Enhanced Percutaneous Absoption of Piroxicam via Salt Formation with Ethanolamines

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Purpose. The aim of this work was to prepare piroxicam-ethanolamine salts (PX-EAs) with improved physicochemical properties for transdermal application.

Methods. The physicochemical properties of prepared salts were investigated by DSC and FT-IR. Their percutaneous absorption characteristics across hairless mouse skin and the effect of various enhancers were studied using a flow-through diffusion cell system.

Results. Three piroxicam-ethanolamine salts were prepared. Piroxicam monoethanolamine salt (PX-MEA) and piroxicam diethanolamine salt (PX-DEA) had higher solubility than piroxicam in most of vehicles tested and a higher permeation rate across the skin. The solubility and permeation rate of piroxicam triethanolamine salt (PX-TEA) was lower than those of piroxicam in most of vehicles tested. However, there was no significant change in octanol/water partition coefficient by salt formation. Salt formation lowered the melting point of piroxicam and, of the systems examined, PX-DEA showed the lowest melting point. When the effect of various enhancers were evaluated, nonionic surfactants having medium HLB, an alkyl chain length of C18 and an ethylene oxide chain were better able to modify the permeability of the stratum corneum and to promote the effective penetration of piroxicam and PX-EAs.

Conclusions. Piroxicam salt formation with MEA and DEA improved the physicochemical properties and enhanced the skin permeability of piroxicam.

KEY WORDS: salt; piroxicam; monoethanolamine; diethanolamine; triethanolamine; skin permeability.

INTRODUCTION

A large number of drugs that are used for either local or systemic effects are weak acid or bases and are thus ionized under normal physiological conditions. Ionized molecules are generally not well absorbed by biologic membranes (1). One possible means of transferring ionizable molecules across biologic membranes is via ion pairing or complexation with large bulky cations or anions (2). Neubert reviewed drugs and counter ions suitable for ion pair formation with a view towards increasing their lipophilicity and transport across the lipid membrane (3). Many researchers have attributed increased drug permeability induced by ion pairing to increased lipophilicity, a direct consequence of attaining electrical neutrality (1–6). This strategy has been used to deliver organic ions across hydrophobic membranes more efficiently. It also can enhance the trans-skin transport of ionic drugs without modifying the molecular structure of a drug, changing the skin's barrier function or using specific devices (7).

Piroxicam is one of the most potent non-steroidal anti-inflammatory drugs. It is ionizable water-insoluble drug at physiological pH. Specifically, piroxicam can be ionized as a zwitterion that has two pKa values (pKa_1 = 1.86, pKa_2 = 5.46) (8). The structure of piroxicam is shown in Fig 1. A zwitterionic drug possesses a large intramolecular multipole moment due to its multiplicity of oppositely charged groups. Consequently, most of these drugs show low solubility in polar and nonpolar media, and low lipophilicity, which result in low skin permeability (7). The relevance of ion pair formation with respect to the membrane uptake of ionized molecules has been widely discussed. However, only a limited number of studies (7,9) have been conducted upon the transport of zwitterionic drugs using the ion pair concept.

In this study, we have prepared the MEA, DEA and TEA salts of piroxicam to improve its permeation properties. Thermal analysis, FT-IR and elemental analysis were used to compare the physicochemical characteristics of piroxicam and its salts. We also investigated the effect of MEA, DEA and TEA piroxicam salts upon the percutaneous absorption of piroxicam from saturated solutions in various vehicles.

MATERIALS AND METHODS

Materials

Piroxicam was a gift from Jeil Pharm. (Seoul, South Korea). Monoethanolamine, diethanolamine and triethanolamine were purchased from Sigma Chemical (St. Louis, MO, USA). PEG-8 glyceryl caprylate/caprate (Labrasol®), PEG-8 glyceryl linoleate (Labrafil® 2609), polyglyceryl-3 oleate (Plurol oleique® CC 497) and propylene glycol caprlyate/caprate (Labrafac® PG) were obtained from Gattefosse Korea (Seoul, South Korea). Peanut oil, cetearyl octanoate/isopropyl myristate (Crodamol® CAP), PEG-12 palm kernel glycerides (Crovol® PK40) and PEG-20 almond glycerides (Crovol® A40) were obtained from Croda (Parsippany, NJ, USA). PEG sorbitan monooleate (Tween® 80) and n-octanol were obtained from Junsei Chemical Co. (Japan). Acrylic pressure sensitive adhesives were obtained from the National Starch & Chemical Co. (Bridgewater, NJ, USA). All other chemicals were of reagent grade or above and were used without further purification.

Preparation and Identification of PX-MEA, PX-DEA and PX-TEA

Preparation of PX-EA Salts

Piroxicam was dissolved in methylene chloride and an equi-molar amount of each ethanolamine was added. The solutions were stirred for 24 h. The salts precipitated and were collected by filtration. The light yellow solid residues [i.e., $(C_2H_8NO)^+(C_{15}H_{12}N_3O_4S)^-$, $(C_4H_{12}NO_2)^+(C_{15}H_{12}N_3O_4S)^-$, $(C_6H_{16}NO_3)^+(C_{15}H_{12}N_3O_4S)^-$] were dried in a vacuum for 3 h. The sum of the weight of piroxicam and ethanolamine added was equal to the weight of the precipitate.

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ABBREVIATIONS: PX-MEA, piroxicam ethanolamine salt; PX-DEA, piroxicam diethanolamine salt; PX-TEA, piroxicam triethanolamine salt; PX-Eas, piroxicam ethanolamine salt; piroxicam diethanolamine salt; and piroxicam triethanolamine salt HLB, DSC, FT-IR.

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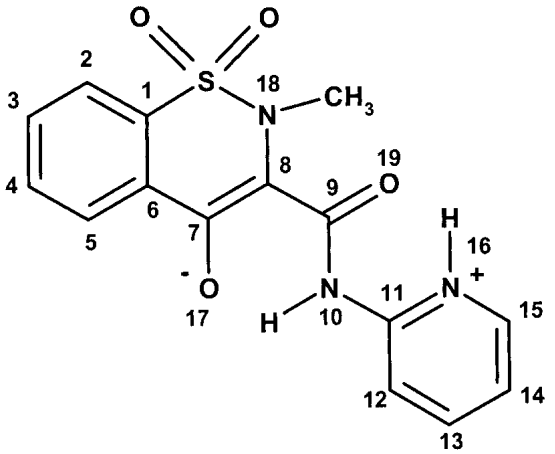

Fig. 1. Permeation profile of piroxicam and piroxicam ethanolamine salts in Crovol® A40 solution as a function of time. mean ± SD ? (n = 3)

Thermal Analysis

Thermograms were recorded using a differential scanning calorimeter (DSC-2010, TA Instrument) at a scan rate of 10°C/min.

IR Spectroscopy

IR spectra were recorded from KBr pellets on a FT-IR spectrophotometer (Magna-IR 550, Nicolet).

Elemental Analysis

Elemental analysis was performed by Chns-o Analyzer Automatic (EA1108, Carlo Erba, Italy). The criterion used for the analysis acceptance was less than 0.3% deviation for each atom (C, H, N, S).

HPLC Methodology

Piroxicam and its salts were analyzed using a HPLC system (Shimadzu Scientific Instruments, MD, USA), consisting of an UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A). The wavelength of the UV detector was set at 320 nm and a reversed phase column (Alltima C8, Alltech associates, IL, USA) was used. The column temperature was maintained at 30°C using a thin foil temperature controller (CH 1445, SYSTEC, MN). The flow rate was 1 ml/min. and methanol/water/phosphoric acid (700/299/1) was used as the mobile phase. The piroxicam salts would be dissociated into piroxicam and ethanolamines under our analytical conditions of pH 3.0. Therefore, we analyzed the salt samples as piroxicam. The retention time of piroxicam was 3.3 min under our experimental conditions.

Solubility and Partition Coefficient

Equilibrium solubility was measured by suspending an excess amount of the drug (piroxicam, PX-MEA, PX-DEA, PX-TEA) in various solvents in screw-capped vials. The solvents used are listed in Table I and Table II. The contents were stirred by an externally driven Teflon-coated magnetic bar at room temperature until equilibrated. The saturated solution was then filtered through 0.45 μm Millipore filter (Millipore, Bedford, MA, USA). The concentration of piroxicam was measured by HPLC after appropriate dilution. The solubilities were measured at 24, 48, and 96 h and they did not change after 24 h.

Table I. Apparent Partition Coefficient and Solubility of Piroxicam or PX-EAs

	APC	Solubility (mg/ml)[a]	Solubility (mg/ml)[b]
Piroxicam	0.590 ± 0.02	0.17 ± 0.068	0.83 ± 0.00
PX-MEA	0.459 ± 0.015	126.2 ± 6.0	1.15 ± 0.03
PX-DEA	0.455 ± 0.007	47.97 ± 0.43	0.78 ± 0.01
PX-TEA	0.477 ± 0.025	13.84 ± 0.42	0.63 ± 0.00

APC: apparent partition coefficient (n-octanol/phosphate buffer 7.4).
[a]Solubility: solubility in phosphate buffer 7.4
[b]Solubility: solubility in n-octanol.
Each value represents the mean ± SD (n = 3).

The apparent partition coefficient of piroxicam or of its salts (PX-EAs) was measured in n-octanol/phosphate buffer (50 mM, pH 7.4). Piroxicam or each PX-EA was dissolved in n-octanol (1 mg/ml) saturated with phosphate buffer (pH 7.4). PX or PX-EA solutions in octanol (10 ml) were equilibrated with 10 ml of phosphate buffer at room temperature. After centrifugation and separation, the piroxicam concentrations in the aqueous and organic phases were determined by HPLC. The pH of the solution was measured by a pH meter (Sartorius Professional Meter PP-15, Sartorius AG, Germany).

In Vitro Transdermal Diffusion Cell System

A flow-through diffusion cell system was used, comprising a multichannel peristaltic pump (IPC-24, Ismatec, Switzerland), a fraction collector (Retriever IV, ISCO, NE), a circulating water bath (Jeio-Tech, Korea), and flow-through diffusion cells. The flow-through cell consisted of two side arms, which enabled conduction of receiver cell media from a peristaltic pump to a fraction collector. Temperature was maintained at 37°C by circulating water at constant temperature through the outer jacket of the receiver cell. The surface area of the receiver cell opening was 2 cm^2, and the cell volume was 5.5 ml.

Procedure and Data Reduction

The preparation of the hairless mouse skins, the penetration study procedure, and data reduction methods have been described in an earlier study (10). Samples were collected every 4 h for 36 h. The saturated suspension in each test vehicle was applied on the skin. If damage had occurred to the skin during 36 h of the experiment, the flux would have continued to increase with time. Figure 2 shows that fluxes remain constant after steady state had been attained. It has been assumed that any physiological change in the skin caused by the vehicle which led to increased flux would function as a constant for that vehicle regardless of the specific solute applied in the vehicle.

RESULTS AND DISCUSSION

Characterization of PX-MEA, PX-DEA, PX-TEA Salts

Piroxicam was completely dissolved in methylene chloride. A precipitate was obtained after ethanolamine was

Table II. Comparison of the Solubility of PX and PX-EAs in Various Vehicles (Measured as the Amount of Piroxicam Dissolved)

Enhancer	Solubility (mg/ml)			
	Piroxicam	PX-MEA	PX-DEA	PX-TEA
Crodamol®	0.54 ± 0.01	0.16 ± 0.008	0.12 ± 0.05	0.026 ± 0.005
Labrafac® PG	2.94 ± 0.5	0.23 ± 0.03	0.16 ± 0.04	0.124 ± 0.039
Peanut oil	1.52 ± 0.63	0.13 ± 0.015	0.08 ± 0.02	0.06 ± 0.02
Labrafil® 2609	4.89 ± 0.4	38.78 ± 0.97	6.72 ± 1.15	1.47 ± 0.05
Plurol oleique®	2.53 ± 0.64	15.97 ± 0.45	6.74 ± 1.13	1.26 ± 0.1
Crovol® A40	7.96 ± 1.46	143.26 ± 3.66	59.12 ± 5.93	9.88 ± 0.74
Crovol® PK40	8.62 ± 1.14	51.34 ± 5.27	14.58 ± 2.71	3.13 ± 0.08
Labrasol®	14.91 ± 1	226.75 ± 19.8	30.99 ± 1.64	3.18 ± 0.33
Tween® 80	13.54 ± 2.29	157.53 ± 3.65	41.97 ± 1.94	5.1 ± 0.35

Note: Each value represents the mean ± SD (n = 3).

added into the methylene chloride solution, which is an indirect evidence of complex formation. PX-EAs have very low solubility in methylene chloride. Elemental analysis also confirmed the formation of PX-EAs. The IR spectra for piroxicam and the PX-EAs are shown in Fig 3. The most obvious difference between the spectrum of piroxicam and those of its salts was found in the O-H and N-H stretching regions. Piroxicam showed a strong narrow signal at 3338 cm^{-1}, which is characteristic of the O-H and N-H vibrations of the cubic form of piroxicam (11). PX-MEA showed no sharp peak in the same region. In the cases of PX-DEA and PX-TEA, the peak was shifted and broadened. The missing or broadened peak observed in this region in the case of the PX-EAs is indicative of the strong intermolecular interaction between piroxicam and the EAs. Piroxicam is known to exist in several different isomers, such as the enol, zwitterionic, and anionic forms and the crystal structure of the piroxicam ethanolamine salt showed that piroxicam was present in the anionic form (12). The negative charge on O(17) of piroxicam and the positive charge on the N atom of the EAs would be expected to interact electrostatically.

The melting point of such salts are expected to be lower than that of the corresponding parent compound due to the

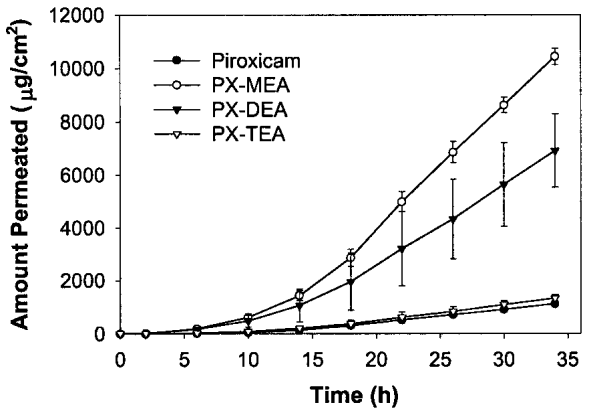

Fig. 2. The structure of the zwitterion form of piroxicam.

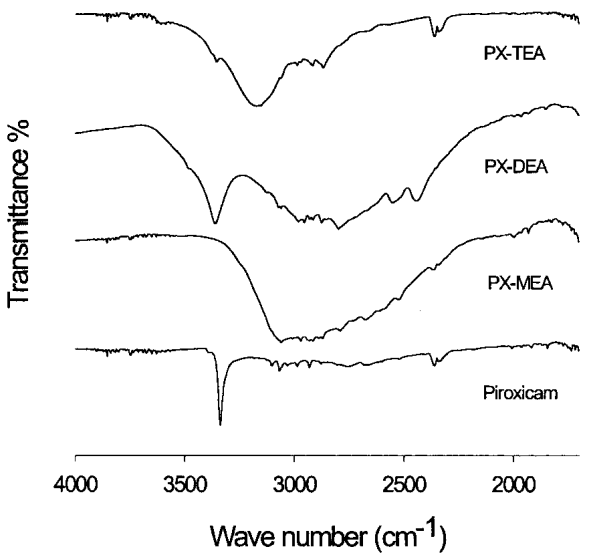

Fig. 3. FT-IR spectra of piroxicam and piroxicam ethanolamine salts.

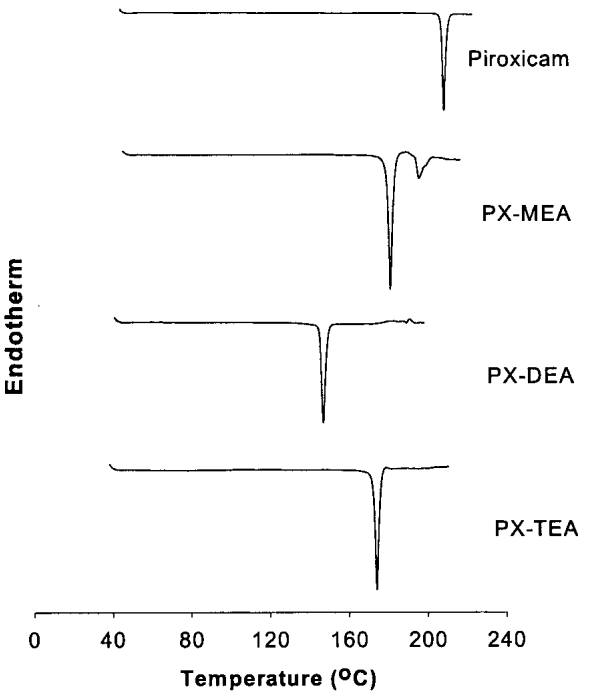

Fig. 4. DSC thermograms of piroxicam and piroxicam ethanolamine salts.

Table III. Melting Points of Piroxicam and PX-EAs

	Melting point (°C)
Piroxicam	202.7
PX-MEA	175.3
PX-DEA	144.1
PX-TEA	173.8

lower crystalline lattice energy (13). The DSC thermograms of piroxicam, PX-MEA, PX-DEA and PX-TEA are shown in Fig 4. Table III shows the melting points of the compounds shown in Fig 4. The melting peaks of PX-EAs were all shifted to lower temperatures with respect to the characteristic endothermal melting peak of piroxicam at 202.7°C. It was not possible to observe any trend in the decreased melting points of the salt forms of piroxicam vs. the counter ion change from MEA to TEA. In the case of the hydrohalide salts of phenylalanine, the melting points decreased as the ionic radius and polarizability of the counter ion increased down the periodic table, while no clear trend was observed in a series of baclofen alkyl sulfonate salts (13). It is hard to predict the melting point lowering effect of EAs since it is complicated to rank the EAs in terms of molecular size, polarizability, and lipophilicity. The dielectric constants of MEA, DEA, and TEA were 31.94, 25.75, and 29.36, respectively (14). Although it may be too early to draw a conclusion due to limited number of counter ions tested, the melting points of PX-EAs decreased as the dielectric constant decreased.

The partition coefficient in n-octanol/phosphate buffer (pH 7.4) and the solubility of piroxicam and the PX-EAs in phosphate buffer (pH 7.4) and n-octanol are summarized in Table I. Table II shows the solubilities of piroxicam and the PX-EAs in various vehicles. In contrast to small differences in the partition coefficients of piroxicam and the PX-EAs, large differences in aqueous solubility were observed. This result is in contrast with the findings of Kadono *et al*, Shim *et al*, and Takács-Novák *et al.* (6,15,16). Kadono *et al.* found that the partition coefficient of the ion pair formed between alkylamine and salicylate increased as the alkyl chain length of the alkylamine increased. Shim *et al.* and Takács-Novák *et al.* also reported that the formation of an ion pair led to the increase in the partition coefficient. One of the factors that should be considered when studying the partition coefficients of salts is the pH of the aqueous phase. If the counter ion used changes the pH significantly, the solubility of the parent compound in the aqueous phase may also change (it usually increases) due to the ionization of the corresponding parent compound. As can be seen in Table I, the solubilities of PX-EAs in phosphate buffer (pH 7.4) were higher than that of piroxicam due to the pH effect, since the final pH values after the solubility study for PX-MEA, PX-DEA, and PX-TEA were 9.2, 9.09, and 8.69, respectively. As the final pH of the solution increased, the solubility in phosphate buffer increased. The solubility of PX-MEA in octanol was slightly higher than piroxicam and those of PX-DEA and PX-TEA were slightly lower than piroxicam. The minimal difference between the solubility of piroxicam and the PX-EAs in octanol and the slight increase in the pH of the aqueous phase in the partition coefficient study resulted in a slight decrease in the partition coefficients of the PX-EAs. The pH values of the aqueous phase from the determination of the partition coefficients of PX, PX-MEA, PX-DEA, and PX-TEA were 7.34, 7.47, 7.46, and 7.45, respectively. It should be noted that the partition coefficient of the PX-EAs is a function of the initial concentration. The higher the concentration of the PX-EAs used, the higher the pH of the aqueous phase becomes, which results in higher solubility in the aqueous phase and a lower partition coefficient.

Salts of zwitterions generally have lower crystalline lattice energy due to the removal of the large zwitterionic dipole, and this results in higher solubilities than the parent zwitterion in both polar and nonpolar solvents (13). PX-MEA and PX-DEA had higher solubilities than piroxicam in the most of vehicles tested with some exceptions, namely, peanut oil, Labrafac® PG, and Crodamol®. Both piroxicam and the PX-EAs had lower solubility in peanut oil, Labrafac® PG, and Crodamol® than in the other vehicles tested. These are the more hydrophobic vehicles and the results indicate that piroxicam might be slightly more hydrophobic than PX-EAs. It should be noted that the solubility of PX-TEA was lower than that of piroxicam in all the vehicles tested except Crovol® A40. Although PX-DEA showed the lowest melting point among the PX-EAs, PX-MEA had the highest solubility, and this was followed by PX-DEA and PX-TEA in all the vehicles tested including water. Although the melting point is a useful predictor of solubility, the trend of solubility of PX-EAs did not correlate with the order of melting points (primary >tertiary >secondary). The order of solubility in water correlated with the basicity of the counter ions, i.e., the pH of

Table IV. Effect of Various Enhancers on the Permeation of PX-EAs and PX

Enhancer	Piroxicam	PX-MEA	PX-DEA	PX-TEA
	flux ($\mu g/cm^2/h$)			
Crodamol®	2.30 ± 0.20	6.87 ± 0.78	5.72 ± 0.25	1.12 ± 0.23
Labrafac® PG	4.52 ± 2.40	10.61 ± 0.68	7.70 ± 1.24	2.24 ± 0.25
Peanut oil	0.90 ± 0.28	9.09 ± 1.59	4.40 ± 0.83	0.68 ± 0.18
Labrafil® 2609	7.54 ± 1.44	49.22 ± 17.6	19.78 ± 4.55	10.7 ± 4.48
Plurol oleique®	4.55 ± 1.64	11.43 ± 3.67	5.36 ± 0.76	2.23 ± 0.15
Crovol® A40	16.60 ± 0.54	153.53 ± 4.54	101.64 ± 20.35	19.59 ± 1.99
Crovol® PK40	11.39 ± 6.73	44.68 ± 5.92	12.71 ± 2.09	6.93 ± 1.29
Labrasol®	0.46 ± 0.09	0.99 ± 0.10	0.39 ± 0.05	0.09 ± 0.003
Tween® 80	0.15 ± 0.02	1.97 ± 1.25	1.34 ± 0.45	0.25 ± 0.06

The amount permeated is expressed as the amount of piroxicam in both cases.
Each value represents the mean ± SD (n = 3).

Table V. Physicochemical Information of the Enhancers Used in This Study

Enhancer	HLB	EO chain length	Hydrophobic portion
Crodamol®			C18/C16, C14
Labrafac® PG	2		C8/C10
Peanut oil			C18:1–56% C18:2–26%
Labrafil® 2609	6	8	C18:2
Plurol oleique®	6		C18:1
Crovol® A40	10	20	C18:1
Crovol® PK40	10	12	C12–46% C18:1–18%
Labrasol®	14	8	C8/C10
Tween® 80	15	20	C18:1

a 0.1N aqueous solution of MEA, DEA, and TEA are 12.05, 11.0, and 10.5, respectively. It is evident that more piroxicam will be solubilized in an aqueous solution of MEA since the solubility of piroxicam increases with increasing pH resulting from the basic character of the ethanolamines.

Effect of the Enhancers

To develop a transdermal delivery system for a drug, appropriate enhancers are usually required to increase the permeation rate or solubilize the drug. To compare the enhancing effect of various vehicles, the permeation of piroxicam and PX-EAs from a saturated solution in various vehicles across hairless mouse skin was investigated. It has been reported that nonionic surfactants and fatty acid esters or fatty acids are capable of increasing the fluidity of the stratum corneum and thereby improve drug partitioning to the stratum corneum (17–19). The average flux of piroxicam over 36 h across hairless mouse skin is summarized in Table IV, and the physicochemical properties of the enhancers used are shown in Table V. Crovol® A40 (PEG-20 almond glyceride) provided the highest skin flux of all compounds tested. The order of the enhancing effect of the various vehicles tested was very similar for all of the compounds tested, indicating that their enhancing mechanism for piroxicam and PX-EAs is similar. The MEA salt of piroxicam had a higher skin flux than piroxicam by 2- to 13-fold, depending on the vehicles used. DEA salt formation increased the flux 2- to 9-fold. However, the TEA salt of piroxicam delivered piroxicam at a lower rate than the parent drug, except in the presence of Crovol® A40, Labrafil® 2609 (a PEG-8 glyceryl linoleate), and Tween 80. These results are largely in accord with our solubility study results where PX-MEA showed the highest solubility followed by PX-DEA, PX, and PX-TEA with some exceptions.

Although the HLB of the surfactant alone is not a reliable predictor of its enhancing capability, it is one of the factors that affects percutaneous absorption (20). The permeation study results showed higher skin permeabilities in the presence of Crovol® A40 (HLB 10), Labrafil® 2609 (HLB 6) and Crovol® PK40 (HLB 10) than in the presence of the more hydrophilic surfactants, Labrasol® (HLB 14) and Tween® 80 (HLB 15). In addition, they were more effective than the more hydrophobic vehicles, such as Labrafac® PG (HLB 2), Peanut oil and Crodamol® (a mixture of cetosteary octanoate and isopropyl myristate).

The size and the nature of both the alkyl chain and the polar group of PEG alkyl esters also influenced piroxicam absorption enhancing ability. The results showed that Crovol® A40 (C18:1) and Labrafil® 2609 (C18:2) showed higher enhancement effects than Labrafac® PG (C8/C10) and Labrasol® (C8/C10). It has been shown in the literature that enhancers with alkyl chain lengths of 16 to 18 and EO chain lengths of 2 to 5 are more effective (20). Although the chain length of Peanut oil or Plurol oleique® is C18, their enhancement effects were lower than that of Crovol® A40 (C18:1). While the EO chain length of Crovol® A40 is 20, Peanut oil and Plurol oleique® do not have an EO chain. Crovol® A40 (PEG-20, C18) which has the same HLB of 10, had a more effective enhancement effect than Crovol® PK40 (PEG-12, C12-46%, C18:1-18%). Also, at a same HLB of 6, Labrafil® 2609 (PEG-8, C18:2) with 8 EO chain was more effective than Plurol oleique® (C18:1), which has no EO chain. These results suggest that the nonionic surfactants with medium HLB, an alkyl chain length of C18 and an EO chain have a better ability to modify the permeability of the stratum corneum and to promote the effective penetration of piroxicam and PX-EAs.

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