25 ± 1.97
	1200 000 M_w	10.0 ± 0.89	1075.82 ± 178.60	4.30 ± 0.48
MC	20–30 cps	8.20 ± 0.33	709.27 ± 91.12	2.41 ± 0.80
	80–120 cps	8.52 ± 1.21	707.04 ± 96.95	2.78 ± 0.95
	350–550 cps	15.6 ± 2.76	742.09 ± 151.50	1.98 ± 0.99

Each value represents the mean $\pm$ S.D. ($n = 3$) except pH value of donor.

was carried by ionized enoxacin. The low penetration capacity was also observed for anodal iontophoresis of CMC in which sodium cations act as the competitive ion. In conclusion, for maintaining a high flux of enoxacin, ionizable polymers should be avoided for transdermal iontophoretic delivery.

Iontophoretic transport across skin is the cumulative result of four factors: the inherently passive diffusion of the permeant, J_o; a direct effect of the field on charged permeant, $J_{\Delta v}$; an increase in skin permeability induced by the field, J_s; and the electroosmotic flow induced by current, J_{osm}. Subsequently the total flux is determined to the following equation:

$$J_{total} = J_o + J_{\Delta v} + J_s \pm J_{osm} \quad (1)$$

The sign $\pm J_{osm}$ depends on the membrane and electrode polarity. The upper layers of skin are known to have an isoelectric point of pH 4, so the pores in stratum corneum will have a negative charge in a solution with pH 4 or higher. Accordingly the direction of electroosmotic flow for pH 5.0 proceeds from anode-to-cathode. In other words, the sign of J_{osm} was positive in Eq. 1. Electroosmosis can affect of iontophoretic flux. It causes migration of neutral solutes such as enoxacin in pH 7.5 solution. The factor of $J_{\Delta v}$ in Eq. 1 does not exist when enoxacin is neutral. However, the electrochemical decomposition of water with production of hydronium ions and a reduction of pH value at anode when using platinum wires as electrodes, could cause the transformation of a proportion of neutral solutes into charged ones. The cathode was placed in donor at pH 10.0 since enoxacin is the negatively charged in this condition. Cathodal iontophoresis is less effective than anodal iontophoresis, as expected from the imposed direction of electroosmotic flow. Subsequently the sign of J_{osm} in Eq. 1 should be negative for cathodal iontophoresis.

Viscosity can act as a reference to evaluate the strength of the gel although it is not sufficiently comprehensive for full characterization of the gel structure. The viscosity of Carbopol 940® was significantly reduced (t-test, $p < 0.05$) after cathodal iontophoresis. It is our viewpoint that water moves from anode (receptor) to cathode (donor) due to the direction of electroosmotic flow which results in the reduction of Carbopol 940® viscosity by dilution. This also explains the slight increase

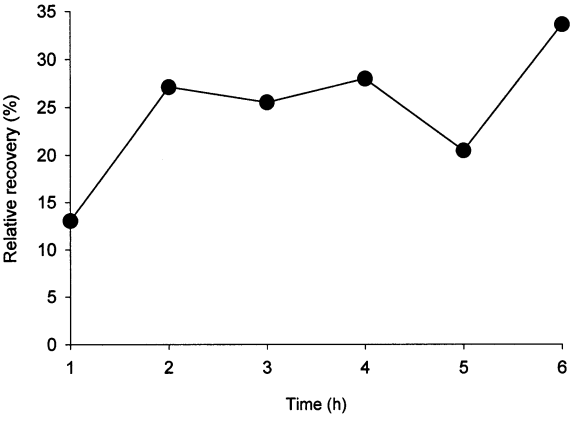


Fig. 4. In vivo relative recovery of intradermal enoxacin during microdialysis at various periods. All data represent the means of three experiments $\pm$ S.D.

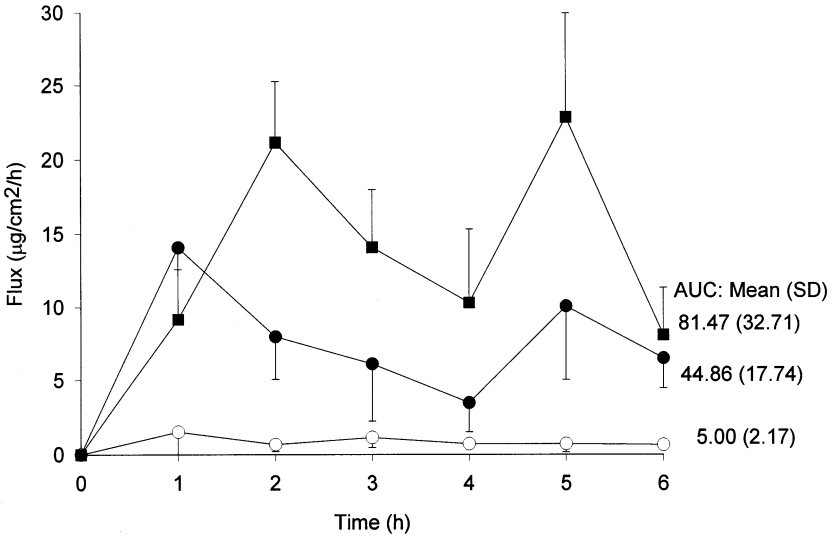


Fig. 5. Plot of flux versus time for enoxacin from various PVP hydrogels at 0.3 mA/cm^2 using in vivo microdialysis as the sampling technique: (●) PVP with deionized water, (○) PVP with pH 5.0 buffer, (■) PVP with deionized water combined with 5% Azone. All data represent the means of three experiments ± S.D.

of polymer viscosity after anodal iontophoresis such as PVA, PVP, MC and HPMC although the significant difference (t-test, $p < 0.05$) was not always detected between initial and final viscosity for these four hydrogels.

To minimize the shift of pH during iontophoresis, deionized water was replaced by buffer as aqueous phase in hydrogels. As expected, the permeability of enoxacin was decreased after replacement due to the competitive effect of current density with buffer species. As observed in Fig. 2, the flux of enoxacin gradually rises at the end stage of application at pH 7.5. The pH value of vehicle reduced to a more acidic condition for pH 7.5 buffer during iontophoresis which could cause the transformation of a proportion of neutral solutes into charged ones resulted in the enhancement of iontophoretic delivery. Judging from the enoxacin retained in skin, at pH 5.0 the skin was observed to have an excess of anionic sites, so the cationic enoxacin attached to skin reservoir extensively as expected. The intradermal enoxacin concentration was the lowest at pH 7.5, which may be due to the fact that in the presence of an electrical field the stratum corneum lamellae interior becomes more accessible to ions but not to neutral solutes (Pechtold et al., 1996).

The iontophoretic penetration determined by AUC and enoxacin accumulation in skin was significantly reduced (t-test, $p < 0.05$) after combination with benzalkonium chloride at pH 5.0 buffer and deionized water. Nevertheless, the cathodal iontophoresis at pH 10.0 showed enhancing effect when incorporating with benzalkonium chloride. During the first 6 h of passive penetration profile, benzalkonium chloride did not appreciably enhance the delivery of enoxacin (Fang et al., 1998). However, the transport of enoxacin across skin was significantly increased when the data were analyzed beyond 6 h. This demonstrated the iontophoretic treatment duration of 6 h was not enough for benzalkonium chloride to exert its enhancing effect on skin structure. In addition, the benzalkonium ion also played a role of strong competitive ion for the reduction of transport of enoxacin. Another possible mechanism cited by Santi and Guy is the shielding of the net negative charge on skin (Santi and Guy, 1996a,b). Benzalkonium chloride possesses an obvious lipophilic 'anchor' in its structure that it can use to associate strongly with skin. As the number of ions such as benzalkonium chloride in the system increases more of the skin's

inherent charge is effectively shielded or reversed in the ion-conducting pathways. Thereby the level of electroosmotic flow decreases or drives in the opposite (cathode to anode) direction which resulted in diminished flux of enoxacin. This mechanism also explains the increased penetration capacity after addition of benzalkonium chloride for cathodal iontophoresis at pH 10.0 because of the change of electroosmotic direction. The alteration in the barrier properties of the skin at pH 10.0 also contributes to the enhancing effect of enoxacin when incorporating with benzalkonium chloride (Fang et al., 1998). The AUC of enoxacin decreased drastically when incorporated with benzalkonium chloride in MC-deionized water formulation. This may be due to MC interacting with the cationic surfactant (Doelker, 1987).

Azone is a non-polar material which dramatically affects the lipid structure of skin (Barry, 1987). However, there was no effect or a negative effect on the iontophoretic transport of enoxacin after incorporation of Azone. There is always a great volume of water employed in hydrogel formulation which exhibits a high electrical conductivity. The addition of 5% Azone, a neat liquid with high octanol/water partition coefficient, would reduce the conductivity of the system and the penetration capacity of enoxacin. Although the iontophoretic transport was much lower after the addition of Azone, the local enoxacin concentration in skin was slightly increased (t-test, $p < 0.05$) for the PVP formulation. However, this phenomenon could not be detected for the MC formulation. According to the theory of partitioning (Okabe et al., 1992; Schuckler and Lee, 1993), a difference in partition coefficient for the two polymers with the stratum corneum would have consequences for the uptake into stratum corneum. As PVP is relatively hydrophilic, the partition coefficients of Azone and enoxacin would be expected to favour the stratum corneum. In conclusion, physical iontophoresis combined with chemical enhancers does not produce enhanced effects on enoxacin delivery. It may be that the enhancement due to iontophoresis is so large that the enhancement due to penetration enhancers is negligible in comparison.

In the microdialysis study, the need for characterizing recovery in vivo, rather than relying upon values of recovery determined in vitro stemmed from observations by a number of investigators that differences in diffusion coefficients in vitro and in vivo are large (Elmquist and Sawchuk, 1997). The recovery of enoxacin was relatively low during the first 1 h. There is a rapid fall in the concentration of most substances in the perfusate during the initial phase of microdialysis, which is probably due to the establishment of a new steady-state level of most extracellular substances because of drainage through the probe (Amberg and Lindefors, 1989). So a period of tissue 'equilibration' after insertion may be necessary. The length of this period in this present study is about 1 h. For in vivo microdialysis of enoxacin iontophoretic delivery, the formulations with deionized water showed higher flux than that with buffer in accordance with the result of in vitro study.

The intradermal microdialysis not only detects the transdermal amount of substance, but also the substance accumulated in skin, since the probe is contacted with dermis sampling substance in the extracellular space of skin. Since the skin residual of enoxacin was enhanced after the incorporation of Azone in PVP for in vitro study, the same phenomenon was also observed for in vivo microdialysis. This effect was more significant for in vivo than in vitro, and suggests the clinical feasibility of using Azone for iontophoretic delivery. In conclusion, the present study demonstrates that relevant pharmacokinetic data may be obtained in the skin using in vivo microdialysis.

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References

Amberg, G., Lindefors, N., 1989. Intracerebral microdialysis. II. Mathematical studies of diffusion kinetics. J. Pharmacol. Methods 22, 157–183.

Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. J. Control. Release 6, 85–97.

Doelker, E., 1987. Water swollen cellulose derivatives in pharmacy. In: Peppas, N.A. (Eds.), Hydrogels in medicine and pharmacy, vol. II: Polymers. CRC Press, Boca Raton, FL, pp. 115–160.

Dzau, Y.I., 1991. Master Thesis, Kaohsiung Medical College, Kaohsiung, Taiwan.

Elmquist, W.F., Sawchuk, R.J., 1997. Application of microdialysis in pharmacokinetic studies. Pharm. Res. 14, 267–288.

Fang, J.Y., Huang, Y.B., Wu, P.C., Tsai, Y.H., 1996. Transdermal iontophoresis of sodium nonivamide acetate. II. Optimization and evaluation on solutions and gels. Int. J. Pharm. 145, 175–186.

Fang, J.Y., Lin, H.H., Hsu, L.R., Tsai, Y.H., 1997. Characterization and stability of various enoxacin liposome-encapsulated formulations. Chem. Pharm. Bull. 45, 1504–1509.

Fang, J.Y., Lin,H.H., Chen, H.I., Tsai, Y.H., 1998. Development and evaluation on transdermal delivery of enoxacin via chemical enhancers and physical iontophoresis. J. Control. Release 54, 293–304.

Ganga, S., Ramarao, P., Singh, J., 1996. Effect of Azone on the iontophoretic transdermal delivery of metoprolol tartrate through human epidermis in vitro. J. Control. Release 42, 57–64.

Hadgraft, J., 1996. Pharmaceutical aspects of transdermal nitroglycerin. Int. J. Pharm. 135, 1–11.

Henwood, J.M., Monk, J.P., 1988. Enoxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 36, 32–66.

Kalia, Y.N., Guy, R.H., 1997. Interaction between penetration enhancers and iontophoresis: effect on human skin impedance in vivo. J. Control. Release 44, 33–42.

Langer, R., 1990. New methods of drug delivery. Science 249, 1527–1533.

Lonnroth, P., Smith, U., 1990. Microdialysis—a novel technique for clinical investigations. J. Intern. Med. 227, 295–300.

Martin, A., Swarbrick, J., Cammarata, A., 1983. Polymer science. In: Physical Pharmacy. Lea and Febiger Press, pp. 592–638.

Matsuyama, K., Nakashima, M., Ichikawa, M., Yano, T., Satoh, T., Goto, S., 1994. In vivo microdialysis for the transdermal absorption of valproate in rats. Biol. Pharm. Bull. 17, 1395–1398.

Megrab, N.A., Williams, A.C., Barry, B.W., 1995. Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation. J. Control. Release 36, 277–294.

Mitchell, K., Ford, J.L., Armstrong, D.J., Elliott, P.N.C., Hogan, J.E., Rostron, C., 1993. The influence of drugs on the properties of gels and swelling characteristics of matrices containing methylcellulose or hydroxypropyl methylcellulose. Int. J. Pharm. 100, 165–173.

Okabe, H., Takayama, K., Nagai, T., 1992. Percutaneous absorption of ketoprofen from acrylic gel patches containing D-limonene and ethanol as absorption enhancers. Chem. Pharm. Bull. 40, 1906–1910.

Pechtold, L.A.R.M., Abraham, W., Potts, R.O., 1996. The influence of an electric field on ion and water accessibility to stratum corneum lipid lamellae. Pharm. Res. 13, 1168–1173.

Santi, P., Guy, R.H., 1996a. Reverse iontophoresis—Parameters determing electroosmotic flow: I. pH and ionic strength. J. Control. Release 38, 159–165.

Santi, P., Guy, R.H., 1996b. Reverse iontophoresis—Parameters determing electroosmotic flow: I. Electrode chamber formulation. J. Control. Release 42, 29–36.

Schuckler, F., Lee, G., 1993. Measuring the uptake of Azone into excised human stratum corneum from thin polymer films. J. Pharm. Pharmacol. 45, 162–165.

Schulz, M., Schmoldt, A., 1997. Therapeutic and toxic blood concentrations of more than 500 drugs. Pharmazie 52, 895–911.

Sekikawa, H., Nakano, M., Arita, T., 1978. Inhibitory effect of polyvinylpyrrolidone on the crystallization of drugs. Chem. Pharm. Bull. 26, 118–126.

Sims, S.M., Higuchi, W.I., Srinivasan, V., 1991. Skin alteration and convective solvent flow effects during iontophoresis: I. Neutral solute transport across human skin. Int. J. Pharm. 69, 109–121.

Sorgel, F., Jaede, U., Naber, K., Stephan, U., 1989. Pharmacokinetic disposition of quinolones in human body fluids and tissues. Clin. Pharmacokinet. 16 (Suppl. 1), 5–24.

Ungerstedt, U., 1991. Microdialysis—principles and applications for studies in animals and man. J. Intern. Med. 230, 365–373.

Vazquez, M.J., Perez-Marcos, B., Comez-Amoza, J.L., Martinez-Pacheco, R., Souto, C., Concheiro, A., 1992. Influence of technological variables on release of drugs from hydrophilic matrices. Drug Dev. Ind. Pharm. 18, 1355–1375.