Drug Development and Industrial Pharmacy, 31:405–416, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500214605

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Enhanced In Vitro Percutaneous Absorption and In Vivo Anti-Inflammatory Effect of a Selective Cyclooxygenase Inhibitor Using Microemulsion

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ABSTRACT Celecoxib, a specific COX-2 inhibitor, was recently approved for the treatment of rheumatoid and osteoarthritis, acute pain, familial adenomatous polyposis and primary dysmenorrhea. Oral administration of celecoxib is effective against ultraviolet B radiation (UVB)-induced skin carcinogenesis; however, its clinical use is restricted because of its failure to block the characteristic cutaneous inflammatory response and lower availability at the site of inflammation. Topical application of celecoxib has been effective compared with oral in certain clinical conditions. The present study was undertaken to develop and investigate the development of microemulsion system (isopropyl myristate/medium-chain glyceride/polysorbate 80/water) for topical delivery of celecoxib. The pseudoternary phase diagram was constructed with constant surfactant concentration, and several compositions were identified and characterized by using dynamic light scattering. The in vitro permeation rate of celecoxib through rat skin was determined for microemulsions, microemulsion gel, and cream by using the modified Franz-type diffusion cell. In all formulations tested, celecoxib permeated more quickly, and the microemulsions increased the permeation rate of celecoxib up to 5 and 11 times compared with those of microemulsion gel and cream, respectively. Increasing the concentration of medium-chain mono-/di-glyceride in microemulsion imparted increased droplet size and viscosity and decreased diffusion coefficient. In vivo anti-inflammatory study suggested that the developed microemulsion formulations might serve as potential drug vehicle for the prevention of UVB-induced skin cancer.

KEYWORDS Celecoxib, Microemulsion, Topical delivery, Dynamic light scattering, Arachidonic acid

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INTRODUCTION

Prostaglandins (PGs) are local mediators for biological responses such as pain, fever, and inflammatory symptoms (Davies et al., 1984; Needleman et al., 1986). Cyclooxygenase enzyme (COX) catalyzes the conversion of arachidonic acid to PGs, a key step involved in the generation of proinflammatory mediators. Cyclooxygenase enzyme exists in two isoforms. The constitute form (COX-1, found in healthy tissues) produces physiologically important PGs, whereas the inducible form (COX-2) is expressed in inflammatory conditions (Kujubu et al., 1991; Masferrer et al., 1992; Seibert et al., 1994; Xie et al., 1991). Conventional nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both forms of COX enzyme, preventing the production of PGs, thereby reducing pain and inflammation (Marnett, 1992). Treatment with NSAIDs also decreases COX-mediated inflammation, inhibits tumor formation, and induces regression of established tumours. However, concurrent inhibition of COX-1 by conventional NSAIDs inhibits PG-dependent physiological mechanisms, such as gastroduodenal mucosal defense and platelet aggregation (Fischer et al., 1999; Fulton, 1984; Narisawa et al., 1983; Pentland et al., 1999; Reddy et al., 1993). The significant side effects associated with long-term use of nonselective NSAIDs necessitate the development of selective COX-2 inhibitors (Oates et al., 1988a, 1988b; Patrono & Dunn, 1987).

Celecoxib, a diaryl substituted pyrazole derivative containing a sulfonamide substituent, is a specific COX-2 inhibitor, which was approved recently by several countries for the treatment of rheumatoid arthritis, osteoarthritis, acute pain, familial adenomatous polyposis, and primary dysmenorrhea (Krishnan et al., 2003; Marshall, 1999; Simon et al., 1998). Celecoxib demonstrated significant chemo-preventive activity in colon carcinogenesis and ultraviolet B radiation (UVB)-induced skin cancer and breast cancer (Fischer et al., 1999; Harris et al., 2000; Kawamori et al., 1998). Topical application of celecoxib suppresses UVB-mediated cutaneous inflammation (Wilgus et al., 2000). Long-term topical treatment with celecoxib has been also found effective in blocking the formation of UVB-induced tumors because inhibition of COX-2 leads to inhibition of angiogenesis and tumor cell proliferation as well as the induction of apoptosis (Parrett et al., 1997; Pentland & Needleman, 1986; Reddy et al., 1996). Although systemic oral administration of celecoxib has been effective against UVB-induced skin carcinogenesis, its clinical use is restricted because of its failure to block the characteristic cutaneous inflammatory response and lower availability of the drug at the site of inflammation. Experimental and clinical data have further demonstrated that treatment with celecoxib may cause fewer serious adverse gastrointestinal effects than treatment with nonselective NSAIDs, although it is still significant in some cases (Everts et al., 2000). Thus, topical delivery becomes an alternative route to the administration for NSAIDs to provide local absorption at the inflammation site without adverse systemic reactions, and such delivery systems are considered advantageous in certain clinical conditions.

Microemulsions as drug delivery vehicles for different routes of administration, such as oral, topical, ocular, and pulmonary, have been reported (Lawrence & Rees, 2000; Tenjarla, 1999). Microemulsions represent a novel type of topical drug delivery system that increases percutaneous absorption of both hydrophilic and lipophilic drugs, compared with conventional vehicles based on the constituents used in the formulation of microemulsions. Furthermore, transdermal drug delivery potential of microemusions is highly dependent on the incorporated ratio of the respective components rather than the individual characteristics of the components.

Microemulsion enhances the transdermal permeation of drugs because of

1. greater solubility potential of microemulsions for both lipophilic and hydrophilic drugs, which may generate an increased thermodynamic activity toward skin;
2. use of components possessing permeation enhancer activity; and
3. possibility to alter the affinity of a drug to the internal phase, thereby favoring partitioning into stratum corneum (Kreilgaard, 2002).

In the present work, an attempt was made to develop a suitable microemulsion system for topical delivery of celecoxib and to investigate the effect of microemulsion composition on such delivery. The permeation profiles of different microemulsion formulations were compared with the permeation profiles of microemulsion gel and cream. Those

microemulsion formulations showing greater permeation were evaluated for topical anti-inflammatory effect on mice.

MATERIALS

Isopropyl myristate (IPM) and polysorbate 80 were obtained from E-Merck, India, and Sigma, USA, respectively. Capmul MCM (caprylic/capric mono-/di-glycerides) was a gift sample from Abitec Corporation, USA. It was approximately a 5:1 mixture of C8/C10 mono-/di-glycerides (MDG) with 2% free glycerol and having the following percentages of fatty acids: caprylic (C8): 83, capric (C10): 15.5, caproic (C6): 1.0, and palmitic (C16): less than 1.0. Celecoxib was received from Zydus Cadila Ltd., India. Solvents used were of HPLC grade, and all other chemicals were of analytical grade. All regents were used as received. Water, doubly distilled in a borosilicate glass apparatus, was used in all experiments.

METHODS

Screening of Oils and Surfactant for Microemulsion

Suitable oil and surfactant that possess good solubilizing capacity on celecoxib were identified by using solubility studies in various oil and surfactant solutions. Solubility of celecoxib in vegetable oils (soya bean oil, olive oil, and castor oil), IPM, MDG, and surfactant solutions was determined by adding excess amount of drug and continuously stirring for at least 72 h at 30°C. The mixtures were centrifuged (2500 rpm for 30 min), and supernatant was filtered through 0.45-μm membrane filter. Drug concentration in the filtrate was determined by using an HPLC after appropriate dilution with acetonitrile.

Formulations

Construction of Phase Diagram and Formulation of Microemulsions

Pseudoternary phase diagram was constructed by using a conventional titration technique with water, IPM, and caprylic/capric mono-/di-glycerides (MDG) at a constant polysorbate 80 concentration. In set 1, the appropriate amounts of IPM, MDG, and polysorbate 80 were taken in different stoppered test tubes and stirred until it becomes clear. These mixtures were then titrated with water by using a microsyringe at a constant temperature until the onset of turbidity or phase separation. In set 2, samples containing IPM, polysorbate 80, and water in different proportions were also prepared and titrated against MDG until phase separation or turbid points were reached. In both cases, the mixtures were stirred vigorously for a sufficient length of time for homogenisation, and the end point was visually monitored against a dark background by illuminating the samples with white light. The experiments were performed in triplicate to check reproducibility. From the end point, compositions of the titrated samples, the mass percent compositions of the IPM, MDG, and water were calculated and plotted on triangular coordinates to construct the pseudo ternary phase diagrams.

Drug-loaded microemulsion formulations were formulated by admixing appropriate quantities of the components and drug in stoppered test tube followed by vortexing vigorously at room temperature for sufficient length of time. All the formulations were examined by polarized light microscopy for lack of birefringence.

Preparation of Microemulsion Gel and Cream

Celecoxib was dissolved in the mixture of IPM and MDG. Carbopol 934 was dispersed in this mixture along with water and stirred magnetically. Polysorbate 80 was added, and the resulting gel was mixed well.

Cream was formulated by dispersing celecoxib in melted anionic emulsifying ointment. Slightly warmed double-distilled water was added to this mixture and stirred gently until it becomes cold to obtain the cream consistency.

Characterization of Microemulsions

The viscosity of microemulsion was measured at 25±0.5°C by using an Ubbelohde viscometer (Remco, India). Refractive index was measured by an Abbe refractometer (Precision Standard Testing Equipment Corporation, India). Drug content of microemulsions

was determined spectrophotometrically (Hitachi, Japan) at 253 nm after dilution with methanol. Solubility of celecoxib in the six-microemulsion systems was determined by adding excess amount of drug and continuously stirring for at least 72 h at room temperature to obtain equilibrium. The supernatant was filtered by using 0.45-μm membrane filter and analyzed by HPLC after appropriate dilution with methanol.

Particle size distribution, diffusion coefficient, polydispersity, and average droplet size of microemulsions were measured at 27±0.5°C by dynamic light-scattering (DLS) spectrophotometer (model DLS 700, Otsuka Electronics Co. Ltd., Osaka, Japan) equipped with He-Ne laser operating at 632 nm monitoring the intensity of the scattered light at 90°.

Microemulsions were clarified by passing through 0.45-μm Millipore filters thrice prior to measurements. The mean hydrodynamic diameter of the droplets (d_h), the polydispersity index (PDI), and the diffusion coefficient (DC) of the dispersed droplets were obtained with the aid of the instrument's software.

Stability Studies

Stability studies of microemulsions were carried out in closed containers at refrigerated and ambient temperatures for 3 months. Samples were withdrawn in duplicate at 0 and after 1, 2, and 3 months, and their chemical and physical stabilities were assessed. Chemical stability was expressed as celecoxib content, which was determined spectrophotometrically. Physical stability was evaluated by visual inspection for physical changes, such as phase separation, flocculation, and/or precipitation.

Preparation of Epidermis

Full-thickness abdominal skin was excised from Sprague-Dawley rats whose hair had been removed previously by an electric clipper. The subcutaneous tissue was surgically removed, and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The full-thickness skin was washed and soaked in 2 M sodium bromide solution in water for 6–8 h. The epidermis was separated from dermis by using swab moistened with water, washed thoroughly, vacuum dried, and stored in a desiccator for later use (Scott et al., 1986).

Partition Studies

Partition coefficient of celecoxib between epidermis and microemulsion was determined in triplicate. The epidermis (1 cm^2) was reconstituted by immersing in water for 24 h. After reconstitution they were blotted dry, weighed, and equilibrated in various microemulsion formulations containing 2% w/w celecoxib for 24 h at room temperature in a shaker. The drug concentration in microemulsions after 24 h was measured, and the difference in concentration was supposed to have partitioned in the epidermis. The partition coefficient (PC) was calculated as follows

$$PC = (C_{initial} - C_{final})/C_{final} \quad (1)$$

where $C_{initial}$ is the initial concentration of celecoxib in microemulsion, and C_{after} is the concentration of celecoxib in microemulsion at the end of equilibration period.

In Vitro Permeation Studies

In vitro permeation studies were performed with the modified Franz diffusion cell with an effective diffusional area of 1.13 cm^2. The epidermis was allowed to equilibrate in receptor fluid for 1 h and then mounted over diffusion cell where the stratum corneum side was facing upward into the donor compartment, and the dermal side was facing downward into the receptor compartment. The receptor compartment was filled with a mixture of 30% polyethylene glycol 400 in normal saline, and it was continuously stirred to ensure uniform distribution and to maintain sink conditions. The temperature of the entire diffusion cell assembly was maintained at 37±0.5°C by using a recirculating water jacket. Drug formulation (1 mL) was applied on the skin surface. Samples were withdrawn from receptor compartment at predetermined time intervals and replenished with the same volume of fresh receptor phase after each sampling. Each experiment was repeated three times, and the receptor samples were analyzed spectrophotometrically at 253 nm.

The cumulative amount of celecoxib permeating across the skin was plotted as a function of time. Drug permeation rate at steady state (μg/h/cm^2) was calculated by dividing the slope of the linear portion of the curve by the exposed area of the skin.

Topical Anti-Inflammatory Effect

Microemulsion formulations B and E (defined in Formulations) were used for this study. The animal protocol was reviewed and approved by institutional animal ethical committee of Jadavpur University, Kolkata. Male Swiss albino mice (20–25 g) were randomly divided into four groups of six mice each. The animals were kept in the animal house of the department of pharmaceutical technology and maintained under standard conditions. They were provided with food and water ad libitum during the quarantine period. Cutaneous inflammation was induced by applying 0.5 mg of arachidonic acid to the inner surface of the right ear of mice. The left ear remained untreated. The arachidonic acid was dissolved in microemulsion formulations. The two control groups received the drug-free microemulsion formulations, whereas the other groups received drug-loaded (2% w/w) microemulsion formulations. One hour after the treatment, the animals were killed by cervical dislocation, and an 8-mm-diameter disk from each ear was removed with a sharp metal punch. The weights of these disks were measured with microgram accuracy, and the inflammatory response (oedema) was evaluated by measuring the difference in weight between the two plugs. The percentage of inhibition was calculated according to the formula:

$$100(W_c - W_t)/W_c \qquad (2)$$

where W_c is mean weight produced by the control groups and W_t is mean weight produced by the test groups.

Analytical Methods

Celecoxib was analyzed by using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) consisted of a LC-10AT model isocratic pump, a SPD-M10 AVP model variable spectrophotometer detector, a CR601 model Chromatopac integrator, a Rheodyne injector (7725i) fitted with a 20-μL loop and a Wakosil II C-18-RS column (250-mm length, 4.6-mm diameter, 5-μm particle size; SGE, Australia). The mixture of acetonitrile, methanol, and pH 3-phosphate buffer (50:10:40) was used as the mobile phase at a flow rate of 1 mL/min, and the detection wavelength was set at 249 nm.

Stock solution of celecoxib was prepared by dissolving appropriate amount of drug in methanol and various dilutions were made with mobile phase to obtain concentrations in the range of 2–50 μg/mL. The peak area of different celecoxib concentrations linearly correlated with correlation coefficient of 0.999.

Statistical Analysis

The significance of the difference between groups was tested by using one-way ANOVA, and Tukey-Kramer multiple-comparisons test was used to compare the groups. The difference at $p < 0.05$ was considered significant. Topical anti-inflammatory effect was analyzed by Student's t-test at 5% significance level.

RESULTS AND DISCUSSION

Screening of Oils and Surfactant for Microemulsion

Microemulsions prepared by using only pharmaceutically acceptable ingredients are limited. Therefore, with a view to developing a suitable microemulsion system for topical delivery of celecoxib, the solubility of celecoxib in various oils and nonionic surfactant solutions were determined, and the results are presented in Table 1. Highest solubility of the drug was observed with IPM (6.0 mg/mL) followed by castor oil, soya bean oil, and olive oil. On the other hand, solubility of the drug in polysorbate 80 was greater than that in polysorbate 20 (Table 1); they are known to be unaffected by pH and ionic strength variations. The use of IPM as oil produced appreciable proportions of both oil-in-water and water-in-oil

TABLE 1 Solubility of Celecoxib in Different Oils and Surfactants at 25°C (Mean ± Estimated Error, n = 3)

Oil/surfactant	Solubility (mg/mL)
Soya bean oil	3.6 ± 0.3
Castor oil	4.4 ± 0.8
Olive oil	3.1 ± 0.3
IPM	6.0 ± 0.5
Capmul MCM	45.5 ± 1.8
Polysorbate 80 (25% in water)	12.0 ± 1.1
Polysorbate 20 (25% in water)	7.4 ± 0.8

microemulsions, compared with physiologically tolerable oils (Acharya et al., 2001). Topical delivery of drugs was improved by the addition of penetration enhancers, which are able to disorganize the skin barrier properties in a reversible manner. Medium-chain fatty acids, mono-, di-, and tri-glycerides, particularly caprylic/capric mono-/di-glycerides have been used as oral absorption enhancers for a number of drugs, because they are physiologically well tolerated (Constantinides et al., 1994). Furthermore, mono-/diglycerides or polyol fatty acid esters also exhibit permeation-enhancing properties (Takahashi et al., 1996, 2001). On the basis of results and the above-mentioned prospects, IPM has been used as oil phase with polysorbate 80 and MDG as surfactant and cosurfactant to develop microemulsion systems for further studies.

Formulation and Characterization of Microemulsions

The pseudoternary phase diagram of a system containing IPM, MDG, and water at a constant polysorbate 80 concentration (23% w/w) is shown in Fig. 1. The concentration of polysorbate 80 was fixed at 23% w/w because the microemulsion zone was moderate with this amount and more than 25% w/w polysorbate 80 induced more gel formation and might impart toxicity to the formulation. The present system produced a greater monophasic zone, and six microemulsion compositions (A–F marked in the diagram) were selected in the monophasic region with varying concentrations of IPM and MDG. The selected formulations were essentially oil-in-water type, and their microemulsion characteristics were maintained after incorporation of 2 wt% celecoxib. The detailed composition of microemulsions, microemulsion gel, and cream is given in Table 2.

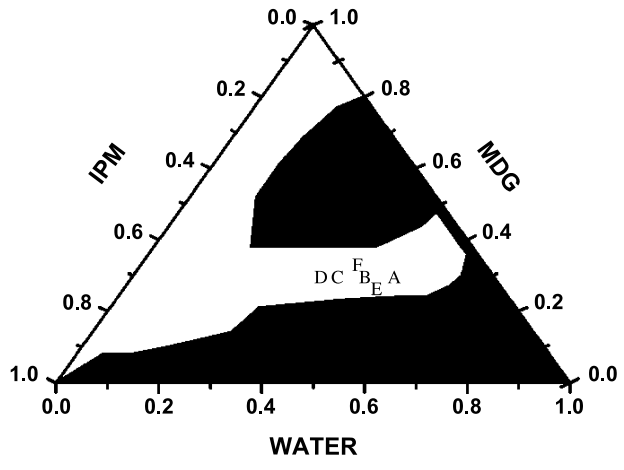

FIGURE 1 **Pseduoternary Phase Diagram of the IPM/MDG/Polysorbare 80/Water System at a Constant Polysorbate 80 Concentration of 23 wt% at 28°C. Clear Region Represents Microemulsion Zone. Scale Magnitude Reduced to 100-Fold.**

The physical properties (viscosity, solubility of drug, particle size, diffusion coefficient, and polydispersity) of microemulsion formulations are presented in Table 3. Drug content in the formulations varied from 1.97 to 2.13 wt%. A previous study reported that microemulsion systems composed of IPM, MDG, polysorbate 80, and water of both water-in-oil and oil-in-water types exhibit Newtonian fluidity (Subramanian et al., 2005). In this study, Newtonian flow was also observed with viscosity between 95 and 118 cp. Newtonian behavior was also reported for microemulsion systems composed of IPM/polysorbate 80/soyabean lecithin/water (Moreno et al., 2001). DLS measurement revealed that microemulsions containing celecoxib possessed droplet size between 104 and 316 nm, whereas the mean droplet size in drug-free microemulsion varied between 79 and 102 nm. Note that the exact values obtained by the DLS instrument's software could often be misleading in comparison with other techniques (SAXS, SANS) (Aswal & Goyal, 1998; Hayter, 1985; Hayter & Penfold, 1981).

It was observed that increasing concentration of MDG increased both droplet size and viscosity and decreased diffusion coefficient of the formulations B, E, and F. The PDI values (i.e., the ratio between the standard deviation in d_h and the average d_h) of the microemulsion dispersion were obtained from DLS measurements by assuming a unimodal log-Gaussian distribution. The PDI value of 0.1 indicates homogeneity in droplet distribution. The studied formulations produced high PDI values indicating appreciable polydispersity of the formulations. The increased addition of celecoxib in microemulsion initially decreased the average droplet size and then made it increase. The minimum average droplet diameter was reached at 0.5 wt% of celecoxib. The mechanism for this behavior was not clear. The influence of the drug on the interfacial activity of the surfactant might have a bearing on the phenomenon. The subsequent

TABLE 2 Compositions (wt%) of Celecoxib Containing Microemulsions, Microemulsion Gel, and Cream

	Microemulsion						Micoemulsion gel	Cream
Components/formulations	A	B	C	D	E	F	G	H
Isopropyl myristate	15	19	23	26	19	19	15	
Mono-/di-glycerides	22	22	22	22	20	24	18	
Polysorbate 80	23	23	23	23	23	23	25	
Water	38	34	30	27	36	32	37	68
Carbopol 934							3	
Emulsifiying ointment[a]								30
Celecoxib	2	2	2	2	2	2	2	2

[a]The composition is emulsifying wax (30%), white soft paraffin (50%), and liquid paraffin (20%) in wt%.

increase in size with increased presence of the drug was as expected. This observation was in line with other reports (Park & Kim, 1999). Refractive indices of the formulations are in the range of 1–1.3.

The solubility of celecoxib in microemulsion and the individual components was determined to investigate the role of microemulsion structure toward solubilization. The results are presented in Fig. 2 along with calculated solubility of drug in individual microemulsion components. The results indicated that microemulsion structure did not contribute toward solubilization of the drug being in agreement with previous findings (Peltola et al., 2003). Generally, drug solubility increases because of formation of an interfacial surfactant film between oil and water by increasing the interfacial area (Tenjarla, 1999). For maximum solubilization, it is desirable to have most of the surfactant at the interface between oil and water, rather than dissolved in oil or water phases or in both. The calculated solubility data have shown that most of the celecoxib was dissolved by polysorbate 80 and MDG.

Favorable partitioning of polysorbate 80 and MDG between the bulk phases reduced their concentration at the interface and, consequently, reduced the interfacial area, causing a reduction in solubility of celecoxib compared with the calculated solubility.

Partition and In Vitro Permeation Studies

The results of partition coefficient and in vitro permeation rate of various formulations are presented in Table 4. Partition coefficient of celecoxib decreased with increasing concentration of IPM in microemulsion, and it was not statistically significant ($p>0.05$) between successive increments. However, partition coefficient at 20% of MDG in microemulsion was significantly ($p<0.05$) higher than that at 22 and 24% of MDG in microemulsion. Partition coefficient decreased with increasing concentrations of IPM and MDG.

The in vitro permeation profiles of various formulations are illustrated in Fig. 3. The permeation

TABLE 3 Physicochemical Characteristics of Microemulsion Formulations

	Dynamic light scattering				
Formulations	d_h (nm)[a,b]	PDI[c]	DC $\times 10^{-8}$ (cm^2/sec)[d]	Viscosity (cp)[a]	Drug solubility (mg/mL)[a]
A	266±4.3	1.14	2.05	114±4.8	38.9±3.0
B	134±1.2	1.14	4.07	101±3.6	39.1±3.7
C	145±1.5	1.11	3.74	95±3.0	41.8±2.5
D	186±2.4	0.42	2.93	102±4.0	42.2±2.6
E	104±1.3	1.06	5.25	96±3.0	39.4±1.6
F	317±5.6	0.32	1.49	118±2.6	42.1±3.7

[a]Each value is the average of three different experiments±estimated error.
[b]Hydrodynamic diameter of the droplets.
[c]Polydispersity index.
[d]Diffusion coefficient.

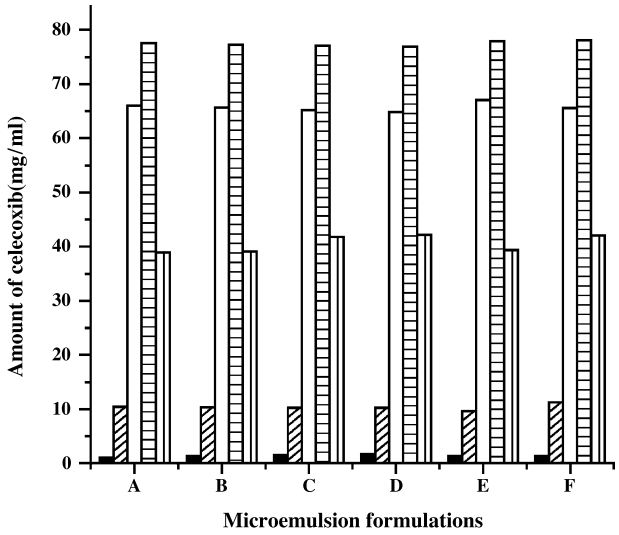

FIGURE 2 Solubility of Celecoxib in Microemulsion Formulations (A–F, ▥) and their Neat Ingredients, IPM (■), MDG (▨), Polysorbate 80 (□), and Total (▤).

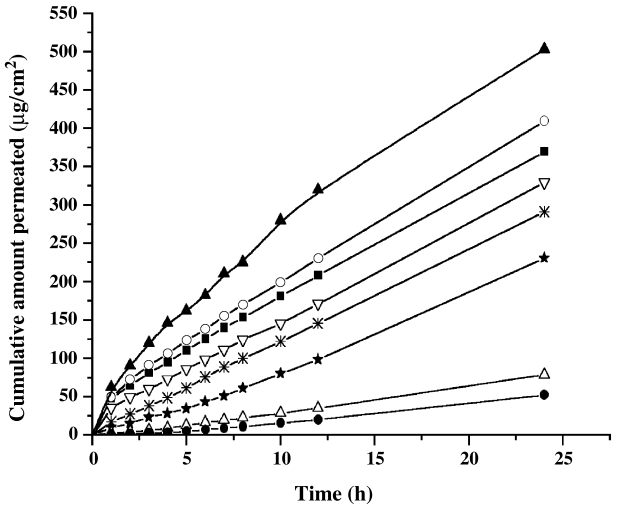

FIGURE 3 Permeation Profile of Celecoxib Through Excised Rat Skins from Microemulsions A (■), B (○), C (▽), D (*), E (▲), F (★), and from Microemulsion Gel (△) and from Cream (●). The Plotted Values were the Mean Value of Three Different Experiments. Estimated Error Varied Between 12 and 26% of the Mean.

rates with microemulsion formulations were 3- to 5-fold and 7- to 11-fold greater than with microemulsion gel and oil/water cream, respectively. In all formulations tested, celecoxib penetrated into rat skin more quickly but largely from microemulsions by virtue of their internal structure/composition. The permeation rate of celecoxib increased with increasing concentration of oil up to 19% and then decreased with further increase in IPM up to 26%. However, the difference in the permeation rates among the IPM concentrations was not statistically significant ($p>0.05$). High surfactant content of microemulsion formulation might have made the effect of oil on the skin permeation less pronounced. This finding was consistent with previous report (Rhee et al., 2001). It has been reported that increasing IPM concentration caused a parallel increase in absorption rate of the drug although the degree of increase for concentrations above a critical level was reduced to some extent (Ozawa et al., 1988). On the other hand, increasing MDG concentration from 20 to 24% decreased the permeation rate of celecoxib. Difference in permeation rate of celecoxib at 20% MDG concentration was statistically significant ($p<0.001$) from that at 24% concentration, but it was not significantly different ($p>0.05$) from that at 22% MDG concentration. Among the microemulsion formulations, formulation E showed the highest permeation rate, and it was significantly different from the rest of the formulations as well as the microemulsion gel and the cream except for formulation B.

Enhancement of skin penetration of drug from a vehicle may be achieved in three ways: 1) by increasing thermodynamic activity, 2) by improving skin/ vehicle partition coefficient, and 3) by altering the barrier property of stratum corneum. The absence of vehicle-skin and/or vehicle-drug interaction and the use of fixed concentration of drug in formulations would establish direct relationship between drug permeation rate and its thermodynamic activity (Twist & Zatz, 1988). A finite concentration of drug was used for in vitro permeation study, and thermodynamic activity of drug in different microemulsion

TABLE 4 Permeation Rate and Partition Coefficient of Celecoxib (Mean ± Estimated Error, n=3) Through Rat Skin from Microemulsion Formulations, Microemulsion Gel, and Cream

Formulations	Permeation rate ($\mu g/cm^2/h$)	Partition coefficient
A	9.9±0.9***	0.79±0.04[a]
B	11.3±1.3***	0.77±0.06[a]
C	9.4±1.0***	0.76±0.03[a]
D	8.7±1.4***	0.69±0.07
E	14.2±1.5***	1.01±0.08[b]
F	8.8±1.0***	0.52±0.03
G	3.1±0.5*	–
H	1.3±0.4	–

[a]Significantly different from the formulation F ($p<0.05$).
[b]Significantly different from the formulation F ($p<0.001$).
*Significantly different from the formulation H ($p<0.05$).
***Significantly different from the formulation H ($p<0.001$).

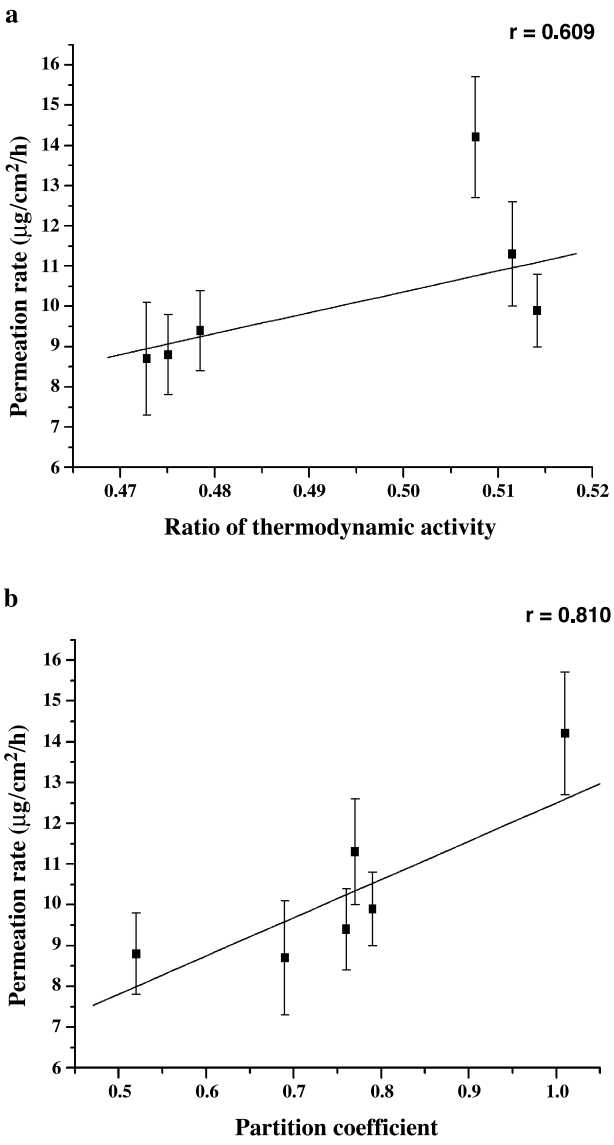

FIGURE 4 **Permeation Rate of Celecoxib from Microemulsions as a Function of Thermodynamic Activity (a) and Partition Coefficient (b). Error Bars Represent Estimated Error (n=3). Solid Line Represents Best Liner Fit.**

formulations was calculated from saturation solubility. The activity of the drug was considered constant in each formulation at its saturated concentration. The ratio of 2% (w/w) drug with its saturated solubility in each formulation was taken, equivalent to its activity in the formulation (Martin et al., 1993; Thomas & Panchagnula, 2003). A comparison of the thermodynamic activity, partition coefficient, and permeation rate for different microemulsion formulations is shown in Fig. 4. The permeation rate of celecoxib has shown moderate correlation with partition coefficient. However, a relationship between permeation rate and the thermodynamic activity was poor. The overall efficacy of a topical formulation depends on the release characteristics of the delivery vehicle and the pharmacokinetics of the drug as it diffuses through skin. In addition, the penetration rate of a topically applied drug is dependent on the thermodynamic activity of the drug in the vehicle if specific penetration enhancers are absent. The drug flux for the formulation containing penetration enhancer is influenced by both penetration enhancement and the thermodynamic activity (Djordjevic et al., 2003). Permeation of the drug is favorable when it is in monomeric form in the formulation. The increase of MDG might have decreased the drug monomer concentration and, consequently, reduced the concentration-driven penetration of the drug into the epidermis. It has been reported that large contents of the components in a vehicle may substantially reduce the partition coefficient of a drug between the skin and the vehicle as well as reduce its overall activity in the vehicle and thereby decrease the transdermal flux (Turi et al., 1979).

The rate of drug release may decrease when the microemulsion is transformed to lamellar structure or an emulsion having a highly ordered microstructure and high viscosity (Trotta, 1999). The partition behavior of celecoxib between epidermis and vehicle may be partially attributed to the viscosity of the vehicle. Increased amount of MDG in the formulations caused increased droplet size and viscosity, which increased diffusional resistance of epidermis. The microemulsions with smaller size showed enhanced permeation of celecoxib through the skin compared with those of larger ones. These results were in agreement with the previous reports (Esposito et al., 1998; Verma et al., 2003). Therefore, decreased permeation rate of celecoxib at higher MDG concentration in microemulsion can be attributed to increased viscosity and droplet size and decreased partition coefficient of celecoxib. Superiority of microemulsions over the other vehicles may be due to the composition of microemulsion and its particle size and viscosity.

Stability Studies

All formulations used for the study showed chemical stability of celecoxib in them having drug content between 93 and 98.9%. No physical change

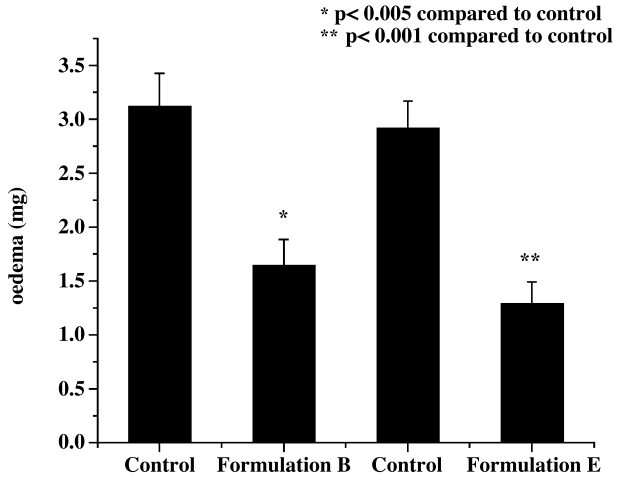

FIGURE 5 **In Vivo Topical Anti-Inflammatory Effect of Celecoxib Microemulsion Formulations B and E. Drug-Free Respective Formulations Acted as Control. The Error Bar Represents SD (n = 6).**

was observed by storing at room temperature, whereas phase separation was observed during storage in a refrigerator but was readily redispersed on shaking at room temperature. The phase separation was due to the presence of nonionic surfactant in the formulations because they are sensitive to changes in temperature (Lawrence & Rees, 2000).

In Vivo Topical Anti-Inflammatory Effect

The arachidonic acid-induced ear edema model was used for the assessment of topical anti-inflammatory effect of celecoxib. Arachidonic acid applied to the mouse ear induced a rapid inflammatory response, and the extent of inflammation can readily be quantified by the wet weight of the edematous ear. This model has been considered suitable for screening anti-inflammatory compounds inhibiting the metabolic arachidonic acid pathway (Ishii et al., 1994; Young et al., 1984). Arachidonic acid-induced inflammatory response in the ear of mice is mainly mediated by leukotriens and prostaglandins and is suitable for evaluating inhibitors of 5-lipoxygenase and cyclooxygenase enzymes (Carlson et al., 1985; Opas et al., 1985). In topical application, celecoxib microemulsions significantly inhibited the edema formation compared with that of control, and the results are shown in Fig. 5. Formulation E inhibited the ear edema by 55.7%, whereas formulation B inhibited it by 47.1%. Exposure of epidermal cells to ultraviolet light B radiation (UVB) has been associated with increased release of arachidonic acid from membrane phospholipids and increased biosynthesis of prostaglandin from arachidonic acid (Hruza & Pentland, 1993). The acute exposure to UVB light induced an inflammatory response and increased the production and release of prostaglandin, and this process may lead to skin cancer on long-term exposure to UVB light. Inhibition of this inflammatory response ultimately is effective in preventing UVB-induced tumor development in the skin (Wilgus et al., 2000). In the present study, celecoxib in the form of microemulsion significantly inhibited the inflammatory response induced by arachidonic acid, and the mechanism of induction is similar to UVB light-induced inflammatory response. Therefore, the topical celecoxib microemulsion formulations may be useful for the prevention of development of skin cancer induced by UVB radiation in the long run.

CONCLUSION

A stable microemulsion system for topical delivery of celecoxib using IPM, Polysorbate 80, MDG, and water has been developed. The droplet size of the dispersion increased with drug addition. Increased MDG in the microemulsion formulation increased droplet size and viscosity with decreased permeation rate of celecoxib in the rat skin. In vitro permeation experiments showed superiority of microemulsions overt microemulsion gel and cream for topical delivery of celecoxib. The permeation rate with the microemulsion is 5- and 11-fold higher than the permeation rates with microemulsion gel and cream. The results of the investigation revealed that superiority of microemulsions over the other vehicles is due to the composition of microemulsion as well as its particle size and viscosity. Anti-inflammatory activity of formulation E on mouse skin was significantly higher than that of formulation B. The developed microemulsion formulations are expected to be potential vehicles for topical delivery of drugs.

ACKNOWLEDGMENTS

N. Subramanian is grateful to All India Council for Technical Education for providing him a research fellowship. The authors thank Abitec Corporation, USA, for the gift sample of capmul MCM and Zydus

Cadila Ltd., India, for providing the celecoxib. S. P. Moulik thanks Indian National Science Academy for a senior scientist position.

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