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Transdermal Delivery of Piroxicam Using Microemulsions

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To improve the skin permeability of piroxicam, a new oil-in-water microemulsion containing 0.5% piroxicam was developed. Among various oils investigated for their suitability as an oil phase for the microemulsion system, oleic acid showed both excellent solubility and skin permeation enhancing effect for piroxicam. Microemulsion existence ranges were identified through the construction of the pseudo-ternary phase diagram. The effect of the content of oleic acid and the ratio of the surfactant/cosurfactant on skin permeation of piroxicam were evaluated with excised rat skins. The optimum formulation with the highest skin permeation rate (47.14 μg/cm^2/h) consisted of 0.5% piroxicam, 10% oleic acid, 60% Labrasol/ethanol (1:5) and water.

Key words: Piroxicam, Microemulsion, Oleic acid, Phase diagram, Skin permeability

INTRODUCTION

Piroxicam is a potent non-steroidal anti-inflammatory drug used for the treatment of acute and chronic rheumatoid arthritis or osteoarthritis. Although piroxicam has strong therapeutic effects, several side effects including gastrointestinal irritation, dizziness, headache and peptic ulcer have been reported following oral administration (Schiantarelli and Cadel, 1981). These side effects may be overcome by transdermal administration of the drug (Schiantarelli *et al.*, 1982). Various transdermal dosage forms such as gels (Santoyo *et al.*, 1995b), creams (Tessari *et al.*, 1995), ointments (Babar *et al.*, 1990; Tsai *et al.*, 1985) and cataplasms (Okuyama *et al.*, 1999) have been tested for this purpose. In an attempt to improve the skin permeation of piroxicam, several physicochemical methods have been evaluated, including the application of iontophoresis (Curdy *et al.*, 2001; Doliwa *et al.*, 2001) and the use of permeation enhancers (Huang *et al.*, 1995; Santoyo *et al.*, 1995).

Microemulsion has been recognized as a good vehicle for the transdermal delivery of drugs. It is defined as an O/W or W/O emulsion producing a transparent product that has a droplet size <0.15 μm and does not have a tendency to coalesce (Kreilgaard, 2002). Several mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of drugs (Delgado-Charro *et al.*, 1997; Kawakami *et al.*, 2002). First, a large amount of drug can be incorporated in the formulation due to the high solubilizing capacity, with increased thermodynamic activity towards the skin (Peltola *et al.*, 2003). Second, the permeation rate of a drug from microemulsion may be increased, since the affinity of the drug to the internal phase in microemulsion can be easily modified, to favor partitioning into the stratum corneum, using different internal phases and changing the composition of the microemulsion. Third, the surfactant and cosurfactant used in the microemulsion may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers (Rhee *et al.*, 2002).

In this study, an optimum O/W microemulsion containing piroxicam was developed after screening various oils to improve the drug solubility and the skin permeability.

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MATERIALS AND METHODS

Materials

The following reagents were used as received without further purification: piroxicam, propylene glycol, triacetin, cotton seed oil, corn oil, soybean oil, sesame oil, olive oil, isopropyl myristate (IPM) (Sigma Chemical Co., U.S.A.), oleic acid (Shinyo Chemica Co., Japan) and HPLC grade acetonitrile (Mallinckrodt Chemical Co. U.S.A.). Caprylic/capric triglyceride (Labrafac lipophile) and PEG-8 glycol caprylate (Labrasol) were kindly donated by Gattefossé,

France. Water was deionized and filtered in house. All other chemicals and solvents were of analytical reagent grade.

Screening of oils for microemulsion

To find out appropriate oils that have good solubilizing capacity of piroxicam, the solubility of piroxicam in various oils was measured. The oils investigated were cotton seed oil, corn oil, soybean oil, sesame oil, olive oil, oleic acid, Labrafac lipophile, triacetine and IPM. An excess amount of piroxicam was added to 5 mL of each selected oil and was shaken reciprocally at 20°C for 72 h. The suspension was filtered with a membrane filter (Nylon Acrodisc, 0.45 μm, Gelman, U.S.A.), and the concentration of piroxicam in the filtrate was determined by HPLC as described below. The effect of oils, except for the vegetable oils, on the skin permeation rate of piroxicam was also investigated. Piroxicam vehicle (0.5%) was prepared by dissolving the drug in propylene glycol containing 5% each of the selected oils. Vegetable oils were not included as they have been shown to have no effect on the skin permeation of the drug (Buyuktimkin *et al.*, 1997).

Construction of phase diagram

The pseudo-ternary phase diagrams were constructed by the dilution method. Oleic acid was used as the oil phase, Labrasol as the surfactant and ethanol as the cosurfactant. Five phase diagrams were prepared at different surfactant/cosurfactant ratios (S/CoS) of 5/1, 3/1, 1/1, 1/3, and 1/5. For each phase diagram at specific S/CoS, mixtures of the oil, the surfanctant and the cosurfactant were prepared. The ratio of oil to the mixture of the surfactant and the cosurfactant was varied as 5, 10, 20, 30, 40, 50, 60, and 70%. The piroxicam-containing formulations were prepared by dissolving 30 mg of the drug into 3 g of the oily mixtures. Water was added drop by drop while mixing on a magnetic stirrer at room temperature, and the samples were marked as being optically clear or turbid. The microemulsion regions were identified as transparent and isotropic mixtures. The phase inversion of the microemulsion from O/W to W/O was determined based on the change of conductivity (Baroli *et al.*, 2000). The conductivity of microemulsions was measured using a conductance meter (Horiba Co., Japan, model D-24) at 20 ± 1°C. The percentage of existence area of the O/W microemulsion in the pseudo-ternary phase diagrams was measured by using the Image Pro-Express software (Media Cybernetics, Inc., U.S.A.).

Determination of droplet size in microemulsion

The size of oil droplets in microemulsion was determined using the dynamic light scattering method employing He-Ne laser (Lexer Laser Inc., U.S.A., Model 127).

Skin permeation study

Various microemulsions of different compositions were prepared and the skin permeation rates of piroxicam were determined to evaluate the effect of the formulation factors. Skins were obtained from male Sprague-Dawley rats weighing 250 ± 20 g. After hair was removed carefully with an electric clipper (Daito Electric Co., Japan, Model 900), a 5 cm × 5 cm patch of skin was excised from the dorsal region from each sacrificed rat and the subcutaneous fat and other extraneous tissues were trimmed. The excised rat skins were stored at -20°C until use. They were used within one week after the skin harvest.

The extent and rate of skin permeation of piroxicam from microemulsions of various compositions were determined using Franz diffusion cells fitted with excised rat skins. The effective diffusion area was 1.77 cm^2. The receptor phase was composed of 10% PEG 400 in pH 7.4 phosphate buffer (0.02 M). The temperature was maintained at 37 ± 0.5°C using a thermostatic water pump (Labfine, Korea, Model FT-101) and it was constantly stirred at 600 rpm throughout the experiment. After 2 mL microemulsion was applied on the epidermal surface of the skin, 200 μL of the receptor phase was withdrawn at predetermined time intervals for up to 10 h. An equal volume of the phosphate buffer was immediately replaced after each sampling. Collected samples were stored at -20°C until HPLC analysis. All skin permeation experiments were repeated four times.

HPLC analysis of piroxicam in receptor phase

The amount of piroxicam in the receptor phase was quantitated with a slight modification of the HPLC method reported previously (Troconiz *et al.*, 1993). The HPLC system consisted of an isocratic pump (Hitachi, Japan, Model L-7110), an autosampler (Hitachi, Japan, Model L-7200), an UV/Visible detector (Hitachi, Japan, Model L-7400) and an integrator (Hitachi, Japan, Model D-7500). The column used was a C_{18} column (Cosmosil, 4.6 × 150 mm, 5 μm particle size, Nacalai Tesque, Japan). The mobile phase consisted of acetonitrile and 8% acetic acid (45:55, V/V) and the flow rate was set at 1 mL/min. The detection wavelength was 365 nm. All operations were carried out at ambient temperature.

Data analysis of skin permeation and statistics

The cumulative amounts of piroxicam permeated per unit skin area were plotted against time. The skin permeation rate at steady-state (J_s) was determined from the slope of the linear portion of the plot. The Student's *t*-test was performed to see any significant difference in the permeation rate of piroxicam between propylene glycol containing 5% each of various oils and the control without containing oil.

RESULTS AND DISCUSSION

Screening of oils for microemulsion

Since only the drug dissolved can permeate through the skin, the solubility of poorly water-soluble piroxicam needs to be increased. To screen appropriate oils for the preparation of microemulsions, the solubility of piroxicam in various oils was measured and the results are shown in Table I. Among the oils tested, triacetin showed the highest solubility (7.23 ± 0.69 mg/g), followed by oleic acid. Vegetable oils resulted in low solubilities ranging from 0.75 to 1.07 mg/g.

The permeation profiles of piroxicam from propylene glycol containing different oils through excised rat skins are shown in Fig. 1, and the calculated skin peremeation rates of the drug are presented in Table II. The skin permeation rate from the vehicle containing triacetin, which was the best solubilizer for the drug, was not significantly different from the control. Among the oils studied, oleic acid and IPM resulted in the significantly higher skin permeation rates of piroxicam compared to the control. Oleic acid showed the highest skin permeation rate which was 77 times higher than that of the control. This may be due to the permeation enhancing effect of the oil used. While triacetin has not been used as a penetration enhancer, oleic acid has been commonly used as a powerful enhancer for many drugs (Buyuktimkin *et al.*, 1997), including piroxicam (Gay *et al.*, 1992; Santoyo *et al.*, 1995; Santoyo and Ygartua, 2000). The enhancing mechanism by oleic acid involves the increased fluidity of lipid portion of the stratum corneum (Kanikkannan *et al.*, 2000). Since oleic acid showed the highest permeation rate and relatively good solubilizing capacity of piroxicam, oleic acid was selected as the oil phase for further studies.

Table I. Solubility of piroxicam in various oils at 20°C

Oils	Solubility (mg/g)
Cotton seed oil	0.99 ± 0.10[a)]
Corn oil	1.07 ± 0.11
Labrafac	0.94 ± 0.12
Oleic acid	4.20 ± 0.25
Olive oil	0.75 ± 0.08
Sesame oil	0.90 ± 0.07
Soybean oil	0.89 ± 0.08
Triacetin	7.23 ± 0.69
IPM	2.52 ± 0.29

[a] Mean ± S.E. (n=4)

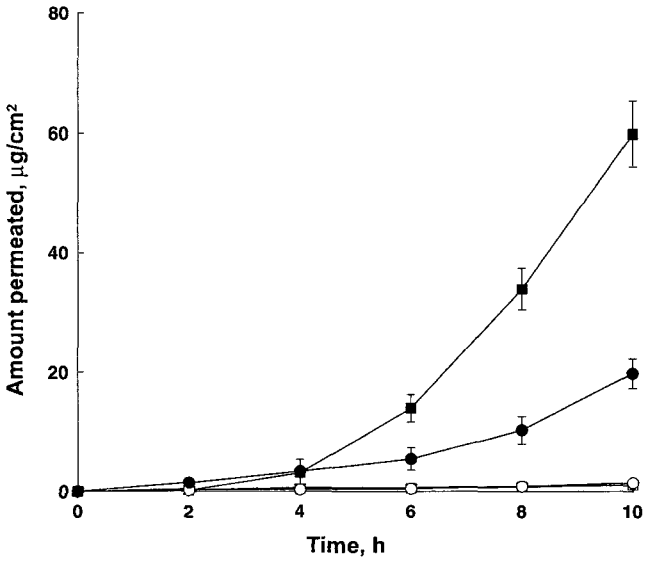

Fig. 1. Permeation profiles of piroxicam through excised rat dorsal skin from propylene glycol containing different olis (Mean ± S.E., n=4). Key: □; Control, ■; Oleic acid, ▼; Triacetin, ○; Labrafac, ●; IPM.

Table II. Permeation rate of piroxicam through excised rat dorsal skin from propylene glycol containing different olis

Enhancer	J_s (μg/cm²/h)[a)]
Control [b)]	0.15 ± 0.05[c)]
Oleic acid	11.48 ± 3.51[†]
Triacetin	0.11 ± 0.01
Labrafac	0.25 ± 0.04
IPM	3.58 ± 1.31[†]

[a)] J_s: permeation rate

[b)] Propylene glycol containing 0.5% piroxicam only

[c)] Mean ± S.E. (n=4)

[†]: Significantly different from the control (P<0.05)

Phase diagram preparation and microemulsion formulation

The construction of a pseudo-ternary phase diagram makes it easy to find out the concentration range of components to form microemulsion. Five phase diagrams obtained at S/CoS of 5/1, 3/1, 1/1, 1/3, and 1/5 are presented in Fig. 2. The translucent W/O or O/W microemulsion area was presented in the phase diagrams.

According to the conductivity measurements, the investigated microemulsion can be separated as W/O or O/W. In the region of low water contents, the W/O microemulsion was formed and the conductivity of the microemulsions remained at around 10 μs/cm. As the fraction of water volume was increased, the O/W microemulsion was formed, and the conductivity was reached above 100 μs/cm. It has been previously reported that O/W microemulsions have relatively high conductivity as compared with W/O microemulsions (Baroli *et al.*, 2000).

The existence areas of O/W microemulsion made with

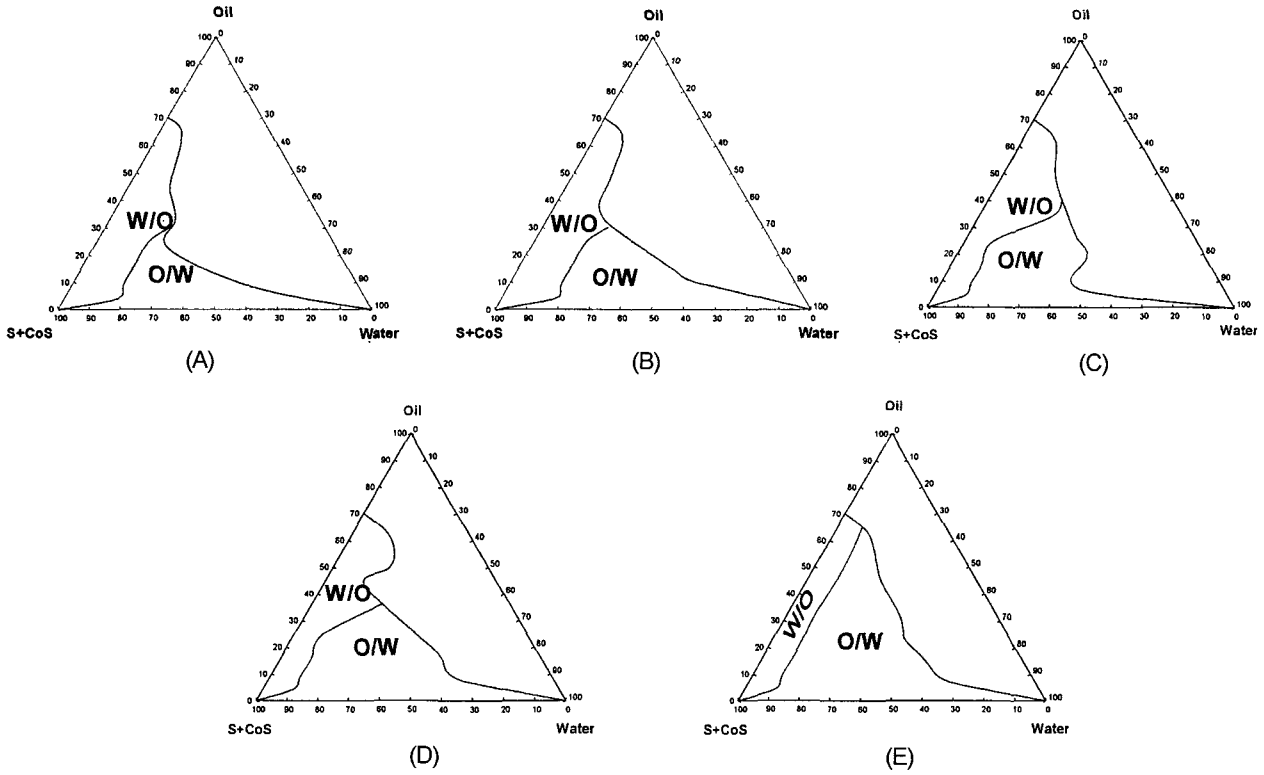

Fig. 2. Pseudo-ternary phase diagrams of microemulsion composed of oleic acid, surfactant (Labrasol®), cosurfactant (ethanol) and water. Key: (A); S/CoS=5/1, (B); S/CoS=3/1, (C); S/CoS=1/1, (D); S/CoS=1/3, (E); S/CoS=1/5

different S/Cos are presented in Fig. 3. Even though five formulations of microemulsions containing piroxicam resulted in similar phase diagrams (Fig. 3), the existence area of O/W microemulsion increased slightly as S/CoS decreased. Increasing the amount of ethanol in microemulsion may also have favorable effects on the skin permeation of piroxicam since ethanol has been used as a permeation enhancer for many drugs (Gao and Singh, 1998).

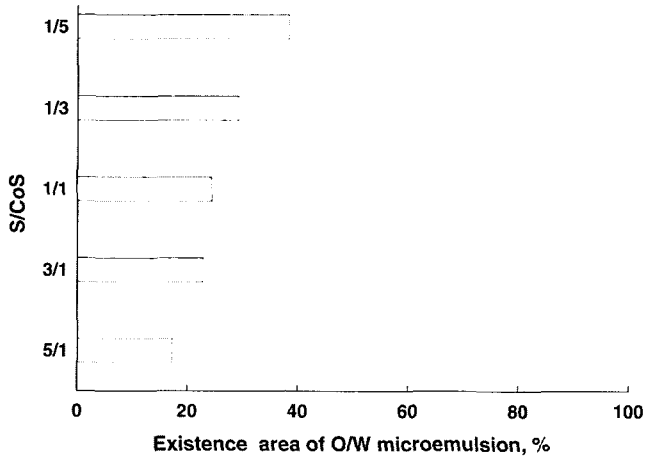

Fig. 3. Existence area of O/W microemulsion formulated with different S/CoSs in the pseudo-ternary phase diagrams

Skin permeation study and optimum formula

To investigate the effect of the content of oleic acid on the skin permeation of piroxicam, various levels of oleic acid were evaluated (Table III), while the content of S/CoS (1/3) mixture was fixed at 40%. The effect of oleic acid was highly significant ($p<0.05$) at the 10% and 15% concentrations compared to 5% concentration. The skin permeation rate of piroxicam was the highest at 15% of oil content. In a previous report, when oleic acid was added to piroxicam gels in a range of 3-10%, the enhancing effect was the highest at 5%, but the value at 10% was less than at 5% (Santoyo *et al.*, 1995). This decrease may have been related to an increase in the lipophilicity of the gel. It was also reported that oleic acid added to the

Table III. Skin permeation rate of piroxicam from microemulsion containing different contents of oleic acid

Content of oleic acid	J_s (μg/cm²/h)[a]
0% (Control)	0.15 ± 0.05[b]
5%	12.32 ± 2.28†
10%	31.79 ± 2.79†
15%	39.64 ± 5.04†

[a] J_s: permeation rate

[b] Mean ± S.E. (n=4)

†: Significantly different from the control ($P<0.05$)

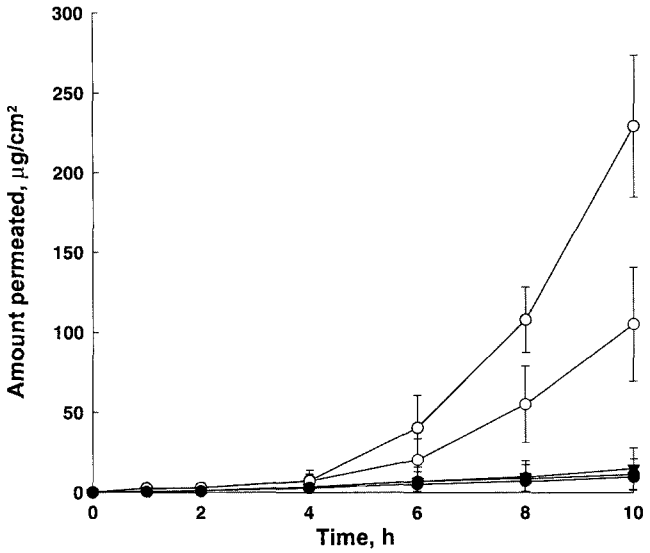

Fig. 4. Permeation profiles of piroxicam through excised rat skins from microemulsions with different S/CoSs (Mean ± S.E., n=4). Key: □; 5/1, ■; 3/1, ▼; 1/1, ○; 1/3, ●; 1/5

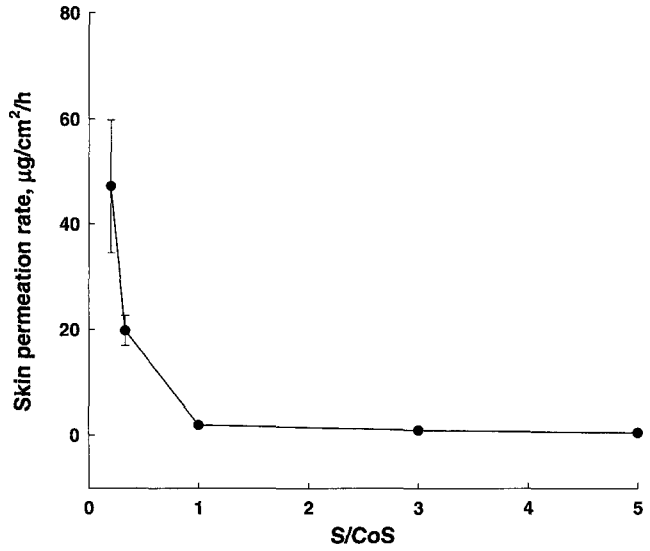

Fig. 5. Skin permeation rate of piroxicam from microemulsions formulated with different S/CoSs (Mean ± S.E., n=4)

piroxicam FAPG ointment increased the lipophilicity of the base and decreased the piroxicam release from the base (Hsu *et al.*, 1994). In contrast, pretreating the skin with oleic acid enhanced the skin permeability of piroxicam. In our study, the skin permeation rates were increased by increasing the oleic acid content over the range of 5-15%. However, the turbidity of microemulsion containing 15% oleic acid was significantly changed after 9 h. This may indicate to the reduction of the drug dissolved in the microemulsion system.

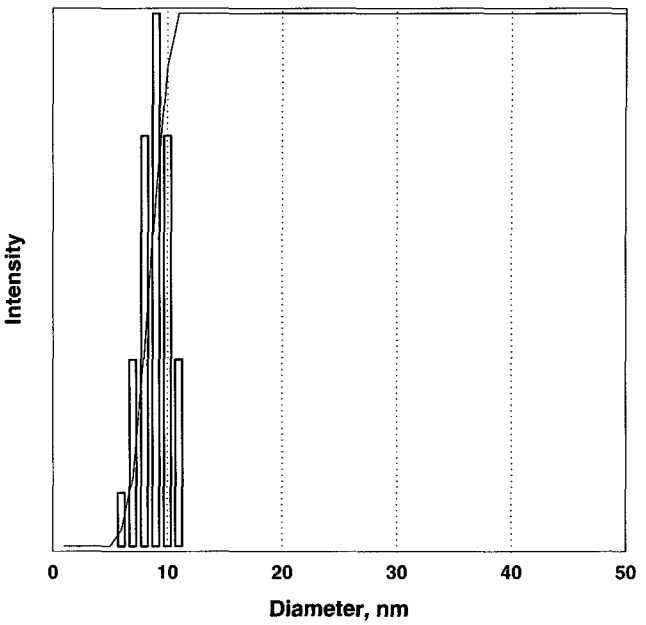

Fig. 6. Histogram of diameter and size distribution of oil droplets in microemulsions containing 10% oleic acid, 10% Labrasol and 50% ethanol measured by dynamic light scattering method.

To investigate the effect of S/CoS on the skin permeation of piroxicam from microemulsion, S/CoSs were varied from 1/5 to 5/1, while the amounts of oleic acid and water phase were fixed at 10% and 28.5%, respectively. The permeation profiles of piroxicam at different S/CoSs are shown in Fig. 4, and the calculated skin permeation rates of the drug are shown in Fig. 5. As shown in these figures, as the S/CoS was decreased, the skin permeation rate of piroxicam was increased. This finding is consistent with a previous report that a higher content of ethanol decreased the oil phase size in microemulsion and increased the skin permeation rate of the drug (Thacharodi and Rao, 1994).

In this study, the optimum formulation exhibiting the highest skin permeation rate (47.14 μg/cm²/h) of piroxicam was found to consist of 0.5% piroxicam, 10% oleic acid, 60% Labrasol/ethanol (1/5) and water. The dynamic light scattering determination revealed that the average size of oil droplets in the microemulsion was 9 nm with narrow distribution (Fig. 6).

CONCLUSION

Various formulation factors were evaluated to find out microemulsions containing piroxicam that provide a large existence area and the high skin permeation of the drug. An optimum formulation of the microemulsion containing 0.5% piroxicam was obtained.

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