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Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies

Jia-You Fang [a,*], K.C. Sung [b], Hung-Hong Lin [b], Chia-Lang Fang [c]

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

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Abstract

The objective of this study was to evaluate the in vitro and in vivo transdermal iontophoresis of various diclofenac sodium polymer formulations. The excised rat skin, human skin as well as cellulose membrane were used to examine the in vitro drug permeation whereas the microdialysis technique was used to monitor the drug concentration in vivo. Polymer solutions based on polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) binary system showed higher drug permeability than that of single polymer vehicle. The effect of formulations on drug permeation through cellulose membrane was quite different from those through rat skin and human skin, which can be explained by the different permeation pathways between them. It appeared to be a membrane-controlled mechanism but not the vehicle matrix-controlled mechanism for diclofenac hydrogels when using skin as the diffusion barrier. The recovery of diclofenac sodium in the in vivo microdialysis was approximately 80–90%, indicating this technique can be used in the intradermal drug monitoring. For all the polymer formulations tested, there was a good relationship between the in vitro and in vivo drug permeation. A synergistic effect on drug permeation was observed when transdermal iontophoresis combined with the pretreatment of cardamom oil as a permeation enhancer.

Keywords: Diclofenac sodium; Transdermal iontophoresis; Polymer; In vivo microdialysis

1. Introduction

Diclofenac sodium (diclofenac) has been widely used, systemically and locally, as anti-inflammatory agent for percutaneous absorption. However,

* Corresponding author. E-mail: fajy@msq.tisnet.net.tw.

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poor penetration of diclofenac through skin is always observed (Nishihata et al., 1987). The pore pathway in skin constitutes an important route for the penetration of sodium salt of diclofenac (Maitani et al., 1996). Based on the literature, the permeation of ionic drugs such as diclofenac can be facilitated by the application of iontophoretic technique via the shunt route (Tyle, 1986; Varghese and Khar, 1996). Iontophoresis is defined as the migration of ions when an external electric field is passed through a vehicle containing charged compounds. Accordingly, transdermal iontophoresis may be suitable for diclofenac to enhance its dermal permeability. Most researchers related to transdermal iontophoresis focus on the discussion of aqueous solution. However, when the transdermal iontophoretic delivery system is administered clinically, the patch or semisolid dosage form may be more applicable than solution. In order to hold promise for future clinical application of diclofenac via percutaneous absorption, this study developed a series of diclofenac loaded polymer formulations. An iontophoresis technique was also used to increase the permeation of diclofenac through skin.

For delivering a drug via transdermal route, an extensive application area may be needed for a desired therapeutic effect. One way to reduce device size is to incorporate penetration enhancers which will improve the permeation characteristic of the skin (Hadgraft, 1996). In previous study, the acetone extracts of Ammonum Cardamomum (Zingiberaceae) was found to enhance the passive permeation of diclofenac across skin in vitro (Huang et al., 1995). Accordingly, cardamom oil was selected to combine with iontophoresis to examine the permeation characteristics of diclofenac.

Microdialysis is a novel, non-traumatic method which allows continuous direct measurements of substances in the interstitial space of a tissue or organ. The most important features of microdialysis are: it samples the extracellular fluid which is the origin of all blood chemistry; it samples continuously for hours without withdrawing blood; and it purifies the sample and simplifies chemical analysis by excluding large molecules such as proteins from perfusate (Elmquist and Sawchuck, 1997). 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. This technique allows the continuous monitoring diclofenac concentrations in the dermal area.

In the present study, the in vitro transdermal iontophoresis of diclofenac released from different polymer formulations through various diffusion barriers including excised rat skin, full-thickness human skin and cellulose membrane were investigted. The in vivo transdermal iontophoresis in rat was also carried out using microdialysis as the sampling technique. The in vitro permeation studies were correlated with the in vivo transdermal results. Finally, the effects of combining cardamom oil and iontophoresis technique on diclofenac permeation were also examined.

2. Materials and methods

2.1. Materials

Diclofenac sodium was a gift from Yung Shin Pharmaceutical Ind. Co., Taiwan. Polyvinylpyrrolidone (PVP; M.W. 360 000) was supplied by Tokyo Kasei Ind. Co., Japan. Hydroxypropyl methylcellulose (HPMC, Metolose®, 4000 cps) was obtained from Shin-Etsu Co., Japan. Cardamom oil was a gift kindly provided by School of Pharmacy, Kaohsiung Medical College, Taiwan. All other chemicals and solvents were of analytical grade.

2.2. Preparation of polymer formulations

The polymers were added into pH 7.4 citrate-phosphate buffer to give a polymer concentration of 5% (w/w) totally. The ionic strength of buffer was 0.06 M. Diclofenac was icorporated into the vehicle to give a concentration of 1% (w/w). The polymer formulations were performed for transdermal experiments after 48 h of preparation since the viscosity and clarity would attain the highest value after this period (Martin et al., 1983).

2.3. Preparation of skin membranes

Samples of whole adult human skin (1.61 ± 10.25 mm) were obtained from breast reduction operations and provided by Kaohsiung Medical College, Taiwan. Subcutaneous fat was carefully trimmed and then rinsed with normal saline. The skin was then sealed in aluminium foil and a plastic bag and stored at -20°C until use. The skin of male Wistar rat (0.37 ± 00.01 mm) was excised from the abdominal region freshly before experiment. The hair of the abdominal region was shaved with electric clippers. The artificial cellulose membrane (Spectra/Por® 2 membrane, Spectrum Medical Ind. Co., USA) was presoaked in pH 7.4 buffer for 45 min to remove extractables before experiment.

2.4. Instruments and in vitro permeation procedures

The skin or membrane was mounted between the two half horizontal glass diffusion cells the same as previously described (Fang et al., 1997). The receptor phase contained 8 ml of pH 7.4 buffer was used. Donor compartment of the cell was filled with 8 g diclofenac polymer vehicle. The available diffusion surface area was 0.785 cm^2. The experiment was carried out at 37°C and the donor and receptor were agitated by magnetic stirrers at 600 rpm. 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 vehicle with the cathode in donor and anode in receptor. The anode and cathode 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 0.2 ml samples were withdrawn from the receptor at regular intervals and immediately replaced by an equal volume of fresh receptor solution.

In the study of penetration enhancer combined with iontophoresis, the 1 ml of cardamom oil was deposited onto the stratum corneum surface after mounting the skin or membrane in a device. Skin samples were pretreated with the cardamom oil for 12 h. After pretreatment, the enhancer solution was removed, and the skin was rinsed with distilled water and the permeation experiments were then started.

2.5. In vivo microdialysis

The microdialysis system consisted of a CMA/102 microinjection pump (Carnegie Medicin, Sweden), which delivers Ringer's solution at a flow rate of 1 ml/min as perfusate to the probe, and a microfraction collector (CMA/142). The microdialysis probe used in this study was custom made, with a membrane length of 10 mm. Male Wistar rat was anesthetized by intraperitoneal injection of 6% sodium pentobarbital. The intradermal microdialysis was modified from the method of Matsuyama et al. (1994). The abdominal fur of rat was shaved and 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 setting the tubing under skin, the needle was withdrawn, followed by insertion of probe and tearing off the tubing. After probe implantation, a glass cylinder with the available diffusion area of 3.8 cm^2 was placed above the tip of the probe on skin with glue (Superglue®, Alpha Tech. Co., Japan). A 8 g polymer formulations was added into the cylinder. The cathode was inserted in the apolymer. The anode 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.

The permeation procedure of in vivo microdialysis was identical to the three-stage experiment modified from our previous study (Fang et al., 1997). In stage I, the initially passive diffusion of diclofenac without applying current density was determined for 6 h. In stage II, a 0.5 mA/cm^2 current density was conducted for 6 h. In the last 4.5 h duration of stage III, the current density was turned off. The samples of either in vitro or in vivo study were assayed by HPLC method as described previously (Huang et al., 1995).

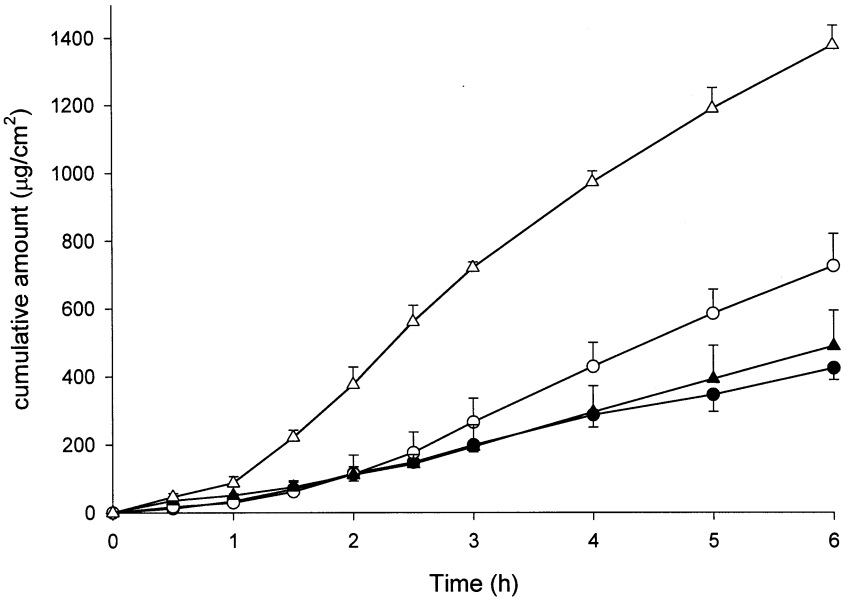

Fig. 1. Cumulative amount of diclofenac sodium detected in the receptor compartment versus time following iontophoresis from various hydrogels through excised rat skin: (●) PVP; (▲) HPMC; (○) PVP-HPMC; (△) PVP-HPMC pretreated with cardamom oil on skin. All data represent the means of three experiments ± S.D.

2.6. In vivo recovery of diclofenac across microdialysis probe

A probe was inserted into dermis as indicated above. Ringer's solution containing 20 m g/ml diclofenac was passed through the probe using an infusion pump. The dialysate samples were collected every 1.5 h for 21 h totally, which was the same as the sampling time plots of in vivo permeation study. The recovery ratio of diclofenac was calculated by the following equation:

$$\text{Recovery } (\%) = 1 - (\text{effluent dialysate amount}/\text{inffluent dialysate amount})$$

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 Sawchuck, 1997).

3. Results and discussion

3.1. In vitro permeation study

Various polymer formulations based on PVP and HPMC polymers with pH 7.4 buffer were performed for iontophoretic delivery for 6 h. The current was set at 0.5 mA/cm^2, which has been reported in the literature to be the maximum acceptable current producing minimal skin damage and irritation (Brand and Iversen, 1996). The cumulative amount-time profiles for various diclofenac polymers through rat skin are shown in Fig. 1. Both polymers show the antinucleating effect which may stabilize some drugs in the supersaturated status and improve the permeability of drugs (Kondo and Sugimoto, 1987; Megrab et al., 1995). The slopes of the resulting plots from 0 to 6 h were computed and the flux (μg) values were calculated from the slopes as shown in Table 1. Those profiles fit well to the zero-order equation. As shown in Fig. 1, the permeability of drug from HPMC-based formulation is

Table 1
In vitro flux of diclofenac through various membrane barriers from four hydrogel formulations

Formulation	Rat skin (μg/cm^2 per h)	Human skin (μg/cm^2 per h)	Cellulose membrane (μg/cm^2 per h)
PVP[a]	74.8 ± 7.75	6.60 ± 1.78	570.32 ± 17.26
HPMC[b]	82.50 ± 19.17	8.13 ± 1.25	454.64 ± 51.52
PVP-HPMC	128.57 ± 19.66	11.71 ± 2.98	789.99 ± 48.76
Cardomom oil[c]	250.84 ± 14.73	104.97 ± 15.94	442.99 ± 45.87

[a] PVP, polyvinylpyrrolidone.
[b] HPMC, hydroxypropyl methycellulose.
[c] Cardomom oil, membrane pretreated by cardomom oil for 12 h for PVP-HPMC hydrogel.

higher than that from PVP-based formulation although there is no significant difference (t-test, $P > 0.05$) between them. This phenomenon can be attributed to the different physicochemical properties between HPMC and PVP. Due to the presence of hydrophobic and hydrophilic groups in structure, HPMC reduced the surface tension of water and interfacial tension of aqueous systems, which may allow good wetting and spreading of drug on skin surface and result in the higher permeability of drugs (Peppas, 1987).

In the development of topical dosage forms, several desirable attributes may increase to the patient comliance and clinical efficacy of the product, including optimal mechanical properties, good bioadhesion and acceptable viscosity. Although HPMC-based formulation exhibited higher drug permeability, it is the most viscous polymer among cellulose derivatives (Vazquez et al., 1992). On the other hand, the mucoadhesive force of PVP is lower as compared to other polymers (Peppas, 1987). In order to obtain an ideal transdermal polymer device, the blends of two or more polymers as delivery vehicles are necessary. Thus, the PVP and HPMC (1:1) were mixed and the in vitro drug permeation studies through rat skin were performed (Fig. 1). The flux of binary vehicle was significantly higher than those of single polymer formulations. This results can be explained by the chemical bonds between these two polymers. Since the physicochemical properties of these two polymers are different, the hydrogen bonding and entanglement density between PVP and HPMC should be lower than the PVP and HPMC polymer alone, which results in faster drug release and the higher permeation rate. However, the detailed mechanism was not studied in this investigation, and further studies are needed to clarify the mechanism.

The excised full-thickness human skin was also used as diffusion barrier in the permeation study as shown in Fig. 2. The trends of drug permeability for various vehicles were the same between human skin and rat skin. Nevertheless, the magnitude of diclofenac flux lfrom three polymer formulations is istatistically insignficantly different (ANOVA test, $P > 0.05$) and greatly reduced by using human skin as the diffusion barrier (Table 1). It is well known that rodent skin is geneally more permeable than human skin (Catz and Friend, 1990). Moreover, human breast skin shows a lower permeation rate than the other anatomic sites (Harada et al., 1993). Although human skin is a direct model to study the actual drug effect for human therapy, the disadvantage of this model is that it cannot distinguish the diversity of different formulations significantly as rat skin (Figs. 1 and 2).

After the pretreatment of rat skin and human skin with cardamom oil for 12 h, the in vitro iontophoretic permeation study was performed. Figs. 1 and 2 show that cardamom oil greatly increases the permeability of diclofenac released from PVP-HPMC binary system. This effect was particularly significant for human skin which showed a 10-fold increase in diclofenac flux compared to the non-treated group. Accordingly, cardamom oil can be combined with iontophoresis to achieve a determined effect of diclofenac with less electrical current, which not only slows the process of polarization of charged molecules but also reduces the possibility of skin damage and im-

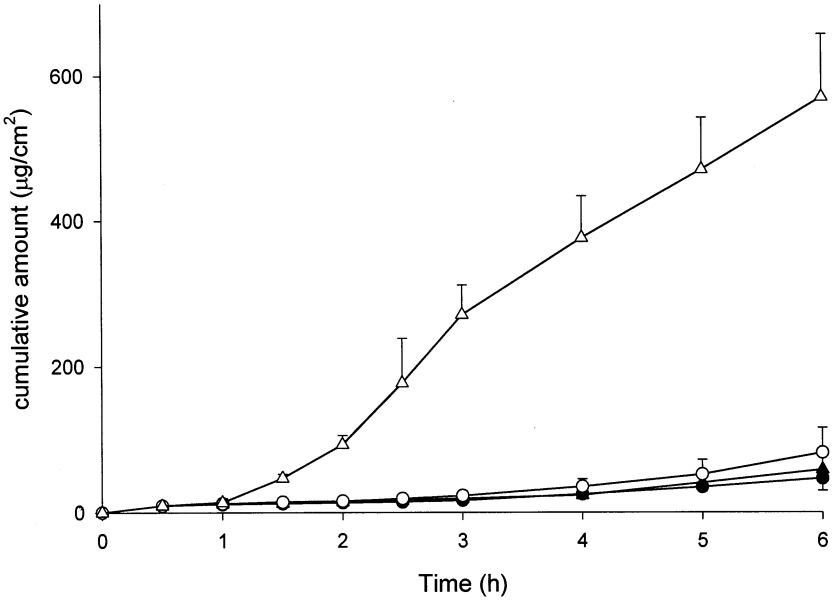

Fig. 2. Cumulative amount of diclofenac sodium detected in the receptor compartment versus time following iontophoresis from various hydrogels through excised human skin: (●) PVP; (▲) HPMC; (○) PVP-HPMC; (△) PVP-HPMC pretreated with cardamom oil on skin. All data represent the means of three experiments ± S.D.

proves the tolerability of skin to the iontophoretic regimen (Rao and Misra, 1994).

To clarify the mechanism for the permeation of diclofenac, the in vitro drug permeation experiment through cellulose membrane was studied. The profiles of diclofenac through the porous membrane are presented in Fig. 3. The permeability of diclofenac through cellulose membrane were higher than that of skin. The higher permeability can be due to the less dense structure of cellulose membrane relative to the skins. Another explanation is that the contribution of electro-osmosis in iontophoresis appears to be negligible due to the lack of fixed charges in the pores of the membrane itself (Santi et al., 1993). The direction of electro-osmotic flow is from receptor (anode) to donor (cathode) in this in vitro study and it may produce a repulsive effect for diclofenac permeated through skins. While considering the structure of the cellulose membrane, the molecular weight cut off of the membrane is $\approx 12\,000-14\,000$. The molecular weight cut off value suggests that there are water-filled pores or channels for drug molecules diffuse freely. Accordingly, the penetration of the drug may depend on the speed of drug partitioning from the polymer matrix into receptor phase (Ho et al., 1994). As shown in Table 1, no relationship can be observed between the permeability of drug through cellulose-based membrane and skin. This result suggests that the diffusion of drug through vehicles is not the predominant mechanism controlling the whole permeation process. This mechanism was also consistent to previous literatures that the release of diclofenac from Carbomer and oleaginous vehicles are not the predominant factor in the whole permeation process (Ho et al., 1994; Takahashi et al., 1995).

The viscosity of polymer formulations was measured by a cone and plate viscometer (Brookfield Co., Model DV-2, USA) (Fang et al., 1996). The viscosity of PVP, HPMC and PVP-HPMC polymers were 10.62, 96, and 61 ($cps \times 10^3$), respectively. Comparing those values to the data of Table 1, the viscosity of the polymer matrices shows no relationship with the drug permeation rates. This result also indicates that the permeation process is consistent with skin-controlled mechanism, since the viscosity of polymer matrix will play an important role in controlling the

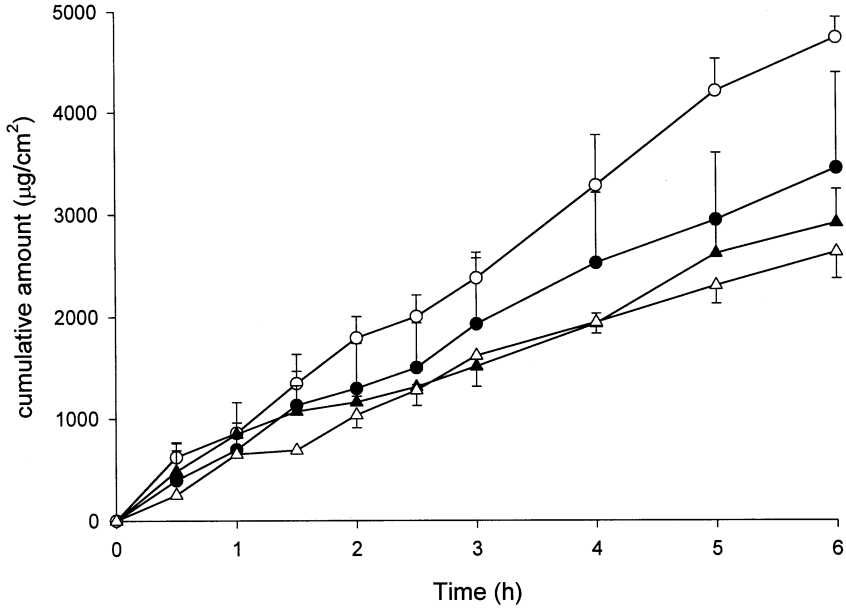

Fig. 3. Cumulative amount of diclofenac sodium detected in the receptor compartment versus time following iontophoresis from various hydrogels through cellulose membrane: (●) PVP; (▲) HPMC; (○) PVP-HPMC (△) PVP-HPMC pretreated with cardamom oil on skin. All data represent the means of three experiments ± S.D.

release of the drug if the diffusion of drug through the polymer matrix is a rate determining step (Ho et al., 1994). Another mechanism to explain the different trends between skin and membrane permeation rate is the difference of their pathways. Solutes without interaction with a polymer matrix primarily permeated through water-filled pores in the artificial membrane according to the free volume theory (Hatanaka et al., 1990). On the other hand, at least two permeation pathways, lipid and pore pathways, exist in the stratum corneum. The ionized diclofenac passes through the pore pathway directly both in skin and artificial membrane. The drug may also distributed to skin first, and then the diclofenac form an ion-pair near skin and permeate through a lipid pathway (Maitani et al., 1994). This mechanism of ion-pair cannot observed in artificial membrane since the pores of cellulose membrane used in this study are large enough for drug molecules of either ionized or un-ionized form to panetrate freely.

Table 1 also shows that the flux of diclofenac significantly reduced after pretreatment of cardamom oil on cellulose membrane. The addition of cardamom oil, a neat liquid with high lipophilicity and relatively lower electric conductivity compared to water, would increase the lipophilicity of membrane and reduce the conductivity of the system. Subsequently the iontophoretic permeation of diclofenac decreased. This result is quite different compared to that of rat skin and human skin which showed the enhancing permeable properties after pretreatment of cardamom oil. It is inferred from these data that a remarkable change in the skin structure can be observed while treated with cardamom oil. In a histological study of skin structure studied as indicated previously (Huang et al., 1993), cardamom oil swells the stratum corneum and epidermic layer by interaction; this interaction may reduce the density of skin and increase its porosity. Accordingly, cardamom oil enhances the permeation of diclofenac through skin either by the lipid pathway or by the pore pathway.

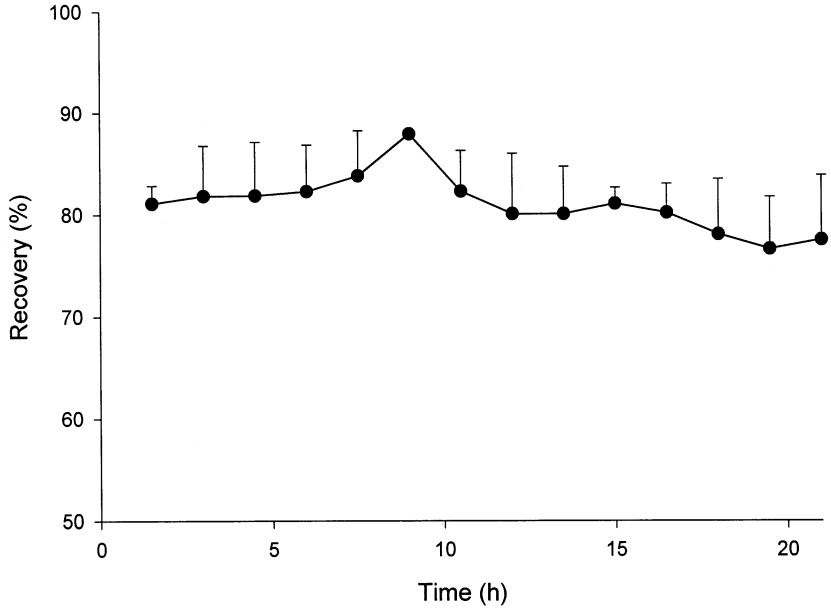

Fig. 4. In vivo recovery of intradermal diclofenac sodium. All data represent the means of three experiments ± S.D.

3.2. In vivo microdialysis and transdermal studies

Recently, in vivo microdialysis has been used as an important technique in the field of pharmacokinetics, especially in the area of drug distribution and metabolism. To apply this technique in obtaining extracellular concentrations of the analytes, the knowledge of the fractional recovery of the solute or analyte is a prerequisite. The relative recovery-time profile of intradermal diclofenac is shown in Fig. 4. The data show that ≈ 80–90% recovery of diclofenac in vivo could be achieved. This recovery is sufficient for practical application. Fig. 5 shows the in vivo permeation results of the four diclofenac polymer formulations. The diclofenac concentration was calibrated with the recovery value of Fig. 4 for all formulations. By comparing the drug permeability of the four hydrogel formulations (Figs. 1 and 5), a good relationship between in vivo and in vitro permeation through rat skin was observed. After the calculation of the area under the curve (AUC) in Fig. 5, the value of cardamom oil-treated formulation (AUC = 175.83 μg × h/ml) was 2-fold compared to that of non-treated group (AUC = 86.98 μg × h/ml). This trend was similar to the in vitro flux between these two formulations through rat skin, indicating a consistency between in vitro study and in vivo study.

The in vivo transdermal studies were divided into three stages: the passive diffusion stage (stage I); iontophoresis stage (stage II); and then stop the applied current (stage III). There were low or negligible diclofenac amount detected at stage I except the formulation preteated with cardamom oil (Fig. 5). This indicates that the permeability of diclofenac is poor when diffuses passively. The concentration of diclofenac extensively increased following the application of current density during stage II. An earlier peak of diclofenac concentration in the first time point of stage II is also observed as shown in Fig. 5. The shunt routes constitute the major permeation pathway for transdermal iontophoresis. Higher amount of drug is obtained through shunts than that through stratum corneum in the early period of applying current (Williams and Barry, 1992). This phenomenon may be amplified due to the higher number of hair follicles in furry rat skin than human skin. After the peak of diclofenac concentration at early stage II, the amount of diclofenac decreased and very low drug concentration was

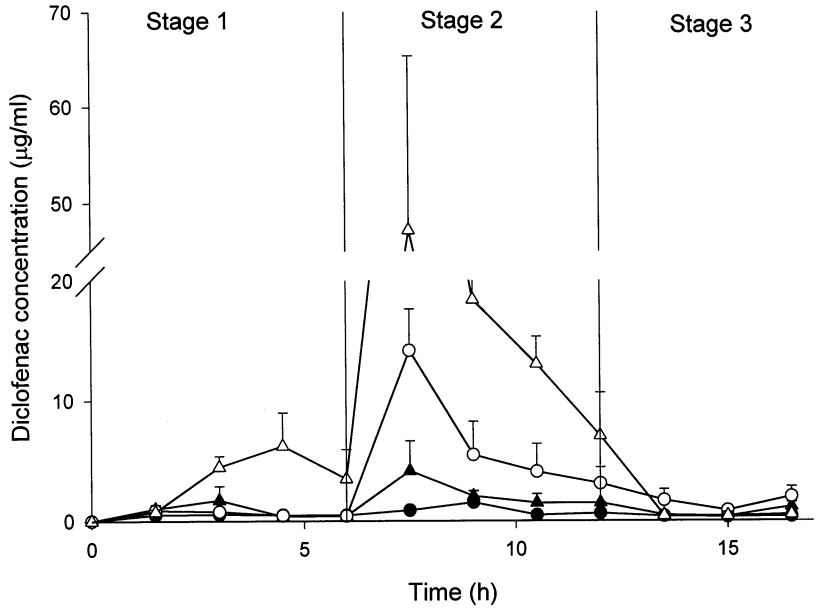

Fig. 5. Diclofenac sodium concentration in dialysate collected from the dermis with hydrogels: (●) PVP; (▲) HPMC; (○) PVP-HPMC; (△) PVP-HPMC pretreated with cardamom oil on skin. All data represent the means of three experiments ± S.D.

detected after the first 2 h of stage III, since the current density was turned off at stage III. It indicates that there is no disruptive effect on the rat skin after the treatment of treatment of 0.5 mA/cm^2 iontophoresis for 6 h.

In order to reveal whether the combination of iontophoresis with cardamom oil may elicit an additive or a synergistic transport-promoting effects in vivo, the AUC of PVP-HPMC binary vehicle at stage II, the AUC of cardamom oil-treated group at stage I and the AUC of cardamom oil-treated group at stage II were calculated; these AUC values can be regarded as the penetration capacity of PVP-HPMC based formulation via iontophoresis, the effect of enhancer on drug diffusion as well as the effect of combining iontophoresis and permeation enhancer on drug permeation, respectively. It is found that the AUC of the combination of iontophoresis and cardamom oil was significantly higher than the sum of the rest two AUCs. This result suggests a synergistic effect is obtained when combining iontophoresis and cardamom oil for transdermal diclofenac delivery.

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