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Development and evaluation of a microemulsion formulation for transdermal delivery of Diclofenac diethylammonium

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Abstract

The aim of the present study was to prepare and evaluate different formulations of Diclofenac diethylammonium in microemulsion base with a view to enhance its permeability through the skin. Various o/w and w/o microemulsions of Diclofenac diethylammonium were prepared by the spontaneous emulsification methods. Isopropyl myristate was used as oil phase, Polyoxyethylenesorbitan monooleate and Sorbitanmonolaurate as surfactants and Isopropyl alcohol, Dioctylsodium sulfosuccinate (AOT) and polyoxyethylene (10) octyl phenol ether as co-surfactants. Microemulsion existence region was determined using the pseudo-ternary phase diagrams for preparing different formulations. The optimized o/w and w/o microemulsions consist of 1.16% w/w Diclofenac diethylammonium as the active ingredient. The microemulsions were characterized for different parameters. In-vitro permeation studies through rat skin showed that permeability parameters like flux (Jss) and permeability coefficient (P) were significantly higher for formulation then reference marketed product (Emulgel) ($p<0.05$). Primary skin irritation studies indicated that optimized formulations are safe for the application on the skin. Stability studies indicated that the physico-chemical parameters of the formulation remain unaffected at accelerated conditions of storage. All the percutaneous parameters as well as the pharmacodynamic anti-inflammatory study parameters were statistically calculated. The results indicated that the microemulsion system studied would be a promising tool for enhancing the percutaneous delivery of Diclofenac diethylammonium.

Keywords: Diclofenac diethylammonium (DDEA), Microemulsion, anti-inflammatory activity, Rat paw oedema, Skin permeation

Introduction

Absorption via the transdermal route is limited by the generally poor penetration of drugs through the stratum corneum. Many methods have been used to decrease the skin resistance to the passage of drugs. These include the pro-drug approach, iontophoresis, phonophoresis, the use of percutaneous absorption enhancers and the use of an appropriate vehicle like microemulsions.[1-4] The concept of microemulsions was first introduced by Hoar and Schulman in the 1940s. They are defined as a system of water, oil and amphiphile which is an optically isotropic and thermodynamically stable liquid solution.[5, 6] Microemulsions are isotropic systems, which are formed spontaneously at a particular ratio of oil, water and surfactant with an average droplet diameter ranging between 30-100 nm.[7-12] These systems require relatively large amount of surfactants to stabilize the large interfacial area created by the nanodroplets.

Often addition of co surfactants such as alcohols is also required in order to attain appropriate fluidity or viscosity of the interface. As compared to conventional formulations microemulsions are better as they have enhanced drug solubility, transparency, good thermodynamic stability, ease of manufacturing and enhancement effect on transdermal delivery.[6, 13-15] This system is suitable for delivery of both water insoluble drugs and water soluble drugs. Water insoluble drugs may be delivered through oil-in-water (o/w) microemulsions[16-18] while water soluble drug may be delivered through water-in-oil (w/o) microemulsions.[19] Recently researchers have focused on microemulsions for transdermal delivery of various drugs of anti-inflammatory[20-26], anesthetics[27, 28], antifungal[29] and steroids.[30] Microemulsions may enhance transdermal drug delivery primarily by the following effects[31]:

- Microemulsions can exhibit a high solubilization capacity for both lipophilic and hydrophilic drugs, thus more drug can be loaded into the microemulsion, which increases the concentration gradient across the skin without depletion.
- The reservoir effect of the internal phase maintains a constant driving force of drug from the external phase to the skin and prolongs absorption. Since the diffusion of the drug into the skin only occurs from the external phase of the microemulsion, the internal phase continually supplies drug to the external phase so that it remains saturated with the drug. The thermodynamic process of drug diffusion across the flexible interfacial surfactant film between the phases of the microemulsion can increase partitioning and diffusion into the stratum corneum.[32]
- The formulation components may affect skin permeability, i.e. surfactants, cosurfactants, and oils may act as permeation enhancers by disrupting the SC lipid organization, thus increasing drug diffusion, or by increasing the partition of the drug in the skin.
- Microemulsion formulations have also been suggested to alter the 'polar pathways' via hydration of the SC because of the presence of a water phase.[33]
- Chemical enhancers may be incorporated in the microemulsion, which will also improve dermal and transdermal delivery of drugs.
- The very low interfacial tension required for microemulsion formation is also responsible for the excellent wetting properties, which ensures excellent surface contact between the membrane and the vehicle.[34]
- There is no clear consensus in the literature regarding the influence of droplet size of the microemulsion on drug permeation. A recent study by Izquierdo et al showed no apparent relationship between droplet size and dermal or transdermal delivery of Tetracaine after 24 h.[35]

Diclofenac diethylammonium (DDEA), a salt of Diclofenac, is widely used in the treatment of a variety of inflammatory and rheumatic disorders. Its gastro-intestinal irritancy, low oral bioavailability (50-60%) smaller dose and short biological half-life (approximately 2 hours) suggest a strong rationale for transdermal drug delivery. Besides improved absorption and penetration properties of Diclofenac diethylammonium[36-38] have been well documented. The transdermal delivery of Diclofenac diethylamine using microemulsion has been reported.[39] Currently, it is marketed as creams, gels and emulgels. The aim of the present study is to investigate the potential of both o/w and w/o type of microemulsion formulations in transdermal delivery of Diclofenac diethylammonium.

Materials and methods

Materials

Diclofenac diethylammonium was obtained as a gift sample from Franco-Indian Pharmaceuticals Pvt. Ltd. Dioctylsodium sulfosuccinate (AOT), polyoxyethylenesorbitan monooleate (Tween 80), Sorbitanmonolaurate (Span 20), Polyoxyethylene (10) octylphenol ether (POPE), Sorbitanmonooleate (Span 80) were also received as gift samples from HICO products Ltd.Isopropyl myristate (IPM), propylene glycol (PG), Polyethylene glycol 400 (PEG 400), glycerine, Isopropyl alcohol (IPA) were purchased from S.D. Fine Chem. Ltd., Mumbai. All other chemicals were of analytical grade.

Screening of Components for Microemulsion

Various combinations of surfactant and cosurfactant with low skin irritation potential were evaluated for microemulsion formation. O/w and w/o microemulsions were screened. The study was carried out at a fixed ratio of surfactant- cosurfactant to oil or water [S-CoS/(O or W)] for different ratios of surfactant to cosurfactant [S/CoS], using the titration method. Thus in no case a microemulsion was formed unless there was a match between the oil and the emulsifier blend. Microemulsions

formed by using the following combination were taken up for further investigations.

- Water-IPM-Tween 80-IPA (O/W TPA ME)
- Water–IPM–Span20–[AOT + POPE (1:1)] (W/O SAP ME)

Pseudoternary Phase Diagram

Once the appropriate microemulsion components were selected, pseudo-ternary phase diagrams were constructed to define the extent and nature of the microemulsion regions for each of the above combination.The data for the construction of pseudoternary phase diagram was gathered by the following titration method.Surfactant and cosurfactant (S+CoS) were mixed in different weight ratios (1:2, 1:1, 2:1). At a fixed S/CoS ratio, the (S+CoS) mixture was mixed with oil phase (in case of W/O ME) or water phase (in case of O/W ME) to give S+CoS : O or W weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. Each mixture was vigorously mixed using a magnetic stirrer until a homogeneous dispersion/solution was obtained. The mixture was titrated with water (in case of W/O ME) or oil (in case of O/W ME) in a covered beaker at ambient temperature while constant stirring. The end point of the titration was the point when the solution became cloudy and/or birefringent. The quantity of the phase added when the mixture became turbid was noted. The percentages of the three different pseudo phases incorporated were calculated. Then the same procedure was also followed with other S/CoS ratios. The point corresponding to the end of the microemulsion area was plotted on the pseudoternary phase diagram with one axis representing the aqueous phase, second axis representing oil (Isopropyl myristate), and the third representing a mixture of S and CoS at fixed weight ratios (S/CoS). The points were joined to define a boundary between clear and turbid regions. The phase diagrams show only the microemulsion regions, no attempt having been made to characterize the phase properties in any further detail. In this diagram, any point of the microemulsion field could be chosen to provide the right mixture of the four components, which led to a clear, transparent and stable microemulsion system.

Characterization of microemulsion

These microemulsions were produced spontaneously simply by blending all the weighed components together with mild agitation using a magnetic stirrer. These were then characterized for their optical properties like transparency and isotropicity between crossed polarizing plates, behavior in a gravitational field and rheological behavior i.e. viscosity.

Optical Birefringence

Optical isotropy of microemulsion formulations was checked visually and also placed between two polarizing plates in series and then observed for light transmittance. After this, one of the polarizing plates was rotated relative to the other through 90° (crossed polarizer) and then examined for birefringence if any. This is to confirm absence of other phases.

Centrifugation

To provide a rapid fool-proof identification of the system as microemulsion, centrifugation technique was employed at 4000 rpm for 30 mins. The systems were then examined for phase separation, gradation of layers, settling or creaming, etc.

Freeze-thaw cycling

5ml microemulsion was subjected for six heating/cooling cycles between 45°C and 5°C temperature with storage at each temperature for not less than 48 hours and assessed for their physical instability like phase separation and precipitation.[40]

Viscosity measurements

Viscosity measurements were carried out by using a Brookfield synchroelectric viscometer (RVT model) with UL adaptor at various speeds of 1, 2.5, 5, 10 and 20rpm.

In-vitro permeation study

Excised guinea pig skin (0.051 ± 0.02 cm thickness) was used for the skin permeation studies using a modified KesharyChien diffusion cell. The skin was mounted carefully between the donor and receptor compartment with the epidermal side facing the donor compartment and was secured in place by means of a clamp. The active diffusion area was 2.01 cm^2. The receiver compartment was then filled with 14 ml of USP phosphate buffer of pH 7.4. The assembly was thermostated by circulating water at 37 ± 1° C in the external jacket of KesharyChien cell to simulate the body temperature. The receiver fluid was stirred for the duration of the experiment. The donor compartment was then charged with 300mg of the formulation and immediately covered with an aluminum foil to prevent loss of volatile components, if any, from the solution. 1 ml of receiver fluid was withdrawn from the

receptor compartment at hourly intervals up to 8 hours and replaced with fresh receiver fluid. The drug concentration in the receiver fluid samples was determined spectrophotometrically (Schimadtzu UV VIS) at 450 nm.

Data Treatment

The in-vitro skin permeation rate or apparent flux, J_{SS} ($\mu g/cm^2/hr$) of Diclofenacdiethylammonium, was calculated from the slope of the linear portion (steady state region) in the plot of cumulative amount permeated per unit area (Q) vs. time (t).[41, 42] The permeability coefficient, K_p was calculated from the following relationship:

$$K_p = \frac{J\,ss}{Cd}$$

Where, C_d is the drug concentration in the donor (delivery system).

Primary skin irritation studies

Skin acceptability of topically applied microemulsions and marketed emulgel was tested on the abraded and intact skin of albino rabbits using the technique suggested by Draize et al.[43] For this study, three rabbits weighing 2 to 3 kg were used. The protocol for these animal studies was approved by the Institutional Animal Ethical Committee (IAEC) no. UICT/PH/IAEC/0109/04. The back of the rabbits was clipped free of hair 24 hours prior to the application of the formulation. Of these, two rabbits were used for the test and one rabbit was exclusively used as the control where no formulation was applied. 0.5 g of each selected microemulsion formulation and marketed emulgel was applied on both intact and abraded skin sites after 24 hours by uniform spreading within the area of 4 cm^2. After 24 hours the skin was observed for any visible change such as erythema (redness) and oedema (swelling) and these reactions were evaluated using the scale given by Draize. The analysis consisted of summing the average scores assigned for erythema (redness) and oedema (swelling) to yield a composite score called primary irritation index which therefore can range from 0 to 8, representing the degree of irritation.

Stability studies

Stability of the selected microemulsions was monitored at 40°C/75%RH, 25°C/60%RH, ambient conditions and at 8 ± 2°C, for a period of three months. The formulations were visually inspected and evaluated for drug content, viscosity, pH and in-vitro permeation across guinea pig skin. Drug content of all the formulations kept at different storage conditions was determined by HPTLC analysis.

HPTLC analysis

0.1 gm of sample was dissolved in 10ml methanol and 5µl of this solution was spotted as bands on precoated silica gel GF254 TLC plate. The plate was then developed in a mobile phase having ethyl acetate: strong ammonia solution: methanol (8:1:1 v/v). The plate was dried and then scanned and peak heights were recorded using Camag HPTLC instrument.

Pharmacodynamic studies

The anti-inflammatory efficacy was evaluated by the carrageenan induced rat paw oedema model by measuring changes in paw volumes with a water plethysmometer. (UgoBasilic Model No. 7140, Italy).[44] The protocol for these animal studies was approved by the Institutional Animal Ethical Committee (IAEC) no. UICT/PH/IAEC/1204/15. Female Charles Foster albino rats weighing about 100 – 130 gm were grouped into seven, each group consisting of six animals. The hair on their back was removed with the help of an electric clipper one day before the experiment. 100 mg of the formulation was applied over a surface area of 8-10 cm2 on the back of the rats. The control group did not receive any application. One hour after drug treatment oedema was produced by injecting 0.05 ml of a 0.5% w/v carrageenan suspension in saline through a 26 gauge needle subcutaneously under the plantar surface of the left hind paw. Immediately thereafter and at the end of 3 and 5 hrs after the carrageenan challenge the oedema volume was monitored by plethysmometer. The left and the right paw volume of each rat measured immediately after the carrageenan challenge was subtracted from the corresponding paw volume at the end of 3rd hour after carrageenan injection. The increase in saline paw volume was then subtracted from the increase in carrageenan paw volume for each rat in a group to give a net increase in paw volume i.e.oedema.The increase in paw volumes of the rats was then averaged for each group. The average paw swelling in the group of drug treated rats (V_t) was compared with that of the control rats (V_c). Percentage inhibition of oedema formation was calculated using the formula (1- V_t/ V_c) x 100.Similarly percentage inhibition of oedema at the end of the 5th hour after carrageenan injection i.e. 6th hour after the application of drug loaded formulation was calculated.

Statistics

The values obtained were expressed as mean ± s.e.m statistical significance of the difference between control and treated groups was calculated using students t-test for unpaired samples and one way ANOVA at confidence limit of 95% i.e. at p= 0.05. Similar statistical treatment was given to find out statistical significance of the difference between marketed emugel formulation and the microemulsion systems $p < 0.05$ indicated a significant difference.

Results and discussion

Important criteria for selection of the materials were that the components be pharmaceutically acceptable, nonirritant and non-sensitizing to the skin, and that they fall under GRAS (generally regarded as safe) category. Most commonly used ester, Isopropyl myristate was selected as the oil phase due to its good solublizing capacity[45-47] and good penetration enhancing capacity. Safety is a major determining factor in choosing a surfactant, as many surfactants may cause skin irritation. Non-ionic surfactants are less toxic than ionic surfactants.[48] The right blend of low and high HLB surfactants leads to the formation of a stable microemulsion formulation. The oils in which the drug is most soluble do not necessarily form microemulsion with the highest drug solubilization capacity.[49] Also the other compounds included in the present microemulsions, Spans and Tweens have been shown to promote drug permeation in the skin.[50, 51] Span 20 was selected as the surfactant for w/o microemulsion. Tween 80 was selected as the surfactant for o/w microemulsion. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of a single surfactant, usually necessitating the addition of a cosurfactant. The presence of cosurfactant decreases the bending stress of interface and provides the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of compositions, thereby increasing the entropy of the system. This increase in fluidity and entropy helps in formation of a thermodynamically stable microemulsion. Thus, the cosurfactants selected for the study were IPA[52] for o/w microemulsion and a mixture of Aerosol OT[53-56] and Polyoxyethylene [10] octylphenyl ether for w/o microemulsion.

Preparation of pseudo-ternary phase diagram

The area of microemulsion existence is represented by the ME side of the phase diagram. The rest of the region on the phase diagram represents turbid and conventional emulsion. The isotropic regions obtained for the o/w Water -IPM -Tween 80- IPA system at various S/CoS ratios are shown in Figure 1. Comparison between the isotropic regions for the system reveals that as the relative concentration of the cosurfactant, isopropyl alcohol increases, the microemulsion region decreases in size. The isotropic regions obtained for the W/O Water–IPM–Span20–[AOT + POPE (1:1)] system are shown in Figure 2.

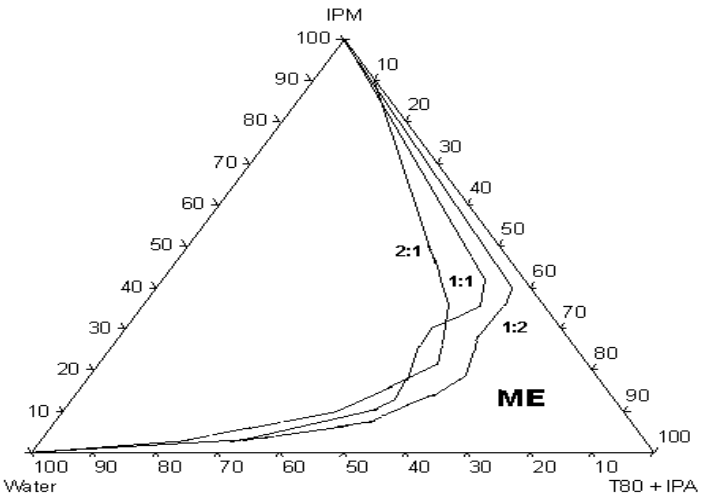

Figure 1: Pseudoternary phase diagrams for TPA system. The Tween 80/Isopropyl alcohol ratios (2:1), (1:1) and (1:2) in the surfactant mixture are shown besides the lines drawn for the respective microemulsion region in the figure. ME represents microemulsion region

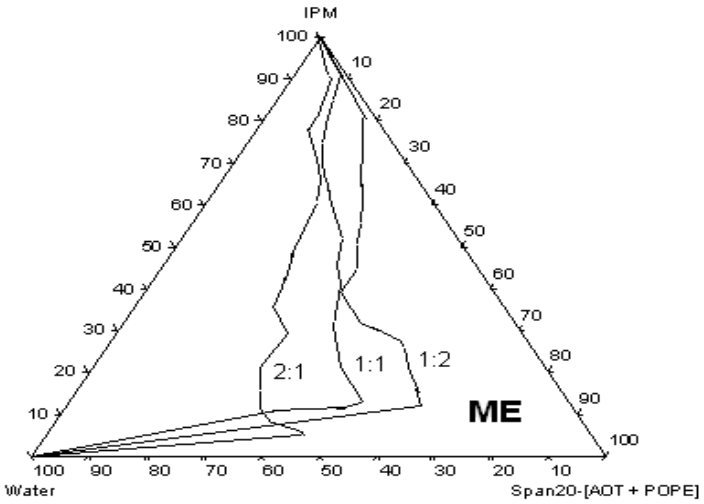

Figure 2: Pseudoternary phase diagrams for SAP system. The Span20–[AOT + POPE (1:1)] ratios (2:1), (1:1) and (1:2) in the surfactant mixture are shown besides the lines drawn for the respective microemulsion region in the figure. ME represents microemulsion region

It was evident that for both types of systems, the isotropic regions at surfactant to cosurfactant weight ratios of 0.5, 1 and 2, the area microemulsion region progressively increased from lower to higher S/CoS ratios. Maximum solubilization occurred when the S/CoS weight ratio was 2. This effect was found to be greater in w/o type of microemulsion and changed slightly in size in case of o/w microemulsion. It is also important to remember that whatever the microstructure, microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating.[57]

Formulation Selection

According to the criteria of the maximum of internal phase added/minimum surfactant needed to obtain the transparent microemulsion compositions, the systems depicted in Table 1 were selected for further investigations from each series. It is well known that a large amount of surfactant causes skin irritation, therefore it is important to determine the surfactant concentration properly and use optimum concentration of surfactant in the formulation. Various microemulsions were selected from phase diagram of S/CoS ratio of 1:2 for o/w water IPM -Tween 80- IPA microemulsion and from S/CoS ratio of 2:1 for w/o Water–IPM–Span20–[AOT + POPE (1:1)] microemulsion as shown in Table 2 and the drug, 1.16% Diclofenac diethylammonium was incorporated.

Table 1: Compositions of microemulsion systems for preliminary screening. O/W TPA ME, S/CoS = 0.5

Code	S:W Ratio	S Tween 80	CoS IPA	O IPM	W Water
TPA 1	2:8	6.65	13.3	78.2	0.65
TPA 2	3:7	9.75	19.5	67.89	1.65
TPA 3	4:6	12.6	25	58	3

Where, TPA= o/w Water- Isopropyl myristate – (Tween 80 – Isopropyl alcohol) microemulsion system, IPA= Isopropyl alcohol IPM= Isopropyl myristate, CoS= Co-surfactant,

Table 2: Compositions of microemulsion system for preliminary screening, W/O SAP ME, CoS = CoS1 + CoS2, CoS1/ CoS2 =1, S/CoS = 2.0

Code	S:O Ratio	S Span 20	CoS_1 AOT solution	CoS_2 POPE	O IPM	W Water
SAP1	3:7	15.69	3.36	3.36	52.32	23.92
SAP2	4:6	18.41	3.94	3.94	39.46	32.88

Where, SAP= w/o Water–IPM–Span20–[AOT + POPE (1:1)] system microemulsion system, AOT= Aerosol OT, POPE= Polyoxyethylene (10) octylphenol ether S – Surfactant

Table 3: Data of physical studies and in-vitro permeation for O/W TPA ME (S/CoS = 1:2) system.

Formulation code	Ratio S:W	pH ± 0.1	Assay ± S.D	Viscosity (cp)	Mean Flux (mcg/cm^2/hr) ± S.D	Permeability coefficient P x 10^{-2} (cm/hr) ± S.D
TPA1	2 : 8	7.0	100.90 ±0.22	2.28	36.78 ± 0.4	2.12 ± 0.02
TPA2	3 : 7	7.0	100.46 ±0.15	4.89	37.85 ± 1.32	2.19 ± 0.15
TPA3	4 : 6	7.1	100.16 ±0.30	6.49	35.06 ± 0.14	2.03 ± 0.01
MF	-	-	-	-	24.43 ± 2.21	1.41 ± 0.13

Where, TPA= o/w Water- Isopropyl myristate – (Tween 80 – Isopropyl alcohol) microemulsion system, MF= Marketed formulation (Emulgel)

Characterization of microemulsion

Optical birefringence

Birefringence[58, 59] is a light scattering phenomenon. It is also called as double refraction and is found in liquid crystals and anisotropic systems. In birefringence, the light passing through a material is divided into two components with different velocities and hence different refractive indices. Thus, when a liquid crystal is observed between crossed polarizer, intense bands of colors are seen which is known as birefringence. In contrast, microemulsion appears completely black. All microemulsions appeared completely dark when observed between crossed polarizing plates because of the inability of the light to pass through. But the systems transmitted the polarized light when the polarizing plates were in series. These observations indicated that all the microemulsions were optically isotropically clear colloidal dispersions.

Physical stability studies

Centrifugation

Centrifugal methods[40] have long been employed by emulsion technologist to induceand accelerate instability by gravitational means. It is commonly accepted that shelf lifeunder normal storage conditions can be predicted rapidly by observing the separation ofdisperse phase when the microemulsion is subjected to centrifugation. The technique to determine behavior of small particles in the gravitational force i.e.their separation rates is quite simple and inexpensive providing a rapid fool-proofidentification of the systems as microemulsions. Brownian movement is associated withparticles smaller than 0.5 μm. The microparticles in this size range are small enough toabsorb kinetic energy from bombardment by the molecules of dispersion medium. It hasbeen calculated that it causes such a particle to change direction 1024 times per second. This keeps the dispersed droplets in a state of violent motion preventing their settlingunder gravitational field. So long as they do not coalesce, it is Brownian movement thatkeeps the droplets of microemulsion droplets from settling or creaming. The reason thatmicroemulsion droplets do not coalesce is due to surface free energy of a

microemulsion system. Just as soon as two droplets coalesce, to form a single droplet of larger size, the interfacial tension of the new droplet becomes negative i.e. the system has negative free surface energy. The larger droplet now spontaneously increases its curvature to effect zero interfacial tension again and two droplets of the original size result. This process appears continuously as does the bombardment of droplets by molecules of dispersion medium. It is this dynamic equilibrium that keeps the microemulsion systems stable. At the end of 30 minutes, the developed microemulsions showed absence of phase separation and drug precipitation after centrifugation at 4000 rpm, verifying the stability of the formulation.

Freeze-thaw cycling

This test induces stress in the microemulsion. At temperature below freezing, theformation of ice crystals in an o/w type microemulsion may cause oil particles to elongate and flatten. In addition, the lipophilic portion of the emulsifier molecule will lose their mobility while the hydrophilic portions are simultaneously "dehydrated" due to thefreezing action of water. As the sample is thawed, water is released and travels rapidlythrough the microemulsion. If the system can "heal" itself before coalescence occurs,then the microemulsion survives the test. However, if the rate of redissolution of theingredients is slow, instability may occur in case of microemulsion which is not related tonormal temperature processes.The developed formulations did not show any evidence of instability, the physicalintegrity of the formulation was maintained at the end of the cycle.

Viscosity measurements

Rheological behavior of these microemulsion systems showed that the systems were Newtonian in nature exhibiting same viscosity at all the different shear rates applied. In general, the viscosity of the microemulsion in each series was higher at higher surfactant concentrations. The low viscosity of the developed microemulsion ensures ease of handling as well as ease of mixing during manufacturing with minimum mechanical agitation.

In Vitro Skin Permeation Studies

The skin permeation profiles of the microemulsion formulations followed both zero order as well as Higuchi model. The results of the in-vitro skin permeation studies were quantify ied on the basis of flux at steady state Jss, permeability coefficient, P.There was a statistically significant increase in the amount of drug permeated from all TPA microemulsions and 4: 6 SAT ME but there was no statistically significant difference in the amount of drug permeated from all other microemulsions as compared to marketed emulgel formulation (MF) at p = 0.05 applying one-way ANOVA. It was observed that in case of W/O microemulsion the flux increased with an increase in the water content of the microemulsion[3], SAP2 > SAP1 as shown in Table 4. The probable mechanism in the increase of percutaneous permeation was the presence of high amount of water in the microemulsion SAP2 which hydrates the skin, causes the corneous cells to swell and thus enhances the permeation.Based on these results, the most active microemulsion compositions from each series were selected and are indicated as follows:

o/w TPA2 ME

w/o SAP2 ME

The permeation profile of selected Diclofenac diethylammonium microemulsion formulations as compared to the marketed emulgel formulation through guinea pig skin is shown in Figure 3.

Table 4: Data of physical studies and in-vitro permeation for W/O SAP ME(S/CoS = 2:1) system

Formulation code	Ratio S: O	pH ± 0.1	Assay ± S.D	Viscosity (cp)	Mean Flux (mcg/cm^2/hr) ± S.D	Permeability coefficient P x 10^{-2} (cm/hr) ± S.D
SAP1	3 : 7	7.5	99.50±0.35	25.60	25.80 ± 1.15	1.49 ± 0.08
SAP2	4 : 6	7.6	100.20±0.44	44.80	37.19 ± 1.24	2.15 ± 0.08
MF	-	-	-	-	24.43 ± 2.21	1.41 ± 0.13

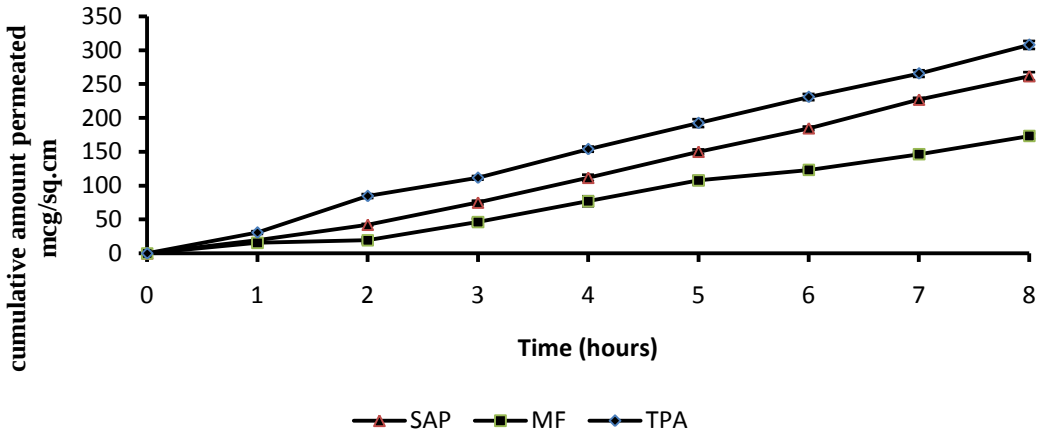

Figure 3: Permeation profile of selected Diclofenac diethylammonium microemulsion formulations as compared to the marketed emulgel formulation through guinea pig skin

Primary skin irritation studies

In the skin irritation study as represented in the Table 5, all the formulations showed no significant skin irritation on both intact and abraded rabbit skin. In no case, oedema formation was seen. The irritation score primary skin irritation index for all the formulations tested using Draize technique was shown in Table 5, thus indicating that the product is only mildly irritating to rabbit skin indicating better skin acceptability for topical application when the product is used on human skin.

Table 5: Irritation test score data

Formulation Code	Irritation score (Mean ± S.D) after 24 hours	
	Intact Skin	Abraded skin
MF	0	0
o/w TPA ME	0	0
w/o SAP ME	1.33 ±0.47	1.33 ±0.47

Stability studies

Drug content by HPTLC analysis was found to lie within ± 2 % of the initial drug content for all the formulations over a period of three months. There was no secondary spot observed during HPTLC analysis indicating there was no degradation of the drug Diclofenacdiethylammonium in these formulations. pH, viscosity and in-vitro permeation profiles were found to be maintained constant throughout the period of three months for all the formulations.

Pharmacodynamic studies

In control animals a significant difference in paw volume was observed between the left and right paw which was an indication of oedema (Figure 4). Any significant reduction in the volume of the paw in the test group as compared to that of control group was considered as anti-inflammatory response.

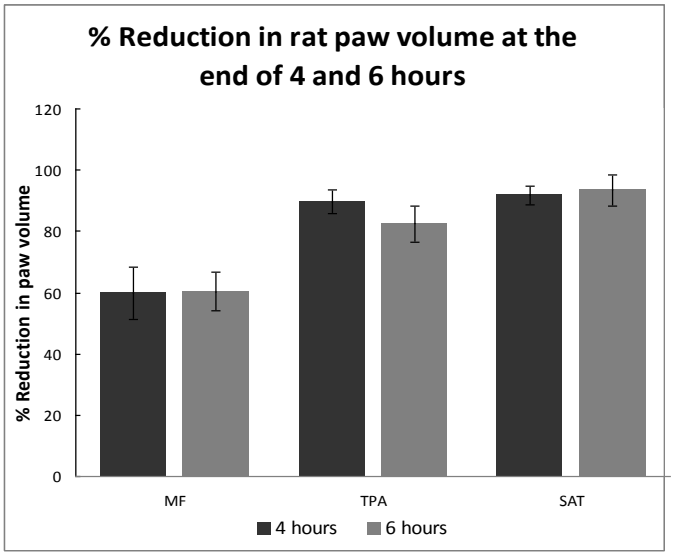

Figure 4: % Reduction in paw volume at the end of 4 and 6 hours. Values are means of 6 determinations.

According to statistical analysis of the obtained results by one way ANOVA and t-test for unpaired samples, a significant inhibitory effect ($p< 0.05$) was observed for each of the formulations tested vs. the control and for o/w TPA2 ME and w/o SAP2 ME test formulations vs. marketed emulgel at the same drug concentration (1.16% w/w). The main difference between microemulsion and commercial emugel formulation is the particle size of droplets of which they are constituted. Apparently an appropriate small droplet size of the microemulsions internal phase contributes to enhanced skin penetration. For drug loaded formulation TPA2, particle size is 85.86 nm ± 3.141, with polydispersity index as 0.309 ± 0.025 indicating homogeneity in globule size distribution. Beckman N4 Plus submicron Particle Size was used. The zeta potential of TPA2 ME is -31.6 ± 2.48 as shown in Table 3. The anti-inflammatory response increased with increase in AOT concentration and with increase in the concentration of water, which could be explained as a result of the interaction of anionic surfactant with the skin and the increased hydration of the Stratum corneum, respectively. It was observed that high water content (~ 30 % w/w) w/o SAP ME had best efficacy. This means that high water content is needed for hydration of the skin. For low water content microemulsions applied, the vehicle actually may be dehydrating the skin sufficiently to increase the skin barrier. The low water content microemulsion also contained water bound primarily to the surfactant head groups. Bound water diffuses within / from the vehicle more slowly than the free water characteristic of the high water content microemulsion. The combination of these two effects, account for low transdermal efficacy from low water content microemulsions. The microemulsion vehicles increased the flux of Diclofenac diethylammonium across guinea pig skin by 1.5 fold relative to a commercially available emulgel. Since both o/w and w/o microemulsions showed good efficacy in vivo it can be considered that the dynamic equilibrium between particles caused the drug to be localized in both the phases so that the solubility of Diclofenac diethylammonium was a less critical and limiting parameter for the bioavailability.

Conclusion

The microemulsion was successfully developed with increased drug solubilization, thermodynamic stability, ease and rapidity of formulation. The in-vitro permeation and in-vivo anti-inflammatory efficacy study demonstrated enhanced transdermal permeation of Diclofenacdiethylammonium from microemulsion formulations in comparison with commercial emulgel preparation used as standard. The developed microemulsions could be used effectively for enhancing the therapeutic efficacy of Diclofenac diethylammonium by transdermal route.

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