Original Article

Formulation of microemulsion gel systems for transdermal delivery of celecoxib: *In vitro* permeation, anti-inflammatory activity and skin irritation tests

Sara M. Soliman[1], Nevine S. Abdel Malak[2,*], Omaima N. El-Gazayerly[2], Abdel Aziz Abdel Rehim[1]

[1] *Department of Pharmaceutics, Faculty of Pharmacy, 6th of October University, 6th of October City, Egypt;*
[2] *Department of Pharmaceutics, Faculty of Pharmacy, Cairo University, Egypt.*

ABSTRACT: The aim of this study was to develop suitable microemulsion gel systems for transdermal delivery that could assist dissolution enhancement of poorly water soluble celecoxib and thus improve its skin permeability. Long term oral administration of celecoxib causes serious gastrointestinal adverse effects, which makes it a good candidate for transdermal formulations, yet its low water solubility (4 mg/L) makes this challenging. Ternary phase diagrams were constructed using isopropyl myristate and oleic acid as oils, Tween 80 as surfactant, and Cremophor RH40 as cosurfactant. Microemulsion areas were identified and two systems each of 36 formulas were prepared and assessed for visual inspection, spreadability, pH measurements, and droplet size analysis. Drug release and *in vitro* permeation of celecoxib from microemulsion formulas through semi-permeable membranes and excised abdominal rabbit skin, respectively, were carried out and compared to celecoxib cream. In all tested formulas, celecoxib was released and permeation was at a higher rate than that from the corresponding cream. The optimized formula (F12) was found to be superior to all other formulas. This formula increased the permeation rate of celecoxib up to 11 times compared to that of the cream. Its stability was retained after one year of storage under ambient conditions and its anti-inflammatory effect was significantly higher than that of celecoxib cream and the oral commercial formula. Skin irritancy and histopathological investigation of rat skin revealed its safety. The results revealed that the developed microemulsion gel has great potential for transdermal delivery of celecoxib.

Keywords: Microemulsion gel, transdermal, histopathology, celecoxib, anti-inflammatory

**Address correspondence to:*
Dr. Nevine Shawky Abdel Malak, Faculty of Pharmacy, Cairo University, Kasr El Ainy Street 11562, Cairo, Egypt.
e-mail: pharmnova@yahoo.com

1. Introduction

Celecoxib was the first synthesized non-steroidal anti-inflammatory drug (NSAID) able to selectively inhibit COX-2 activity (*1*). Celecoxib is used for the treatment of rheumatoid arthritis, osteoarthritis, and acute pain with oral administration (*2*). Long term oral administration of celecoxib causes serious side effects, such as gastrointestinal toxicity, gastric mucosal ulceration, hemorrhage and recently, cardiotoxic effects, that restrict its oral use and make it a good candidate for transdermal administration (*3*). Yet, very poor aqueous solubility of celecoxib in water (4 mg/L) and the excellent barrier function of the skin limit its formulation as a transdermal dosage form and make this challenging. Therefore, formulation of celecoxib in a transdermal dosage form with a high degree of skin permeation and safety could be useful.

One of the most important techniques for enhancement of transdermal permeation of drugs is the use of nanoemulsion and microemulsion vehicles (*4*,*5*). Microemulsion is defined as an oil-in-water (o/w) or water-in-oil (w/o) emulsion producing a transparent product that has a droplet size < 0.2 μm and does not have a tendency to separate (*4*,*6*). It is thermodynamically stable dispersions of oil and water stabilized by an interfacial film of amphiphile blend (surfactants either alone or in combination with co-surfactant) (*7-10*). Microemulsions have received great attention for various applications including, dermal and transdermal drug delivery due to ease of preparation, thermodynamic stability, permeation enhancement activity of their components, and a high solubilizing capacity for various drugs over conventional topical formulation vehicles (*11-16*).

Therefore, the aim of this study was to develop suitable microemulsion gel systems (without addition of gelling agent) after screening of oils, surfactants, and cosurfactants for transdermal delivery of celecoxib to enhance its dissolution and to improve its skin permeability with enhanced safety. Microemulsions were prepared using pharmaceutically acceptable

ingredients without using additional chemical enhancers.

The prepared formulas were subjected to extensive physicochemical evaluation, *in vitro* release, and *in vitro* permeation studies. Candidate formula was subjected to assessment of anti-inflammatory activity using the carrageenan-induced rat's paw edema method in addition to skin irritancy test and histopathological investigation of rat skin to investigate the safety of the microemulsion gel formulas for transdermal use.

2. Materials and Methods

2.1. *Materials*

Celecoxib was obtained as a gift from Amoun Pharmaceutical Co. (Cairo, Egypt). Isopropyl myristate (IPM), oleic acid, Tween 80 (polysorbate 80), and Tween 40 were obtained from Merck-Schuchardt (Hohenbrunn, Germany). Propylene glycol and ethylene glycol were obtained from Fluka AG (Buchs, Switzerland). Chloroform and sodium lauryl sulfate (SLS) were obtained from Adwic, El-Nasr Pharmaceutical Chemical Company (Cairo, Eygpt). Cremophor RH40 (polyoxyl 40 hydrogenated castor oil) was from BASF (Schwarzheide, Germany). Synthetic cellulose nitrate membrane (0.45 µm Tuffyrn membrane filter) and Celebrex® capsules (100 mg celecoxib capsules) were from Sartorius Stedium (Aubagne, France) and Pfizer Egypt (Cairo, Egypt), respectively. Olive oil, white soft paraffin, liquid paraffin, cetostearyl alcohol, and all other chemicals were of analytical grade and used without further purification.

2.2. *Screening of oils, surfactants, and cosurfactants for microemulsion preparation*

Solubility of celecoxib in various oils such as IPM, oleic acid, and olive oil in surfactants including Tween 80 and Tween 40 and in cosurfactants such as propylene glycol, Cremophor RH40 and ethylene glycol was determined to select the appropriate oil phase, surfactant, and cosurfactant.

An excess amount of celecoxib was added to each oil, surfactant and cosurfactant in stoppered vials and was shaken reciprocally at 30ºC for 72 h to reach equilibrium (*17*). The mixtures were removed from the shaker and centrifuged for 30 min at 2,500 rpm to remove excess undissolved celecoxib. The supernatants were filtered through a 0.45 µm Millipore filter and the drug concentration in the filtrate was determined using a UV spectrophotometer (UV-1601 PC, Shimadzu, Kyoto, Japan) at λ_{max} 258.4 nm after appropriate dilution with chloroform (*18*).

2.3. *Construction of microemulsion phase diagrams*

Ternary phase diagrams were constructed to obtain the concentration range of components for the existing microemulsion zones. Mixtures of oil, surfactant, and cosurfactant at certain weight ratios were weighed into glass vials and were shaken to ensure complete mixing. Phase diagrams were constructed by titrating these mixtures with aliquots of distilled water according to the method mentioned by Aboofazeli and Lawrence (*19,20*) in 10% increments in a range from 10-50% (w/w). Following each water addition, the mixtures in the vials were mixed using a vortex mixer (VM-300; Gemmy Industrial Corp., Taipei, Taiwan) for 2-3 min and then incubated at 30°C for 24 h before the next addition, for equilibrium. After being equilibrated, the mixtures were assessed visually as microemulsions, crude emulsions, or gels after each addition of distilled water. Thirty-six samples were prepared for each system, and only clear and transparent mixtures with gel consistency visualized after vortexing and equilibrium were considered monophasic, these samples were marked as points on the phase diagrams and were chosen for further addition of the drug. The area covered by these points was considered to be the microemulsion region of existence. The top apex of the diagram represents the high hydrophile-lipophile balance (HLB) surfactant component and the other two apices represent the oil and the cosurfactant.

2.4. *Preparation of celecoxib microemulsion gel systems*

In order to prepare the drug loaded microemulsions, the appropriate oil, surfactant, and cosurfactant weight ratios were weighed in glass vials. Then, 2% (w/w) of celecoxib was accurately weighed and added to the mixture, vortexed and then water was added drop wise at ambient temperature and vortexing was continued for 5 min. The resultant microemulsions were stored for 24 h at room temperature for equilibrium before further investigation.

2.5. *Evaluation of physical properties of the prepared celecoxib microemulsion gel systems*

The prepared celecoxib microemulsion gel systems were subjected to the following evaluation tests.

2.5.1. *Visual inspection*

The prepared celecoxib microemulsion gel systems were examined for optical clarity, fluidity, homogeneity, and phase separation.

2.5.2. *pH measurements*

pH of 10% (w/w) aqueous solution was measured using a Hanna-213 pH meter (HANNA Instruments, Woonsocket, RI, USA). The solutions were prepared by dissolving 1 g of each prepared formula in 9 g of distilled water using a magnetic stirrer (*21*).

2.5.3. *Test for spreadability*

A spreadability test was conducted by pressing 0.5 g of each prepared formula between two glass slides and left for about 5 min until no more spreading was expected. The diameter of the formed circle was measured and used as comparative values for spreadability (*22*,*23*).

2.5.4. *Droplet size analysis*

Droplet size analysis was performed using a laser light scattering particle size analyzer (Master Sizer 2000; Malvern Instruments Ltd., Worcestershire, UK). Microemulsion samples were diluted with distilled water and charged into a wet sample holder.

2.6. *Drug release studies using a Franz diffusion cell*

For this investigation, static Franz glass diffusion cells (Microette plus; Hanson Research, Chatsworth, CA, USA) were used. These cells consist of donor and receptor chambers between which the cellulose nitrate membrane was positioned. The area for diffusion was 1.7 cm^2 and the receptor chamber volume was 14 mL. The receptor chamber was maintained at 37 ± 0.5ºC in order to ensure a surface skin temperature of 32ºC on the surface of the membrane (*24*). The receptor medium consists of a 1% (w/v) SLS solution. Each cell contains a magnetic stirring bar and was stirred at 100 rpm during the experiment. Weighed amounts of 0.5 g of the microemulsion gel were evenly spread on the surface. Aliquots of 2 mL of the medium were withdrawn at: 0.5, 1, 2, 3, 4, 5, and 6 h and replaced with an equal volume of fresh medium to maintain a constant volume. The concentration of celecoxib was determined spectrophotometrically at the predetermined λ_{max} of 255.2 nm (Shimadzu). The mean percentage of celecoxib released across the membrane was plotted as a function of time. All experiments were run in triplicate and the results were expressed as mean values ± S.D.

2.7. *Release kinetics of celecoxib from the prepared microemulsion gel systems*

The release data were analyzed using linear regression equations and were fitted to zero order, first order, and simplified Higuchi diffusion models. The following linear regression equations were employed:

$$C_t = C_o - K_t \text{ (Eq. 1) for zero-order kinetics}$$

$$\text{Log}C_t = \text{log}C_o - K_t/2.303 \text{ (Eq. 2) for first-order kinetics}$$

$$Q = K_t^{1/2} \text{ (Eq. 3) for Higuchi diffusion model (}25\text{)}$$

where, Q is the amount of drug released per unit area at time t. The coefficient of determination (R^2) was determined and $t_{50\%}$ (time until 50% drug release) was then computed according to the determined order and the release rate of celecoxib was calculated from the slope of the straight line.

For comparison, a 2% o/w celecoxib cream was prepared and subjected to drug release. The cream was composed of 2% (w/w) celecoxib, 30% (w/w) emulsifying ointment, and 68% (w/w) water. The cream was formulated by dispersing celecoxib in melted anionic emulsifying ointment composed of 30% (w/w) emulsifying wax, 20% (w/w) liquid paraffin, and 50% (w/w) white soft paraffin (*26*). Slightly warmed distilled water was added to this mixture and stirred gently until it became cold, to obtain cream consistency (*26*). Based on the results of the release study, candidate formulas showing optimum drug release were subjected to further analysis.

2.8. *Rheological properties measurements*

The selected celecoxib microemulsion gel formulas were tested for rheological behavior at 25°C using a Brookfield digital viscometer (RV-TD; Brookfield Engineering Laboratories, Middleboro, Inc., MA, USA) with a spindle (LV.4).

2.9. *In vitro drug permeation studies through excised rabbit skin*

2.9.1. *Preparation of full excised abdominal rabbit skin barrier membrane*

Ethical clearance was obtained from the institutional animal experimentation committee before the study. Rabbits were sacrificed and the full thickness of rabbit skin was excised from the abdominal region and hair was removed with an electric clipper. The subcutaneous tissue was removed surgically and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The cleaned skin was washed with distilled water and stored in the deep freezer until further use. The skin was brought to room temperature and cut into 5 cm diameter circular patches when used.

2.9.2. *In vitro permeation*

Permeation of celecoxib for the selected microemulsion gel formulas through the skin and drug analysis were carried out according to the procedure adopted for the release study through cellulose nitrate membranes. All experiments were run in triplicate and the results were expressed as mean values ± S.D. The cream containing 2% (w/w) celecoxib was used as a control formula to compare drug permeation through the skin from microemulsion gel formulas with that from the cream.

2.9.3. *Permeation data analysis*

Average values of three readings of *in vitro* permeation data were calculated and the average cumulative amount of celecoxib permeated through the skin per unit surface area ($\mu g/cm^2$) was plotted as a function of time. The drug flux (permeation rate) at steady state (J_{SS}) was calculated from the slope of the straight line. The permeability coefficient (K_P) was calculated using the following equations:

$$K_P = J_{SS}/C_o \text{ (Eq. 4)}$$

where C_o is the initial concentration of the drug. Enhancement ratio (E_r) was calculated by dividing J_{SS} of the respective formulation by J_{SS} of the control formulation (*27*,*28*):

$$E_r = J_{SS} \text{ of formulation}/J_{SS} \text{ of control (Eq. 5)}$$

The coefficient of determination (R^2), $t_{50\%}$ (time to 50% drug permeation), and the percentage of drug permeated after 6 h were also determined.

2.10. *Thermodynamic stability of celecoxib microemulsion*

To assess the thermodynamic stability for the selected microemulsion formulas, the following two tests were carried out for the selected formulas.

2.10.1. *Centrifuge stress test* (*11*)

The selected microemulsion gel formulas were centrifuged at 3,000 rpm for 30 min and then examined for liquefaction and phase separation. Formulas that did not show phase separation were considered for a freeze thaw stress test.

2.10.2. *Freeze thaw stress test* (*29*)

Selected microemulsion gel formulas were submitted to a total of three complete cycles, each cycle consisting of 24 h at 25°C followed by 24 h at –5°C. These cycles were important for determining the ability of the microemulsion to withstand thermal shock, as well as to evaluate physical stability of the microemulsion.

2.11. *Long term stability*

The optimized celecoxib loaded microemulsion gel formula showing satisfactory physicochemical properties, highest release, and permeation rate was stored under ambient conditions for one year. The stored microemulsion formula was re-evaluated regarding visual inspection, pH measurement, and spreadability test. In addition, the morphology and the droplet size analysis of the celecoxib microemulsion formula were observed using a transmission electron microscope (TEM). One drop of diluted sample was deposited on a film-coated 200-mesh copper grid and later stained with one drop of a 2% aqueous solution of phosphotungstic acid (PTA) and allowed to dry and any excess fluid was removed with filter paper before examination using a JEM-100 CX electron microscope (JEOL, Tokyo, Japan).

2.12. *Anti-inflammatory studies in rats*

The study was conducted in accordance with the principles of Laboratory Animal Care and was approved by the Institutional Ethics Committee. The anti-inflammatory activity of the optimized formula was carried out using the carrageenan-induced paw edema method developed by Winter *et al.* (*30*,*31*) in albino rats. Male albino rats weighing 150-180 g were fasted overnight with free access to water and were divided into 4 groups of 6 animals. The dorsal side of the rats was shaved 12 h before starting the experiments except in the control group. The first group (control) received carrageenan only without the drug. The second and third groups received an application of optimized microemulsion, or conventional cream, respectively, at a 11.66 mg/kg dose level (*32*) on the shaved dorsal region of all animals (except in the control group) half an hour before subplantar injection of carrageenan. The last group received an oral treatment of commercial Celebrex® at a dose of 11.66 mg/kg. The animals were injected with 0.1 mL of carrageenan suspension (1%, w/v, in distilled water) in the subplantar region of the right hind paw. Paw edema volume was measured before carrageenan injection as well as after 1, 2, 3, 4, 5, and 6 h following the carrageenan injection using a plethysmometer by the mercury displacement method. The percentage inhibition of edema volume was calculated as follows:

$$\% \text{ Inhibition} = 100 \times [1 - (A - x / B - y)] \text{ (Eq. 6)}$$

where A is paw volume after administration of carrageenan at time t, and x is paw volume before administration of carrageenan. B is the mean paw volume of control rats after administration of carrageenan at time t and y is mean paw volume of control rats before administration of carrageenan.

2.13. *Skin irritation test*

Although all the materials used for preparation of microemulsions fell under the Generally Regarded as Safe (GRAS) category, concentrations of all materials is a very critical issue for these formulations. For example, a large amount of surfactants is usually an irritant to the skin. Therefore, a skin irritation test was performed to confirm that the concentration of materials used for microemulsion preparation was safe.

The skin irritancy test was carried out to determine any possible localized reaction of the optimized

microemulsion formula on the skin of male albino rats (150-180 g) according to the method described by Draize *et al*. (*33*). The animals were divided into three groups: the first group served as control (no treatment), the second group received 0.8% (v/v) aqueous formalin solution as a standard irritant, and the third group received the optimized formula. A dose of 0.5 g of optimized formula or 0.5 mL of formalin solution was applied on a 5 cm^2 area of the shaved dorsal side of the rats daily for three consecutives days (*34*). The development of erythema and edema were monitored daily for 3 days.

2.14. *Histopathological examination of skin specimens*

After three days, the rats were sacrificed and skin samples from treated and untreated (control) areas were taken. Each skin sample was stored in 10% (v/v) formalin saline solution. The skin samples were cut vertically in different sections. Each section was dehydrated using ethanol, embedded in paraffin for fixing, and stained with hematoxylin and eosin and then examined through the light electric microscope (Nikon, Tokyo, Japan) fitted with a Canon power shot G3 digital camera (Canon, Tokyo, Japan) and compared with control sample.

3. Results and Discussion

3.1. *Screening of oils, surfactants, and cosurfactants for celecoxib*

Table 1 summarizes solubility of celecoxib in various solvents such as oils, surfactants, and cosurfactants at 30ºC. Celecoxib showed the highest solubility in oleic acid followed by IPM and olive oil. Among surfactants, solubility of the drug in Tween 80 was greater than that in Tween 40. Celecoxib was found to be more soluble in Cremophor RH40 when compared to propylene glycol and ethylene glycol.

3.2. *Construction of microemulsion phase diagrams*

The aim of the construction of phase diagrams was to find out the microemulsion existence region. Based on the solubility studies, oleic acid and IPM were chosen to represent the oily phases, Tween 80 was used as surfactant, and Cremophor RH40 was used as cosurfactant for constructing phase diagrams.

Oleic acid, an unsaturated C18 fatty acid, has been widely employed as a pharmaceutical excipient for microemulsion preparation (*15*,*35*,*36*). Similarly IPM, a long chain triglyceride, is often used as an oil phase and as a permeation enhancer in transdermal formulations (*37*). It is considered to be the most popular and most biocompatible fatty acid ester used for formulation of pharmaceutically accepted microemulsions (*38-43*). Tween 80 is a non-ionic surfactant with an HLB value of 15 which is widely used in pharmaceutical preparations due to its history of usefulness, safety, and stability (*44*,*45*). Cremophor RH40 is a non-irritant, safe, and good emulsifier; it was used as a cosurfactant in preparing ketoprofen microemulsions (*46*).

Upon increasing the water content from 10-50%, a turbid macroemulsion was formed where the clear microemulsion region was reduced (data not shown). It was found that the microemulsion region obtained using IPM (Figure 1A) was larger when compared to oleic acid (Figure 1B). This may because oleic acid has a larger molecular size compared to IPM and Tween 80, where a low degree of oil penetration was expected to take place in the interfacial surfactant layer. It was previously reported that the phase behavior is strongly influenced by the size of the molecule of the oil used

Table 1. Solubility of celecoxib in various oils, surfactants, and cosurfactants at 30ºC

Solvents	Solubility (mg/mL)
Oleic acid	22.3 ± 5.0
IPM	5.6 ± 1.0
Olive oil	3.4 ± 0.1
Tween 80	158.5 ± 10.0
Tween 40	137.1 ± 4.0
Cremophor RH40	153.3 ± 2.0
Propylene glycol	22.1 ± 5.0
Ethylene glycol	4.1 ± 0.3

Abbreviation: IPM, isopropyl myristate.

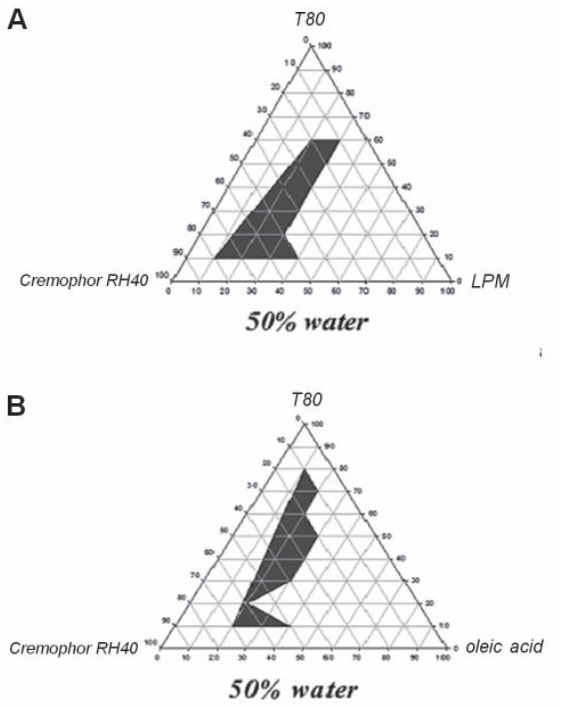

Figure 1. Quaternary phase diagrams of microemulsion containing IPM(A) or oleic acid (B) as oil, Tween80 as surfactant, and Cremophore RH40 as cosurfactant. Water content was 50%.

(*20*). Similarly, these findings were in agreement with those of Yuan *et al*., who compared IPM with an oleic acid microemulsion formulation and reported a large microemulsion existence area with IPM and a small area with oleic acid (*47*).

3.3. *Preparation of celecoxib microemulsions*

As hundreds of formulations could be prepared from the microemulsion region of each phase diagram, a constant point at 50% water was selected for formulation of celecoxib microemulsions for all phase diagrams. This selection was done for the purpose of formation of a microemulsion gel spontaneously without adding any gelling agent and was based on the fact that the higher the water content in the microemulsion systems, the lower would be the solubility of celecoxib in the microemulsion with an expected higher release. It was previously mentioned that, when increasing the water content, celecoxib solubilization capacity in microemulsions decreased and its release behavior was increased (*48*). From each phase diagram constructed, different formulas were selected from the microemulsion region for incorporation of drug into the aqueous phase. Sixteen formulas out of seventy-two were adopted from microemulsion systems S1 and S2 (See Table 2 for the composition of these microemulsion systems). The prepared microemulsions were in the form of microemulsion gels and no liquefaction was observed upon increasing the water content up to 50% and upon addition of celecoxib. All other preparations liquefied upon addition of 2% (w/w) celecoxib, and therefore were excluded. The composition of the selected celecoxib microemulsion formulas for each system is presented in Table 3.

Table 2. Composition of the microemulsion systems

System	Oil	Surfactant	Cosurfactant
S1	Oleic acid	Tween 80	Cremophor RH40
S2	IPM	Tween 80	Cremophor RH40

3.4. *Evaluation of the physicochemical properties of the prepared celecoxib microemulsion gel systems*

3.4.1. *Visual inspection*

Visual inspection of the prepared microemulsions showed clear homogeneous systems of gel consistency and no phase separation was observed (Table 4).

3.4.2. *pH determination*

It was previously reported that, for microemulsions to be non-irritant and safe for transdermal application, their pH has to fall in the physiologic accepted range for transdermal preparations, *i.e.*, pH 4-7 units (*23*). pH measurements of 10% (w/w) aqueous solutions of the microemulsion systems are summarized in Table 4. The pH values were found to be in the range of 3.94-4.44 units for S1 prepared using oleic acid and in the range of 6.20-6.84 units for S2 prepared using IPM. Therefore, the pH of all the prepared formulas was within the required range and was considered to be safe for transdermal application.

3.4.3. *Spreadability measurement*

The spreadability is an important criterion for uniform and ease of application of transdermal preparations. Spreadability of the microemulsions was measured in terms of average diameter of the spread circle. As shown in Table 4, spreadability values for all prepared microemulsion formulas ranged between 2.35 and 4.80 cm. Needless to say, the larger the diameter, the better the spreadability (*49*).

Table 3. Composition (%, w/w) of microemulsion formulas for each system

System	Formulation code	Oil	Surfactant	Cosurfactant	Celecoxib
S1	F1	13.07	6.53	45.73	2
	F2	13.07	13.07	39.20	2
	F3	13.07	19.60	32.67	2
	F4	13.07	26.13	26.13	2
	F5	13.07	32.67	19.60	2
	F6	13.07	39.20	13.07	2
	F7	13.07	45.73	6.53	2
S2	F8	19.60	6.53	39.20	2
	F9	26.13	6.53	32.67	2
	F10	19.60	13.07	32.67	2
	F11	6.53	19.60	39.20	2
	F12	19.60	25.07	20.66	2
	F13	19.60	26.13	19.60	2
	F14	13.07	32.67	19.60	2
	F15	19.60	32.67	13.07	2
	F16	19.60	39.20	6.53	2

All formulas contain 32.67% water.

Table 4. Various physicochemical properties of the prepared microemulsion gel formulas

System	Formulation code	Visual inspection**	pH*	Spreadability (cm)*	Droplet diameter (μm)*	Span
S1	F1	Clear	4.44 ± 0.01	4.50 ± 0.00	0.20 ± 0.04	0.43
	F2	Clear	4.37 ± 0.06	4.50 ± 0.10	0.19 ± 0.03	0.47
	F3	Clear	4.28 ± 0.08	4.15 ± 0.05	0.25 ± 0.09	0.77
	F4	Clear	4.26 ± 0.01	4.25 ± 0.15	0.20 ± 0.11	0.47
	F5	Clear	4.21 ± 0.07	4.80 ± 0.20	0.19 ± 0.08	0.80
	F6	Clear	4.14 ± 0.06	4.20 ± 0.10	0.22 ± 0.01	0.93
	F7	Clear	3.94 ± 0.02	4.30 ± 0.15	0.23 ± 0.06	0.85
S2	F8	Clear	6.38 ± 0.03	2.35 ± 0.05	0.21 ± 0.12	0.85
	F9	Clear	6.59 ± 0.03	2.35 ± 0.05	0.24 ± 0.09	0.73
	F10	Clear	6.55 ± 0.04	2.95 ± 0.15	0.21 ± 0.08	0.67
	F11	Clear	6.53 ± 0.01	3.50 ± 0.00	0.20 ± 0.07	0.60
	F12	Clear	6.20 ± 0.10	3.15 ± 0.05	0.20 ± 0.08	0.30
	F13	Clear	6.70 ± 0.05	2.95 ± 0.05	0.22 ± 0.06	0.60
	F14	Clear	6.70 ± 0.01	4.30 ± 0.10	0.21 ± 0.40	0.86
	F15	Clear	6.82 ± 0.01	2.60 ± 0.10	0.24 ± 0.11	0.83
	F16	Clear	6.84 ± 0.01	2.85 ± 0.05	0.23 ± 0.07	0.67

* Data are shown as mean ± S.D. ** Clear homogeneous systems of gel consistency with no phase separation was observed.

3.4.4. *Droplet size analysis*

Droplet size determination may provide information about the influence of the structure of the surfactant system on microemulsions and drug release from the microemulsion (*50*). A small droplet size makes it an excellent carrier for improving drug percutaneous uptake, thus increasing efficiency in uptake of the drug (*51*). The mean volume of the droplet sizes and span (polydispersity) are shown in Table 4. All the formulas had droplets ranging from 0.19 ± 0.03 to 0.24 ± 0.11 μm with a low polydispersity index, which would indicate uniformity of droplet size within each formula.

3.5. *Release studies*

The release of celecoxib from different microemulsion systems and cream was carried out through a cellulose nitrate membrane over a period of 6 h in 1% SLS solution. SLS was added to the dissolution media to maintain the sink conditions during the release study because the release of poorly soluble drugs requires release media that are different from those normally used for water-soluble drugs (*49*).

Figure 2A shows the release pattern of celecoxib from microemulsion formulas of S1 prepared with oleic acid as oil, Tween 80 as surfactant, and Cremophor RH40 as cosurfactant. The percentage of celecoxib released from S1 could be arranged in a descending order as follows F1 > F2, F3 > F4 > F5 > F6 > F7. This might be due to a difference in surfactant concentration. As surfactant concentration was increased from 6.5% (F1) to 45.7% (F7), the percentage of celecoxib released was decreased from 76.11% (F1) to 44.00% (F7). This may be due to an increased thermodynamic activity of the drug in the microemulsion at a lower content of surfactant (*51*). It was previously found that the increase in surfactant concentration caused a decrease in the release of ketoprofen and carbamazepine from microemulsions (*46*,*52*).

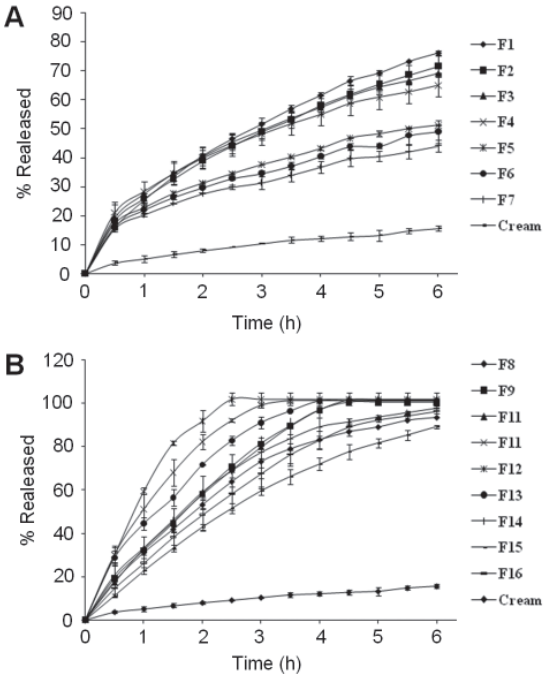

Figure 2. Release pattern of celecoxib from various microemulsion formulas and the corresponding cream. A, S1 prepared with oleic acid as oil, Tween 80 as surfactant, and Cremophor RH40 as cosurfactant; **B**, S2 prepared with IPM as oil, Tween 80 as surfactant, and Cremophor RH40 as cosurfactant.

By applying a one way ANOVA test for the values of $t_{50\%}$ for microemulsion formulas of S1, it was concluded that F1 was superior over all other formulas ($p < 0.05$) except for F2 and F3 where no significant difference was present ($p > 0.05$) (data not shown). There was a high significant difference between all microemulsion formulas of S1 and the corresponding celecoxib cream ($p < 0.05$) (data not shown).

Figure 2B shows the release pattern of celecoxib from microemulsion formulas of S2 containing IPM as oil, Tween 80 as surfactant, and Cremophor RH40 as cosurfactant. Among the microemulsion formulas of S2, F12 showed the highest release where 100% of celecoxib was released within 2.5 h. On the other hand, F15 showed the least drug release. The release of the drug from microemulsion formulas of S2 were 6- to 12-fold greater than drug release from the cream. An ANOVA test revealed that there was a significant difference between the $t_{50\%}$ of celecoxib of different microemulsion formulas of S2 ($p < 0.05$) except F9, F10, and F14 that showed no significant difference between them ($p > 0.05$) (data not shown). The values of $t_{50\%}$ of all microemulsion formulas of S2 were highly significant when compared to the corresponding cream (data not shown).

Inspite of the higher solubility of celecoxib in oleic acid, higher drug release was obtained from IPM microemulsions (S2) than that obtained from oleic acid microemulsions (S1). This could be attributed to decreased thermodynamic activity of the drug in microemulsions prepared with oleic acid due to its high affinity for the oil, therefore slowing release of the drug from the vehicle (*53*). For example, the percent release of celecoxib from F5 of S1 was 51.27% after 6 h (Figure 2A) compared to 97.7% from F14 of S2 (Figure 2B). These results were in agreement with the studies of Ceschel *et al*. who proved that the release of the drug would be favored by selecting the vehicle which had a low affinity for the drug (*53*).

3.6. *Release kinetics of celecoxib from the prepared microemulsions*

According to the values of the coefficient of determination (R^2), the mechanism of drug release was defined. It was found that all the microemulsion formulas and the cream showed a best fit for the Higuchi diffusion model (Table 5). The time required for 50% ($t_{50\%}$) celecoxib to be released from microemulsion formulas of each system and the cream were calculated according to the release model and also the release rate of celecoxib from each formula was determined from the slope of the straight line (Table 5). Based on the results of the release studies, the formulas with the highest release rate namely F1, and F3 of S1 and F11, F12, F13, and F14 of S2 were chosen for further analyses. F5 of S1 was chosen similarly to be compared to F14 of S2 in order to study the effect of changing oil type on the permeation rate because they possessed the same concentration of oil.

3.7. *Rheological properties measurements*

The results revealed that all the selected microemulsion formulas showed non-Newtonian, pseudoplastic flow with thixotropy as the viscosity decreased with increasing shear rates (Figure 3) (F12 is a representative example). Needless to say, thixotropy is a desirable feature for semisolid drug carriers for dermal application (*54*). This result was in agreement with the findings of Ambade *et al.*, who found that the rheological behavior of microemulsions containing flurbiprofen showed non-Newtonian, pseudoplastic flow (*11*).

3.8. *In vitro drug permeation studies through excised rabbit skin*

In vitro drug permeation was examined through excised rabbit skin over a period of 6 h in 1% SLS solution at 37 ± 0.5ºC for the selected microemulsion formulas and the corresponding cream. Figure 4 shows permeation

Table 5. Release rate and $t_{50\%}$ of celecoxib from various microemulsion gel formulas of S1 and S2 and from the corresponding cream

System	Formulation code	Mechanism of release	Release rate (µg/h$^{1/2}$)	$t_{50\%}$ (h$^{1/2}$)
S1	F1	Higuchi	35.2	1.68
	F2	Higuchi	31.3	1.76
	F3	Higuchi	29.0	1.75
	F4	Higuchi	25.7	1.82
	F5	Higuchi	20.1	2.34
	F6	Higuchi	18.7	2.51
	F7	Higuchi	16.3	2.82
S2	F8	Higuchi	45.8	1.35
	F9	Higuchi	61.4	1.26
	F10	Higuchi	62.2	1.26
	F11	Higuchi	63.7	0.97
	F12	Higuchi	82.9	0.91
	F13	Higuchi	58.8	1.07
	F14	Higuchi	46.6	1.26
	F15	Higuchi	46.4	1.56
	F16	Higuchi	50.8	1.42
Cream		Higuchi	6.88	7.50

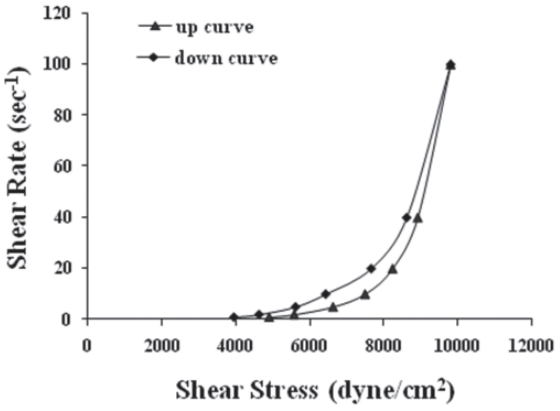

Figure 3. Rheogram of the prepared microemulsion gel system. F12 is a representative example.

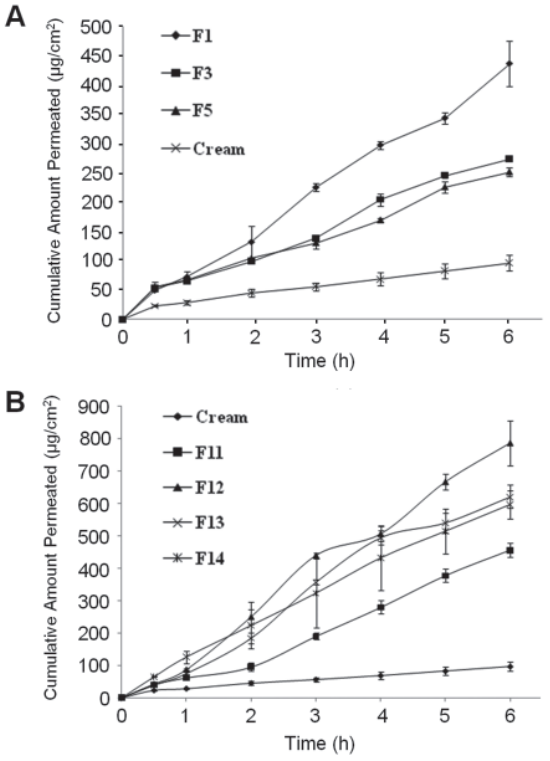

Figure 4. Permeation profiles of celecoxib through rabbit skin from different microemulsion formulas and the corresponding cream. A, S1; **B**, S2.

profiles of celecoxib through rabbit skin from different microemulsion formulas of S1 and S2. Permeation data analysis of S1 and S2 are represented in Table 6.

For microemulsion formulas of S1 (F1, F3, and F5), *in vitro* skin permeation was the highest from F1 and the lowest from F5 (Figure 4A). This might be due to differences in surfactant concentration. As surfactant concentration increased from 6.5% (F1) to 19.6% (F3) and to 32.67% (F5), the permeation rate of celecoxib decreased. This may be due to decreased thermodynamic activity of the drug in the microemulsion at high surfactant content (*51*). It is known that the thermodynamic activity of the drug in a formulation is a significant driving force for its release and penetration into skin (*55*). A similar result was reported by Chen *et al*. who found that the increase in surfactant concentration causes a decrease in the permeation rate of ibuprofen (*56*).

An ANOVA test revealed that there was a significant difference between the $t_{50\%}$ results of the microemulsion formulas of S1 ($p < 0.05$) and there was a significant difference between all microemulsion formulas of S1 and cream ($p < 0.05$) (Table 6).

Results of the celecoxib permeation study from microemulsion formulas of S2 (F11, F12, F13, and F14) and cream are shown in Figure 4B. Among the microemulsion formulas of S2, F12 showed the highest permeation rate and the least $t_{50\%}$. On the other hand, F11 showed the lowest drug permeation rate and highest $t_{50\%}$. There was a significant difference between the $t_{50\%}$ results of the microemulsion formulas of S2 ($p < 0.05$) and there was a significant difference between microemulsion formulas of S2 and the corresponding cream ($p < 0.05$) (Table 6).

The release and permeation of celecoxib from F14 of S2 containing 13.07% IPM showed a higher rate than F5 of S1 containing 13.07% oleic acid in spite of the higher solubilizing capacity of the latter for celecoxib (Table 6). The possible reason is that drug release and permeation are influenced by the solubility of the drug in the vehicle. If the vehicle can increase the solubility of the drug, then the drug itself would be retained in the vehicle after application on the surface of skin, which results in reduced partition into the skin (*54*). The same

Table 6. Permeation data parameters of some selected celecoxib microemulsion gel formulas of S1, S2, and the corresponding cream

System	Formulation Code	R^2	Flux (J_{SS}) (µg/cm^2/h)	Kp (cm/h)	E_r	$t_{50\%}$ (h)	% Permeated after 6 h
S1	F1	0.994	70.5 ± 3.2	3.53 ± 0.16	5.32	5.05 ± 0.21	60.3 ± 3.9
	F3	0.988	42.6 ± 1.2	2.13 ± 0.06	3.22	7.93 ± 0.29	37.8 ± 3.0
	F5	0.990	36.6 ± 2.3	1.83 ± 0.12	2.77	9.02 ± 0.39	34.7 ± 1.2
S2	F11	0.981	78.5 ± 0.9	3.92 ± 0.04	5.93	4.94 ± 0.02	63.2 ± 2.1
	F12	0.986	137.6 ± 7.7	6.88 ± 0.38	10.39	2.82 ± 0.12	102.0 ± 2.8
	F13	0.977	112.7 ± 5.5	5.64 ± 0.27	8.51	3.35 ± 0.12	86.1 ± 3.6
	F14	0.997	97.3 ± 6.8	4.86 ± 0.34	7.35	3.51 ± 0.51	82.7 ± 4.3
Cream		0.988	13.2 ± 2.1	0.66 ± 0.10	–	27.66 ± 2.82	13.3 ± 1.4

result was obtained by Zhu *et al*., who found that the skin permeation of penciclovir in a microemulsion was significantly increased, while the solubility of penciclovir in the microemulsion was decreased (*57*). Our findings agree with previously reported results regarding the higher permeation ability of IPM than oleic acid for transdermal delivery of some drugs (*13*,*58*).

3.9. *Thermodynamic stability studies*

None of the selected microemulsion formulas showed phase separation using both tests and no changes in physical appearance such as turbidity or creaming was observed (data not shown). These results indicate that all the selected formulas showed good physical stability.

F12 of S2, which showed small droplet size, good spreadability, and the highest release and permeation rates was subjected to long term stability, anti-inflammatory activity, and the skin irritation test.

3.10. *Long term stability studies*

The optimized microemulsion formula (F12 of S2) was stable when stored under ambient conditions, where there was no change in visual appearance or phase separation, and no significant change in droplet size, pH, and spreadability values (data not shown) which declares the ability of the microemulsion formula to withstand thermal shock (*29*). The morphology and droplet size analysis of the fresh and stored microemulsion formula were observed using TEM. TEM photographs depicted in Figure 5 reveal that all droplets after storage possessed nearly the same size and spherical shape as freshly prepared ones.

3.11. *Anti-inflammatory studies in rats*

Figure 6 shows that F12 of S2 produced maximum anti-inflammatory activity where about 83.2 ± 1.4% inhibition in rat's paw edema volume when compared to conventional cream and oral Celebrex® where the percentage of inhibition 6 h after application were 24.8 ± 2.8% and 43.1 ± 7.2%, respectively. The enhanced anti-inflammatory effects of the microemulsion formula could be due to the enhanced permeation of celecoxib through the skin.

3.12. *Skin irritation test*

The skin irritancy test was performed to confirm the safety of the optimized microemulsion formula (F12 of S2). The results are shown in Table 7. Draize *et al*. (*33*) mentioned that a value of the primary irritancy index (PII) < 2 indicates that the applied formulation is a non-irritant to human skin. Therefore, F12 of S2 was considered to be a non-irritant as PII was < 2.

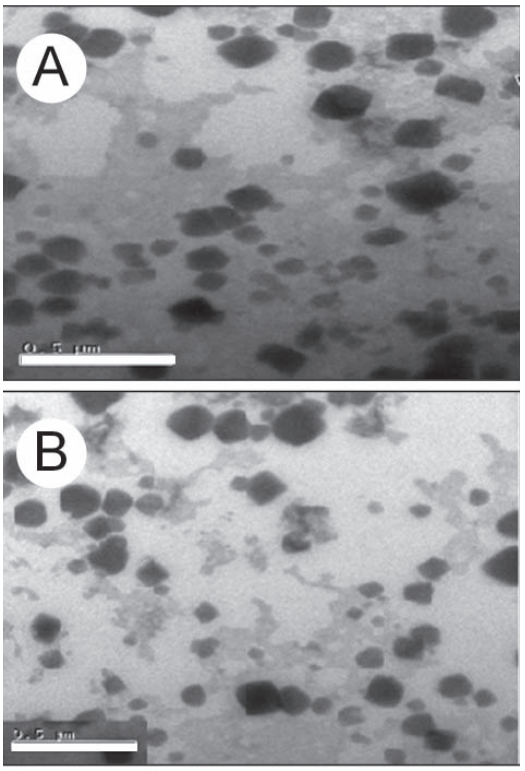

Figure 5. TEM photographs of Celecoxib microemulsion Formula F12 of S2. A, freshly prepared; **B**, after storage for one year. Bars, 0.5 µm.

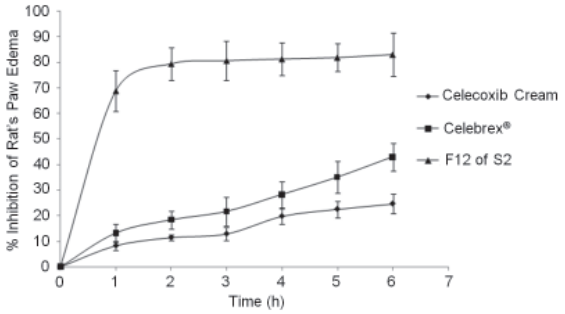

Figure 6. Anti-inflammatory activity of optimized microemulsion formula (F12 of S2), conventional cream, and Celebrex® using the carrageenean-induced hind paw edema method.

3.13. *Histopathological examination*

Histopathological examination of the microemulsion treated and control rat skin was performed using a Nikon light microscope and is illustrated in Figure 7. The photomicrographs of control rat skin (untreated) group I showed normal skin with well defined epidermal and dermal layers as shown in Figure 7A. While the second group (formalin solution as standard irritant), the photomicrograph showed inflammatory cell infiltration and blood capillary dilatation in the dermis (Figures 7B and 7C). When the skin was treated with the optimized microemulsion formula (F12 of S2) for 72 h, the dermis did not show any inflammatory cell infiltration. There was no histopathological alteration or

Table 7. Data of the skin irritation test

Rats	First group (Control)		Second group (Formalin solution)		Third group (F12 of S2 microemulsion)	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0.00	0.00	3	1	0.00	0.00
2	0.00	0.00	4	2	0.00	0.00
3	0.00	0.00	4	3	0.00	0.00
4	0.00	0.00	4	3	0.5	0.00
5	0.00	0.00	3	2	1	0.00
6	0.00	0.00	3	3	1	0.00
Mean	0.00	0.00	3.5	2.33	0.42	0.00
S.D.	0.00	0.00	0.5	0.75	0.45	0.00
PII	0.00 ± 0.00		5.83 ± 1.17		0.42 ± 0.45	

Abbreviation: PII, primary irritancy index.

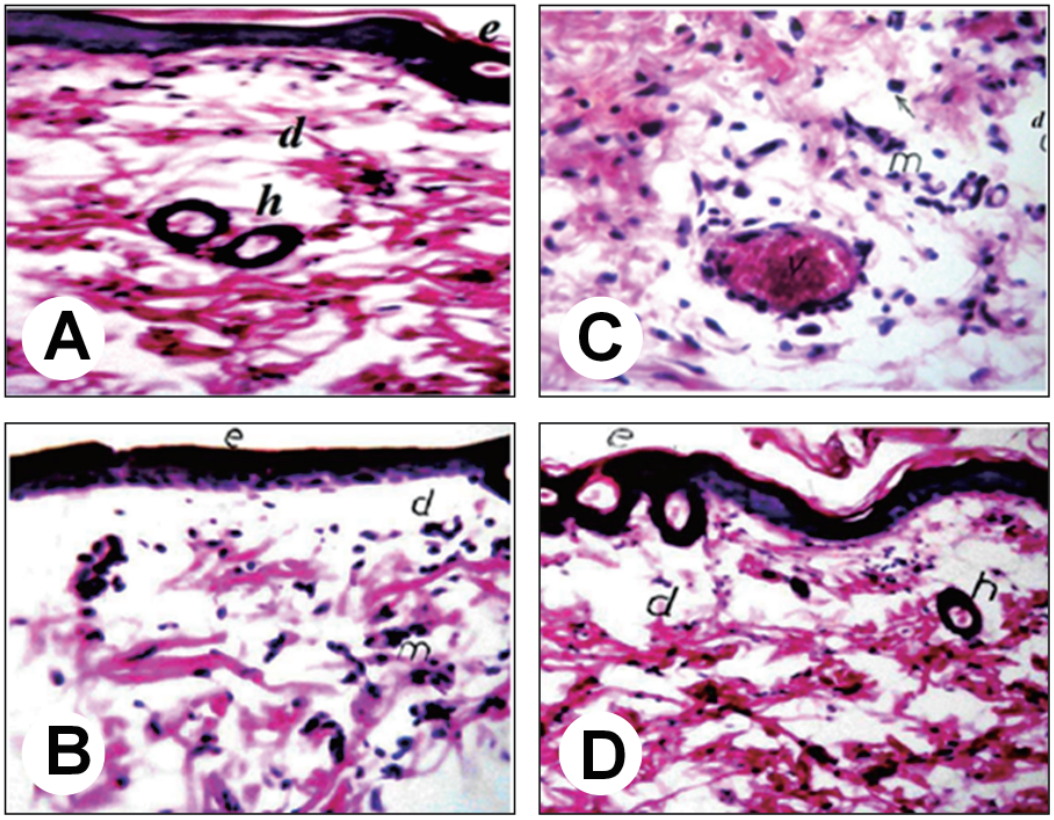

Figure 7. Light micrographs of rat skin untreated (A), treated with standard irritant (B and C) and treated with F12 of S2 microemulsion (D).

apparent signs of skin irritation (erythema and edema) observed on skin specimens indicating the absence of any skin irritation as a consequence of microemulsion treatment (Figure 7D). These results indicated that the developed microemulsion is safe for transdermal delivery of celecoxib.

4. Conculsion

In this study, celecoxib microemulsion systems were prepared and evaluated. All the formulas showed good release profiles and exhibited a rapid rate of permeation when compared to conventional celecoxib cream. The optimized formula (F12 of S2) consisting of 2% (w/w) of Celecoxib, 19.6% (w/w) of IPM, 25.07% (w/w) of Tween 80, 20.66% (w/w) of Cremophor RH40, and 32.67% of distilled water was found to be superior to all other formulas. It increased the permeation rate of Celecoxib up to 11 times compared to the conventional cream. Its stability was maintained after one year of storage at ambient conditions. The anti-inflammatory studies revealed a significant increase in percent inhibition of F12 as compared to the commercial oral formula ($p < 0.05$) and celecoxib cream. This indicated that the developed microemulsion was efficacious, and the small droplets have enormous interfacial areas, thereby enhancing the solubility of a poorly soluble drug, and influencing its transport properties. Results of

the skin irritation test indicated that F12 could be safe for human use. From these results, it can be concluded that the developed microemulsion gel has great potential for transdermal application of celecoxib.

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