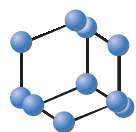


RESEARCH ARTICLE

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Development and Characterization of Water-in-Oil Microemulsion for Transdermal Delivery of Eperisone Hydrochloride



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Abstract: Background: Eperisone hydrochloride possesses short biological half-life due to first pass metabolism resulting in low bioavailability and short duration of response with toxic effects, ultimately limits its utilization for treatment of muscle spasm.

Objective: In view of this background, current study was designed for the development of Eperisone hydrochloride-loaded microemulsion and Eperisone hydrochloride-loaded microemulsion based cream for topical delivery and compared it with conventional cream.

Methods: Firstly, water-in-oil microemulsion was prepared by spontaneous emulsification method. The concentration of components was found out from existence of microemulsion region by constructing pseudoternary phase diagram. The oil was selected on the basis of drug solubility effect on the drug release, whereas surfactant and cosurfactant were screened on the basis of their efficiency to form microemulsion region. The influence of components on microemulsion formation, drug release capacity, permeation was studied by differential scanning calorimetry, X-ray diffraction, *in-vitro* release and *ex-vivo* drug permeation studies respectively. By using microemulsion, the cream was prepared for proving optimum structure for topical application. Microemulsion was evaluated for droplet size, zeta potential, pH, viscosity and conductivity. Besides the cream was characterized for pH, rheology and stability. Permeation of EPE from microemulsion across the rat skin was evaluated and compared with conventional cream.

Results: The microemulsion consisting Isopropyl Myristate/Water/Span 80:Tween 80 (50/8/42% by weight) possessed droplet size of 95.77nm, zeta potential of -5.23 mV with 7.25 pH and conductivity near to zero ($<0.05\text{mScm}^{-1}$). Physical parameters of the cream were satisfactory, also 2.33-fold higher permeation and 1.57-fold higher release observed as compared to conventional cream.

Conclusion: It can be concluded that Eperisone hydrochloride-loaded microemulsion and its cream is being effectively used for muscle spasticity by topical route.

Keywords: Eperisone hydrochloride, transdermal delivery, water-in-oil microemulsion, sustained release, Permeation, metabolism.

1. INTRODUCTION

Eperisone hydrochloride (EPE) is widely used for the treatment of plasticity to relieve muscle stiffness [1, 2] because of its low incidence of central depression. EPE decreases the muscle tonus by

inhibition of both mono- and polysynaptic pathways [3]. EPE has been shown to have very low bioavailability after oral administration due to extensive first-pass metabolism by the liver at a considerably high ratio [4] before it exhibits a pharmacological effect at an affected area, resulting very short biological half-life around 1.6-1.8h [5] along with low and variable plasma level concentration [6] and extensively high oral clearance in humans [4]. Since a considerable amount of a drug gets absorb within a short duration of time, need to

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maintain plasma concentration by increasing dose and dosing frequency *i.e.* 50 mg thrice a day causes adverse reactions [7]. Due to the gastrointestinal side effects, poor bioavailability and short biological half-life, alternative route of administration such as topical route are preferred over oral route.

Nowadays, topical route has been considered as one of the most relevant routes to target transdermal delivery of drug more effectively [8]. The main advantage of topical drug delivery lies in targeting the drug action directly to the site of the disorder by allowing accumulation of high drug concentration within the tissue and around its vicinity for sustained delivery of drug to provide steady plasma concentration profiles, particularly for drugs with short biological half-lives [9]. Other advantages include ease of administration which increases patient acceptability (non-invasive method), the possibility of immediate withdrawal of treatment in the case of any adverse event and relatively large area of application [6].

Although the great potential of topical delivery, relatively very few formulations are commercially available due to the barrier function of Stratum Corneum (SC) which limits permeation of most drugs [10]. Thus, the problem which needs to be noticed is to overcome the barrier function of SC and enhance drug delivery through the skin.

Therefore, with an aim to improve the efficacy and compliance of treatment eventually with transdermal route, microemulsion (ME) formulation was designed to increase penetration and permeation of drug, also promoting lymphatic absorption bypassing metabolism. Owing to the facile and low cost preparation ME system was opted over other colloidal systems *i.e.* liposomes, niosomes, nanoparticles [11]. MEs are thermodynamically stable and isotropic system composed of oil, water and amphiphile(s), droplet range of 20-200nm [12]. Numerous investigations have revealed the pharmaceutical significance of MEs for dermal [13] and transdermal [14] administration of a variety of drug molecules. The components of ME can interact with lipid layers of SC and change its structural integrity leading to enhanced permeation of drug(s) [15-17]. As in case of o/w MEs, water acts as a dispersed phase, but water can not permeate through lipids of SC [18]. This is evidenced from the fact that o/w ME type commercial formulation delivers very low dose through skin

even with the maximum drug loading in oil phase. Therefore, w/o type ME has been proposed based on assumptions that oil can penetrate into lipids of SC as well as act like a carrier of drug dissolved in aqueous droplets, dispersed in penetrating oil.

In view of all the above-mentioned features of w/o MEs, this system was explored for topical delivery of EPE by constructing phase diagrams with various types of oils, mixed surfactant, ratifying the effect on ME region and considering a combination with the minimum amount of surfactant and a maximum amount of water content. Further, the optimized ME formulation was evaluated for physical evaluation and *ex-vivo* drug permeation.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

EPE was received as a gift sample from Sharon Bio-Medicine, Navi Mumbai, India. Capryol 90, Labrafac, Labrafil M 1944, CS were obtained from Gattefose, France whereas Cremophor RH 40, Cremophor EL from BASF, Germany respectively. Ethyl oleate, Isopropyl myristate, Isopropyl palmitate, polyoxyethylene sorbitan trioleate (Tween 85), sorbitan trioleate (Span 85), Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80), Plurol oleic, olive oil were purchased from S.D. Fine Chemicals, Mumbai, India. Other chemicals used were of analytical grade.

2.2. Animals

Male Sprague Dawley (SD) rats (7 weeks old, 250-300 g) were obtained from Bharat serum and vaccines Mumbai, India and maintained at 22 ±2°C with free access to food and water. Approval of this study was obtained from the Animal Ethics Committee of CPCSEA, Ministry of cultures, Government of India.

2.3. Analytical Methodology

EPE was assayed by reversed-phase high-performance liquid chromatography (RP-HPLC) method using a HPLC system (HP 1200 series,

Agilent Technologies, Shimadzu) equipped with UV variable wavelength detector. The analysis was carried out on a 4.6_250mm Hypersil C-18 column (Macherey-Nagel, Bethelhem, PA). The mobile phase was composed of 90%v/v methanol and 10%v/v of double distilled water (pH 3 maintained by acetic acid). A flow rate of 1.0ml/min used and EPE was monitored by UV detection at 255 nm. The calibration curve was constructed for testing linearity. The method was found linear over the examined concentration range of 10-90µg/ml. The average calibration equation could be given by: $y = 47412x - 131018$, with a correlation coefficient of 0.9942, where y is the ratio of peak area of EPE and internal standard and x is the concentration of drug (µg/ml). The limit of detection was 0.645µg/ml. The retention time was 2.9 min. The area under peak was used to calculate the concentration of EPE. Validation studies were executed according to the ICH and USFDA validation guidelines.

2.4. Formulation Development

2.4.1. Screening of Components

High drug loading and stability are important criteria while screening of components for formulation of microemulsions. The oil, surfactant and cosurfactant for developing MEs of EPE were selected on the basis of solubility by employing shake flask method [19]. 3ml of selected oils [Capryol 90, Ethyl oleate, Labrafac Lipophile WL 1349, Labrafac M 1944, Isopropyl palmitate, Isopropyl myristate, Olive oil, Oleic acid] and surfactants [Cremophor RH 40, Cremophor EL, Tween 85, Span 85, Tween 20, Tween 40, Tween 60, Tween 80, Span 20, Span 80, Plurol oleic] were taken in vials and excess amount of drug was added and kept in mechanical shaker (Remi Instruments) for 72h at a room temperature to reach to an equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 10,000rpm for 30min (Remi Instruments). The supernatant was taken and filtered through a 0.45µm membrane filter and concentration of drug was determined by taking absorbance using spectrophotometric technique at 255nm after dilution with methanol.

2.4.2. Spontaneous Emulsification Method

The pseudoternary phase diagrams were constructed using the selected oils, surfactants and co-surfactants by aqueous phase titration method. Nine combinations of oil and Smix ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were made so that maximum ratio could be covered to describe the boundaries of phases for ME region. Each weight ratio of oil Smix were vigorously shaken by vortexing after each addition of titrating water, phase clarity and flowability were assessed visually. Samples that were transparent and had low viscosity were considered as MEs [20]. Further, it left for 24h to attain equilibrium and was confirmed by visual observation.

2.4.3. Preparation of EPE-loaded Microemulsion

EPE loaded w/o ME was prepared by using a blend of surfactant and co-surfactant (Smix) was added to oil phase and mixed with the aid of vortex mixer as follows in Table 1 [21]. Double distilled water containing dissolved drug was added dropwise slowly to above mixture with continuous vortexing to yield ME. No phase change was observed after the addition of drug solution. MEs were then subjected to thermodynamic stability tests.

2.4.4. Thermodynamic Stability Studies

To overcome the problem associated with metastable and unstable formulations during storage, the MEs were subjected to thermodynamic stability tests to assess their physical stability like centrifugation stress (3500rpm, 30min), heating cooling stress (4 and 45°C, eight cycle) and freeze-thawing stress (-21 and 25°C, > 48h) [22].

2.5. Design of Experiment

ME was optimized with respect to the effect of different oil, mixed Smix, their ratios and concentration of components on *in-vitro* release and *ex-vivo* permeation characteristics. Therefore, different pseudoternary phase diagrams were plotted with three different oil phase *i.e.* Cap 90/OO/IPM with varying Smix systems, Span 80 as a surfactant and different cosurfactants *i.e.* Tween 85/80/60 which is shown in Table 2.

Table 1. Composition of w/o microemulsion formulations.

Batch No.	Oil (%)	Smix (%) (1:1)	Drug (%)	Water (%)
M1 (Cap ST80)	52.5	40	2.5	5
M2 (Cap ST80)	40.5	49	2.5	8
M3 (Cap ST60)	57.5	35	2.5	5
M4 (Cap ST60)	47.5	45	2.5	5
M5 (OO ST80)	47.5	45	2.5	5
M6 (OO ST80)	32.5	58	2.5	7
M7 (OO ST60)	42.5	50	2.5	5
M8 (OO ST60)	31.5	59	2.5	7
M9 (IPM ST80)	46.5	46	2.5	5
M10 (IPM ST80)	47.5	42	2.5	8

Cap 90- Capryol 90; OO- Olive oil; IPM- Isopropyl myristate; ST80- Span 80:Tween 80; ST60- Span 80:Tween 60.

Table 2. Design of experiment.

System	Oil	Smix
1	Cap 90	Span 80: Tween 85/80/60
2	OO	Span 80: Tween 85/80/60
3	IPM	Span 80: Tween 85/80/60

Table 3. Composition of cream base.

Sr. No.	Ingredients	Quantity
1.	Stearic acid	15g
2.	Potassium hydroxide	0.50g
3.	Sodium hydroxide	0.18g
4.	Cetyl alcohol	0.5g
5.	Propylene glycol	3g
6.	Glycerin	5ml
7.	Steraryl alcohol	0.17g
8.	Methyl paraben	0.1
9.	Propyl paraben	0.05g
10.	Water	q.s to 100g

2.6. Preparation of EPE-loaded Microemulsion Based Cream

The pharmaceutical dermal cream base was prepared by o/w emulsion technique. In brief, the required quantity of lipid soluble components, as specified in Table 3, were taken in a china dish

and mix together by melting on a hot plate. Subsequently, water soluble components were taken in another china dish and heated on the hot plate.

The melted organic phase was then mixed to aqueous phase with regular mixing and stirring was continued in a uniform direction to form oil-

in-water emulsion based dermal cream base and EPE-ME then admixed into cream base applying gentle stirring. Different ratio of cream base and EPE-ME *i.e.* 5:1, 7:1 and 9:1 were prepared and evaluated for desired physical properties.

2.7. Characterization of EPE-loaded Microemulsion

The optimized ME was characterized for droplet size, size distribution profile (NANOPHOX particle size analyzer Symphatec, Germany) at 25°C at a 90° angle and zeta potential (Malvern Zetasizer, Malvern Instruments Ltd.) by applying an electric field across the dispersion.

ME was inspected for optical transparency and homogeneity by visual observation against strong light [23] and optical birefringence using crossed polarizers for optical isotropy to confirm the absence of other phases by placing ME between two polarizing plates in a series and then observed for light transmittance. After this, one of the plates was rotated relative to the other through 90° and then examined [24, 25]. Also transparency of ME was confirmed by measuring % transmittance (%T) at 632nm by spectrophotometrically (Shimadzu 1800 Series).

The effect of dilution of ME was assessed by visual inspection. Conductivity measurement was done to assist the formation of w/o ME using digital conductivity meter (Equip-tronics conductivity meter) having a cell constant of 1 cm⁻¹ [24].

Viscosity and refractive index (RI) of ME was determined by Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc. with TexturePro CT software) and Abbe's refractometer respectively at temperature, 25±0.3°C [24]. The ME was characterized for drug content and pH (Digital pH meter-Systronics).

2.8. *In-vitro* Release Study

The dialysis bag diffusion technique was used to study the *in-vitro* release of EPE-ME [26]. 1ml sample of EPE-ME was placed in the dialysis bag and immersed into pH 7.4 phosphate buffer solution (PBS). The entire system was kept at 32±0.5°C with continuous magnetic stirring at 300 rpm/min. Samples were withdrawn from the receptor compartment at predetermined time intervals

and replaced by fresh medium. The amount of drug dissolved was determined with HPLC on Agilent 1200 system. Data obtained from *in-vitro* release was studied to fit various kinetic equations to find out the mechanism of drug release.

2.9. Thermal Analysis

Thermal analysis was carried using SII Nanotechnology (SEIKO) and DSC 6220 differential scanning calorimeter (DSC). Accurately weighed samples were sealed in flat bottom aluminium pans and heated from ambient to 300°C at a rate of 10°C/min in a nitrogen atmosphere (flow rate, 50-60 ml/min).

2.10. X-ray Diffraction Study

X-ray diffraction studies of EPE, EPE-ME and blank ME were performed by a diffractometer to observe the physical state of EPE in the ME. The instrument details are as follows:

- Manufacturer: Pan-analytical
- Model: Xpert PRO MPD
- Anode: Copper K-alpha
- Wavelength: 1.5405 angstrom
- Power: 45KV and 40mA
- Detector: Xcelerator with diffracted beam monochromator.

2.11. Evaluation of EPE-loaded Microemulsion Based Cream

The EPE-loaded microemulsion based cream (EPE-MEBC) was characterized for total drug content, pH.

EPE-MEBC was subjected to texture analysis for assessment of different rheological properties like work of shear (Brookfield Cap 2000 L cone and plate viscometer, software: TexturePro CT) and spreadability, stickiness, firmness (Brookfield Texture Pro CT V1.8 Build 31 texture analyser, software: Capcalc V3.0 Build 20-0).

A simple method was adopted for determination of force of extrusion in terms of weight in grams required to extrude a 0.5cm ribbon of cream in 10 seconds from the collapsible tube.

2.12. *In-vitro* Release Study

In-vitro release studies of EPE-MEBC and conventional cream (CC) were carried out for 24h using dialysis membrane. A weighed amount 250 mg of each formulation was packed into dialysis bag and suspended in a beaker containing pH 7.4 PBS (20ml) at with constant stirring (300 rpm) for 2h. Samples (2.0ml) were withdrawn at predetermined time interval and replaced with an equal quantity of fresh medium. The samples were then analyzed for drug content using suitable analytical technique.

2.13. *Ex-vivo* Drug Permeation and Skin Retention Studies

2.13.1. Preparation of Full Thickness Rat Skin

The studies were conducted on a modified Keshary Chien-diffusion cell having an effective diffusion area of 3.14cm² and 17ml of receiver chamber capacity, using abdominal skin of male Sprague Dawley rat. The full thickness of rat skin was excised from the abdominal region after hair was removed by trimming carefully as short as possible using scissors, without harming the skin surface. In the following stage, the skin was cleaned of muscle, subcutaneous tissue to dermal side or vasculature surgically and the dermal side was wiped with isopropyl alcohol to remove adhering fat. The cleaned skin was washed with pH 7.4 PBS and stored at 4°C in 10% glycerin solution act as cryoprotective until further use for experiment.

2.13.2. Experimental Setup for *Ex-vivo* Drug Permeation and Skin Retention Studies

The skin specimens were thus mounted between the donor and receptor compartments with the SC facing upwards into the donor side, with the assembly set up at 32±2°C using a thermostated water bath. Assurance was made that the skin was completely in contact with the receptor phase, eliminating any air bubbles. A pH 7.4 PBS was used as the receptor medium and the temperature maintained at 32±2°C. Receptor phase was stirred constantly at 300 rpm throughout the experiment [27]. The donor was filled uniformly with formulation, such that 5mg/cm² of the excised section of rat skin. At set intervals of time (0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 11, 12 and 24h), 2.0 ml of the re-

ceptor phase was withdrawn and immediately replenished by the equal volume of PBS (pH 7.4). At the end of the experiment (24 h), the amount of formulation remaining on the skin was collected carefully and weighed further the skin was washed briefly with water and methanol. The skin was cut with scissor and transferred to a test tube, subjected to 3 cycles of vortexing for 5min and ultrasonication for 30min each. Thus, the resulting solution was filtered through a 0.45µm membrane and diluted suitably with the mobile phase. All samples were quantified by HPLC to estimate the amount of EPE released into the receptor phase from the formulation. The cumulative percent of EPE permeated *versus* time (h) graph was plotted, with the determined amount of EPE released at different time intervals. Another graph of amount of EPE diffused per unit area (Q/A) *versus* time (h) was plotted. Using linear regression of statistical analysis, linearity of this region was checked. With confirmation of linearity for this region, the slope of the line (*i.e.* flux) was figured out. Also the amount of EPE that remained on the skin (*i.e.*, in the donor compartment) and deposited within the skin was calculated by HPLC. The dermal/transdermal selectivity index (S value) was represented by an equation of skin accumulation/flux or CSA/PC.

The EPE-MEBC was filled in aluminum collapsible tubes and stored at different storage conditions, 30±2°C/ 65±5% RH and 40±2°C/ 75±5% RH for a period of 3 months. Samples were withdrawn after specified intervals and evaluated for total drug content, pH, consistency, color change and drug release pattern.

3. RESULTS AND DISCUSSION

3.1. Formulation Development

3.1.1. Screening of Components

A microemulsion is formed when the interfacial tension at the water/oil interface is brought to a very low level and the interfacial layer is kept highly flexible and fluid [11]. This condition is usually met by a careful and precise choice of components and their respective proportions [28].

While selecting surfactants, safety is an important criterion followed by the selection of the right blend of low and high hydrophile lipophile

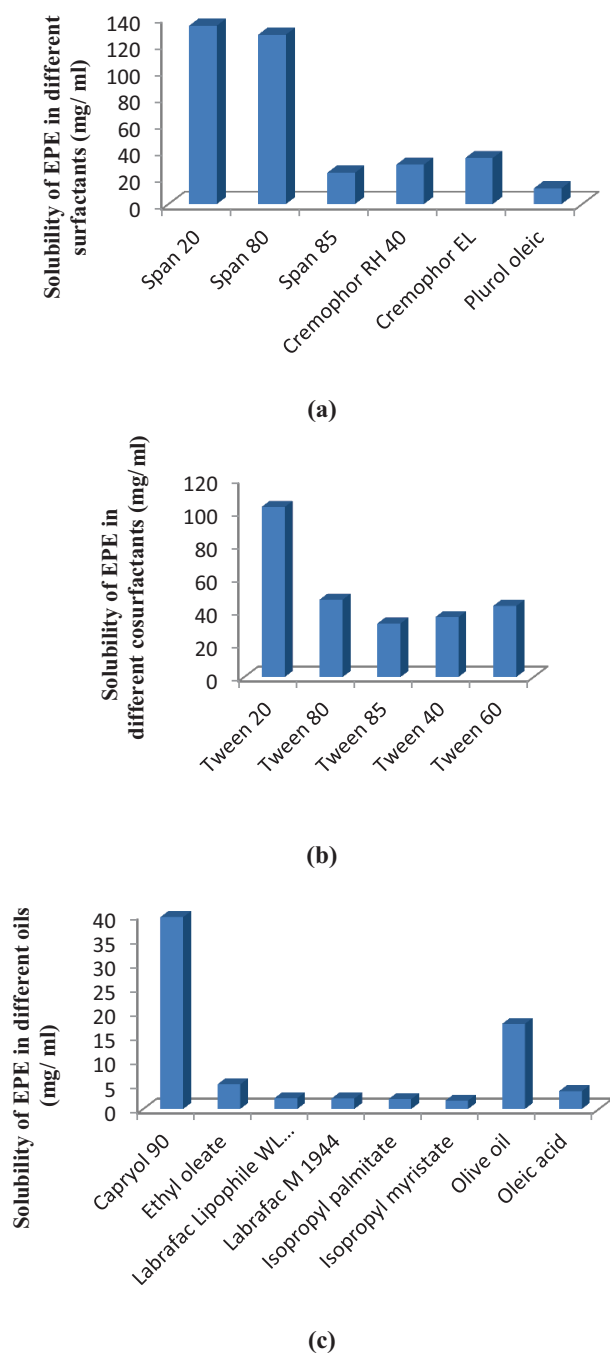


Fig. (1). Solubility of EPE in (a) surfactants, (b) cosurfactants and (c) oils.

balance (HLB) surfactant, leads to the formation of a stable ME. Therefore, the solubility of EPE was performed with different surfactants and cosurfactants (Fig. 1a and b), thus sorbitan esters and polyethoxylated sorbitan esters both nonionic surfactants were selected because nonionic surfactants are unlikely to react with other ingredi-

ents, less toxic than ionic surfactant, highly stable to changes in pH and are nontoxic, non-irritating, biodegradable and biocompatible.

Release profile is a very crucial design factor in the development of w/o ME for water soluble drugs which depends on the solubility of the drug in dispersed phase *i.e.* oil phase. However, the solubility of EPE was determined with different oils (Fig. 1c) and was found to be significantly more soluble in Capryol 90 than in other oils whereas slightly soluble in olive oil and sparingly soluble in isopropyl myristate. These oils were used to explore the effect on the release profile of formulation.

3.1.2. Preparation of EPE-loaded Microemulsion

3.1.2.1. Spontaneous Emulsification Method

The construction of phase diagram makes it easy to find concentration range of components from the existence of ME region. The pseudo-ternary phase diagrams for all three systems showed that the w/o ME form arc-shaped regions at low water content in the presence of surfactant ranging between 20 and 90% (w/w). MEs formed within a narrower region between 20 and 50% using Span 80 with Tween 85 mixed surfactant (Fig. 2a, a1 and a2) and within a broader range between 30 and 90% using Span 80 with Tween 60 or 80 mixed surfactants. This is plausibly due to the HLB of the surfactant, whereby Tween 85 is more hydrophobic than the other two Tween surfactant grade with a similar headgroup. Thus, ME stabilized by Span 80 and Tween 85 is restricted to a smaller region compared to the other two and hence ST85 Smix was rejected. Therefore for other Smix, a surfactant range between 30 and 50% is the most suitable composition range that appears to fit all the surfactant combinations. In order to solubilize the required amount of drug, minimum water content was selected. Only 5% of water was the minimal concentration required for solubilization of EPE.

ME formulations were prepared from all constructed phase diagrams except system 3 with ST60 (Fig. 2c2), because low ME region was obtained with extreme low water content in the presence of surfactant ranging between 40 and 90% (w/w) where higher oil concentration was observed but batches could not be tried with it due to limitation on usage of IPM (NMT < 50%).

Table 4. EPE-ME formulations and physical stability studies.

Batch No.	Observation (Immediate)	Observation (after 24 h)	Physical Stability			Inference
			H/C	Cent	F/T	
M1 (Cap ST80)	Turbid	-	-	-	-	-
M2 (Cap ST80)	Clear	Clear	√	√	√	Passed
M3 (Cap ST60)	Turbid	-	-	-	-	-
M4 (Cap ST60)	Clear	Phase Separation	-	-	-	-
M5 (OO ST80)	Turbid	-	-	-	-	-
M6 (OO ST80)	Clear	Clear	√	√	√	Passed
M7 (OO ST60)	Turbid	-	-	-	-	-
M8 (OO ST60)	Clear	Clear	√	√	√	Passed
M9 (IPM ST80)	Clear	Clear	√	√	√	Passed
M10 (IPM ST80)	Clear	Clear	√	√	√	Passed

H/C, Heating Cooling Cycle; F/T, Freeze Thaw; Cent, Centrifugation.

Preliminary batches with 5% water concentration in Table 1 were found to be turbid because it might be at the edge of ME region hence a rapid increase in particle size due to coalescence followed by phase separation was observed. While batches with 8% water concentration were found to be clear even after 24h and were subjected to thermodynamic stability as described in Table 4.

ME batches which exhibited no phase separation or breaking or drug precipitation indicating thermodynamic stability against centrifugation and freeze thaw cycles were taken for further physical characterization.

From detailed physical characterization (Table 5), it was ratified that among the prepared MEs, M10 (IPM ST80) was selected for further study as it not only gave the desired pH for topical delivery, but also a smaller particle size for faster partition and faster onset with sustained release as compared to remaining MEs.

As EPE is sparingly soluble in IPM, had a higher release rate from ME compared to OO and Cap 90, as the solubility of drug in oil phase decides the performance of the system. So system 1 with ST80 Smix ratio (1:1) was selected and further influence of Smix ratio on ME region were evaluated. But the amount of cosurfactant could not increase as compared to surfactant because

then required HLB value would not be obtained. In contrast, when surfactant concentration of Smix was increased from 1:1 (Fig. 2a3) to 2:1 (Fig. 2b3) and 3:1 (Fig. 2c3), depletion in ME region was observed. It might be due to insufficient cosurfactant concentration, required to reduce interfacial tension and provide flexibility of the interfaces. The literature also supports that the Smix 1:1 possesses maximum ME region as compared to the other ratios indicating that Smix ratio has a pronounced effect on phase properties.

Therefore, further batches were tried with IPM ST80 (1:1) system by considering that the amount of oil should not cross the limit in the composition of ME. Firstly, batch tried with an increase in water concentration to 10% was found to be turbid because it might be at the edge of ME region hence a rapid increase in particle size due to coalescence was followed by phase separation. Hence, it is evident that increase in percent concentration of water leads to turbidity at minimal Smix. Another effect to be evaluated was increase in Smix concentration. Therefore, the batch was tried with increased concentration of water and Smix, but no significant improvement in physical characteristics was observed. So the Smix concentration was fixed at the lowest value *i.e.* 42% w/w. So the final concentration fixed at 8% w/w of water, 42% w/w of Smix and 50% w/w of IPM (M10).

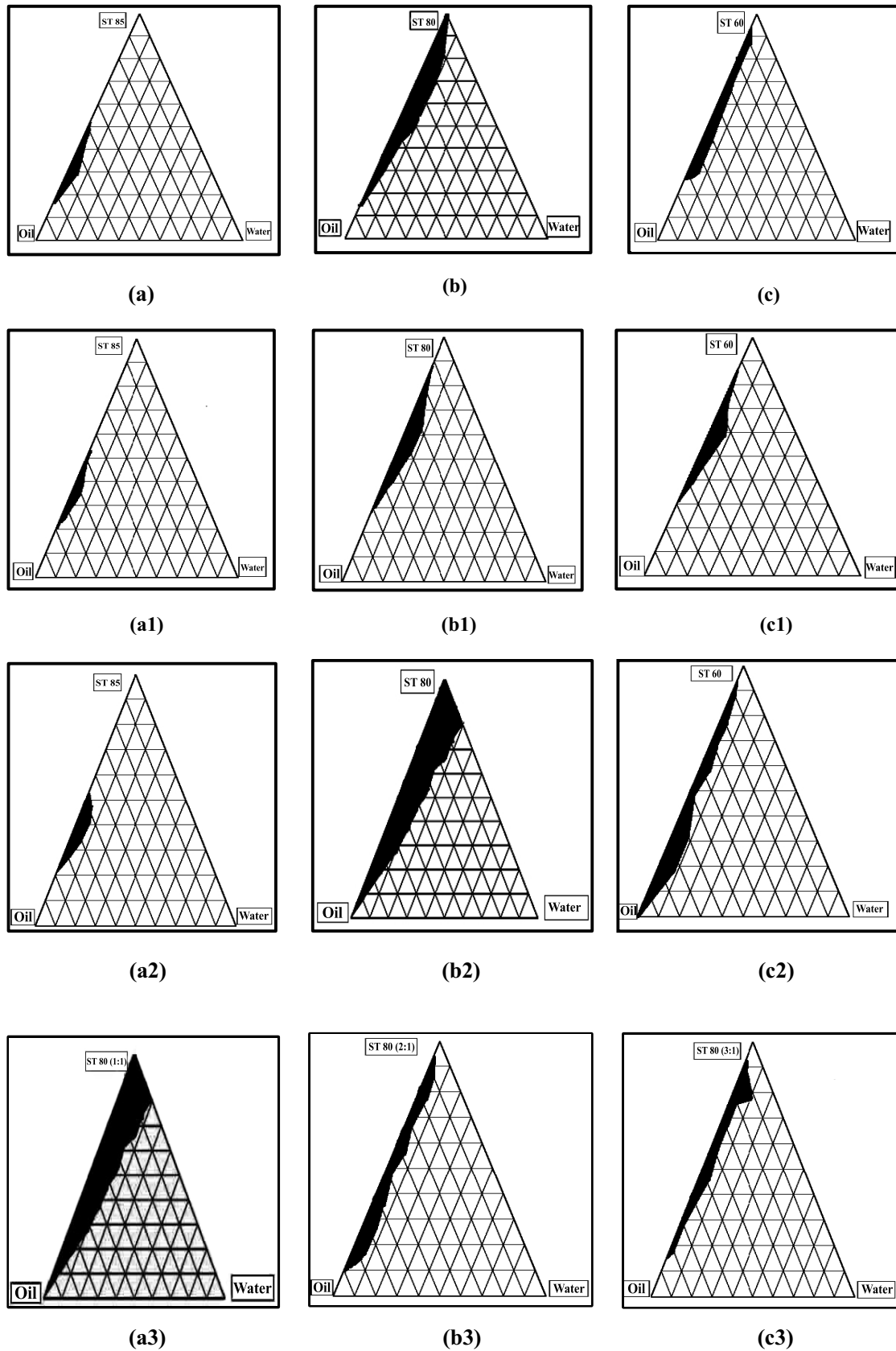


Fig. (2). Pseudoternary phase diagrams showing microemulsion region: **a)** Cap90:ST85(1:1): Water **b)** Cap90:ST80(1:1): Water **c)** Cap90:ST60(1:1): Water **a1)** OO:ST85(1:1): Water **b1)** OO:ST80(1:1): Water **c1)** OO:ST60(1:1): Water **a2)** IPM: ST85(1:1): Water **b2)** IPM: ST80(1:1): Water **c2)** IPM:ST60(1:1):Water **a3)** IPM:ST80 (1:1): Water **b3)** IPM:ST80 (2:1): Water **c3)** IPM:ST80 (3:1): Water.

Table 5. Physical properties of EPE-ME formulations.

Batch No.	pH	Particle Size (nm)	Conductivity (mS cm ⁻¹)	% Transmittance (%)	Refractive Index	% Drug Release (%)
M2 (Cap ST80)	6.23	87.59	<0.05	97.91	1.446	68.79
M6 (OO ST80)	6.96	102.44	<0.05	96.75	1.467	79.63
M8 (OO ST60)	6.99	114.21	<0.05	95.45	1.463	71.23
M9 (IPM ST80)	7.25	94.06	<0.05	97.45	1.435	88.21
M10 (IPM ST80)	7.29	95.77	<0.05	97.89	1.438	90.73

Table 6. Trails for MEBC with different ratio of cream base.

Parameter	Ratio of Cream Base:EPE-ME (gm)		
	5:1	7:1	9:1
Consistency	Fluidy	Fairly Fluidy	Better
Spreadability (mm)	-	-	54
% Drug release (%)	-	-	81.46

3.2. Preparation of EPE-loaded Microemulsion Based Cream

Dermal cream provides optimum structure and viscosity to ME for topical application. EPE-MEBC is very effective in treatment due to the smaller droplets size, which allows cream to spread and deposit smoothly and uniformly onto the surface of skin and increases drug release [29]. Further, cream retains on skin for a longer time as compared to gel [30, 31]. This cream can be formulated by high-energy techniques like ultrasound generators, high-pressure homogenizers or high shear stirring on large scale [31].

Various ratios of cream base were incorporated with EPE-ME to evaluate for proper physical characteristics as follows in Table 6. Depending upon parameters considered, cream base with 9:1 ratio was able to yield proper acceptable appearance, consistency, spreadability and drug release.

3.3. Characterization of EPE-loaded Microemulsion

The optimized ME possessed mean droplet size of 95.77nm with a PDI of 0.24, which ratified the homogeneity and stability of formulation. The zeta potential of ME obtained -5.23mV that was being towards neutral. Moreover, the zeta potential is

efficiently diminished to nearly neutral values when the drug is loaded into the nano formulation [32]. It is reported that the stability of ME and lipid emulsions containing non-ionic surfactants does not depend on zeta potential [33].

ME appeared to be transparent and homogeneous liquid against strong light because its droplets being smaller than $\frac{1}{4}$ th the wavelength of visible light, permits white light to pass through the dispersed system making it transparent [34]. In the optical birefringence study, ME appeared completely dark when observed under cross polarizer indicating ME is optically isotropic colloidal dispersion [29]. %T of ME was 97.86% which approaches 100%, defining its isotropicity and also gives an idea about the size of droplets as droplet size is directly proportional to %T.

Upon dilution with distilled water, ME formed opaque, layered appearance, indicating that it loses original structure as the dispersed phase is oil. Although when diluted with an oily solution it remained stable which confirms that the formation of w/o ME. ME had conductivity value of 0.0291 mS/cm, near zero which certifies formation of w/o ME and do not inverse to o/w or to bicontinuous form. The literature reported that the MEs stabilized by a nonionic surfactant has a negligible charge which results in low electrical conductivity

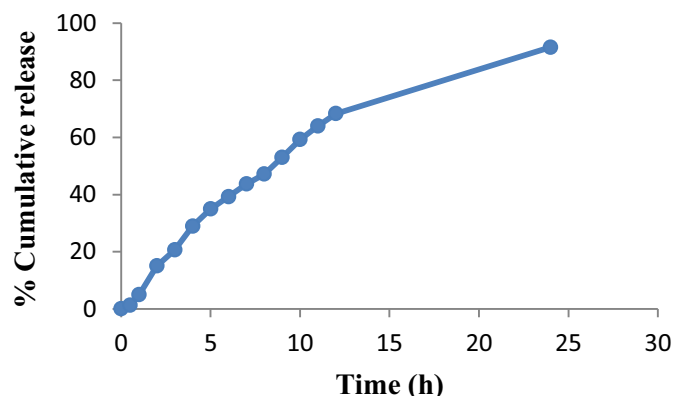


Fig. (3). Cumulative percentage release of EPE from the ME for over 24 h.

[35, 36]. Also, low conductivity describes that most of the ionized drug remains loaded in the water droplets [37].

RI reflects an optical property which is used to characterize the isotropic nature of ME and basically signifies the chemical interaction among drug and excipients. The RI of ME was found to be 1.438. No significant difference observed in RI's of EPE and all excipient, so it concludes that the ME is chemically stable and remains isotropic in nature, thus showing no interaction between excipients and drug.

The viscosity of ME was less than 19 CPS, which is very low as expects one of the characteristics of ME, so it can easily spread on the skin. However, high fluidity makes it difficult to control the application during use. Therefore, ME with a higher viscosity is the most preferred for topical application. Most MEs exhibit Newtonian fluid as a change in the shear rate has minimal or no effect on the measured shear stress [35]. The viscosity of ME depends on a number of factors including the type and concentration of surfactant, oil, water, cosolvent, ionic strength, pH and temperature [38]. Also, it reveals that the viscosity is directly proportional to the concentration of oil and surfactant used.

One of the main factors to be focused in the topical formulations is choosing the right pH, for its efficacy in the delivery of drug as well as to minimize any irritation to skin. From this point of view, a neutral pH is a preferred condition for topical applications. The pH of ME formulation was found to be 7.25 which is suitable for application to the skin. Tween 80 in formulation, possesses single aliphatic tail, have hydroxyl values around

65-80, indicative of the amount of ethylene oxide present in the surfactants. And higher the hydroxyl value of sample, higher is the acid value. This is in agreement with results whereby the pH is towards neutral [39].

The absorbance for drug content evaluation was taken by spectrophotometrically and the % drug content for the ME was observed within limits *i.e.* 98.86%.

3.4. *In-vitro* Release Study

In-vitro release study was performed to investigate the release pattern of EPE from ME system. When graph was plotted against percent cumulative release and time (Fig. 3), drug release was observed 68.32% within 12h, which may have been because of small droplet size and low PDI value leading to high surface area, thus permitting faster rate of drug release. However, the total % cumulative drug release at the end of 24h was found to be 90.03%. This study confirms a significant enhancement of drug release in sustained manner through ME system.

Various models such as a zero-order model, first order model, Higuchi model, Hixon Crowell model and Korsmeyer Peppas model were used to analyze the data obtained from the *in-vitro* release study. Best fit model with the highest coefficient of regression was obtained with zero order model followed by Hixon Crowell model. The linearity of the method was carried out with the construction of a five-point calibration curve and was found to be linear. The average calibration equation could be described by: $y = 4.6658x - 95362$, with a correlation coefficient of 0.9598, suggests

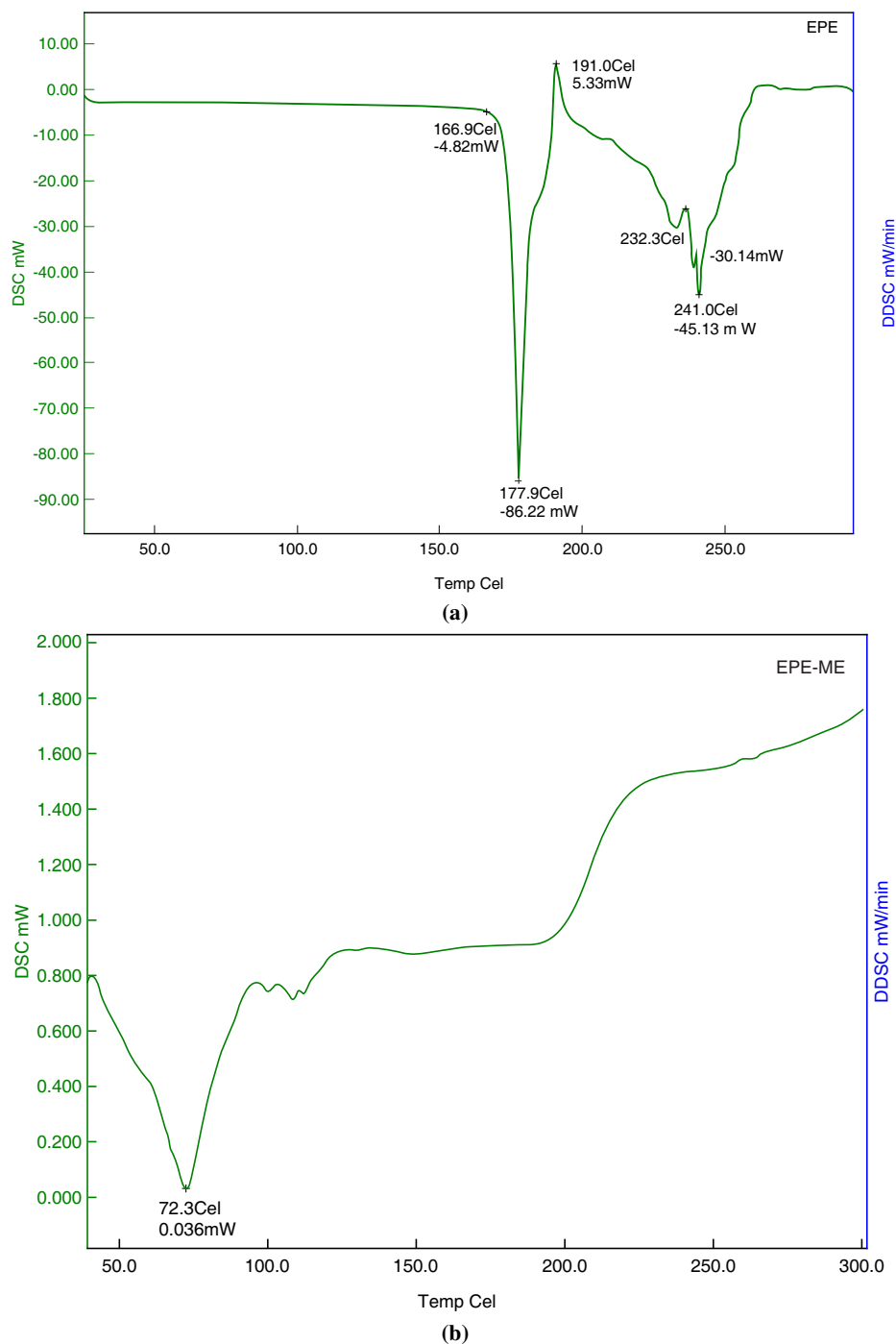


Fig. (4). DSC of: (a) EPE; (b) EPE-ME.

that the drug release from ME formulation is independent of concentration.

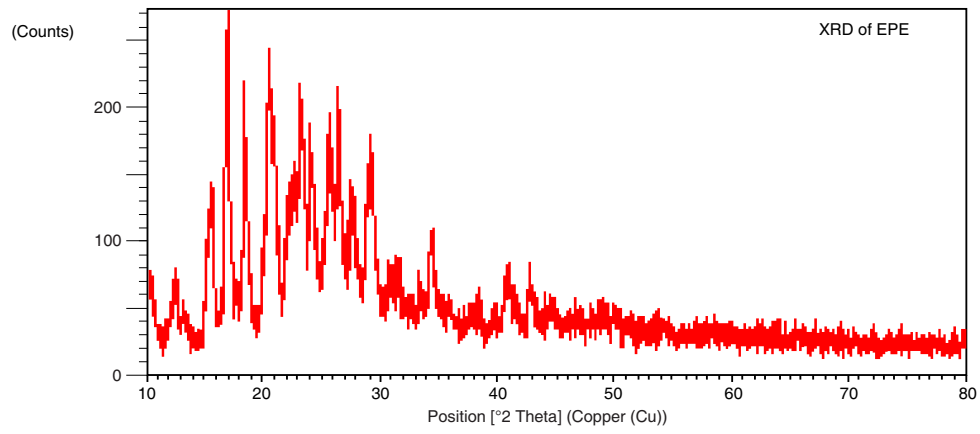
3.5. Thermal Analysis

DSC (Fig. 4a and b) thermogram represents the change in thermal behaviour as a result of interaction between excipients during preparation. For pure EPE, a sharp melting peak at 177.9°C was observed. The DSC of EPE-ME did not show any

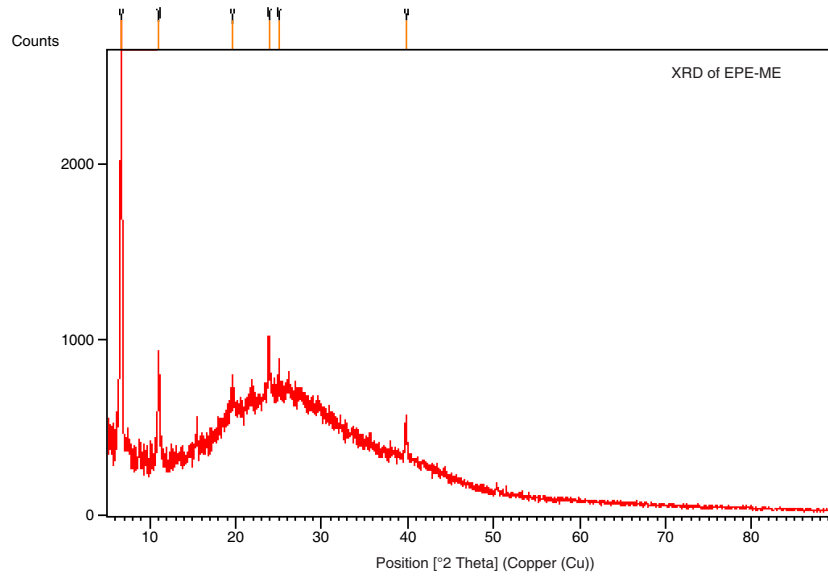
other significant melting peak which indicates complete incorporation of EPE in the ME and possible reduction of drug crystallinity.

3.6. X-ray Diffraction Study

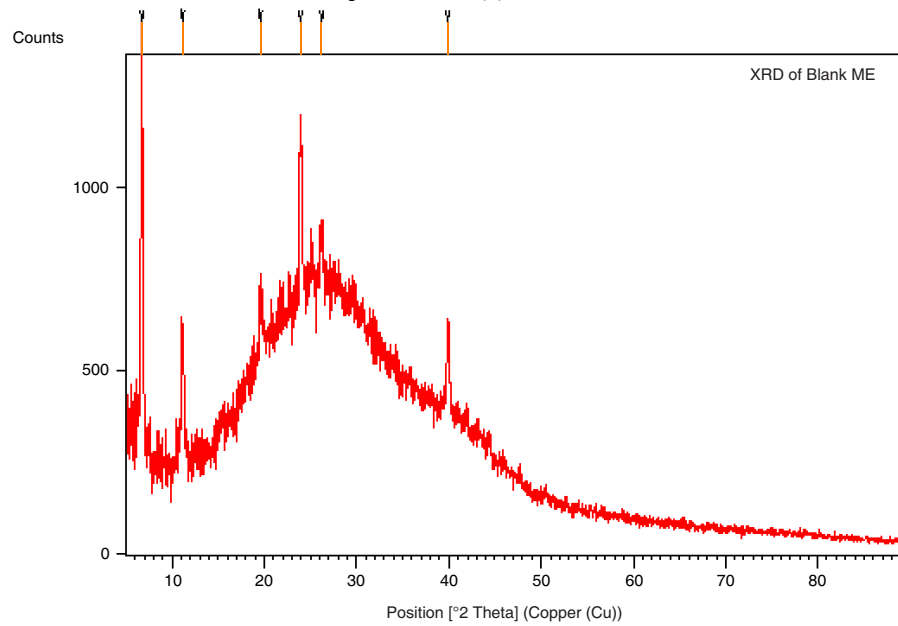
As shown in Fig. (5a-5c), the XRD pattern of EPE-ME was different from that of pure EPE. From XRD patterns, it was observable that the EPE exhibited crystalline characteristic peaks



XRD patterns of : (a) EPE



XRD patterns of : (b) EPE-ME

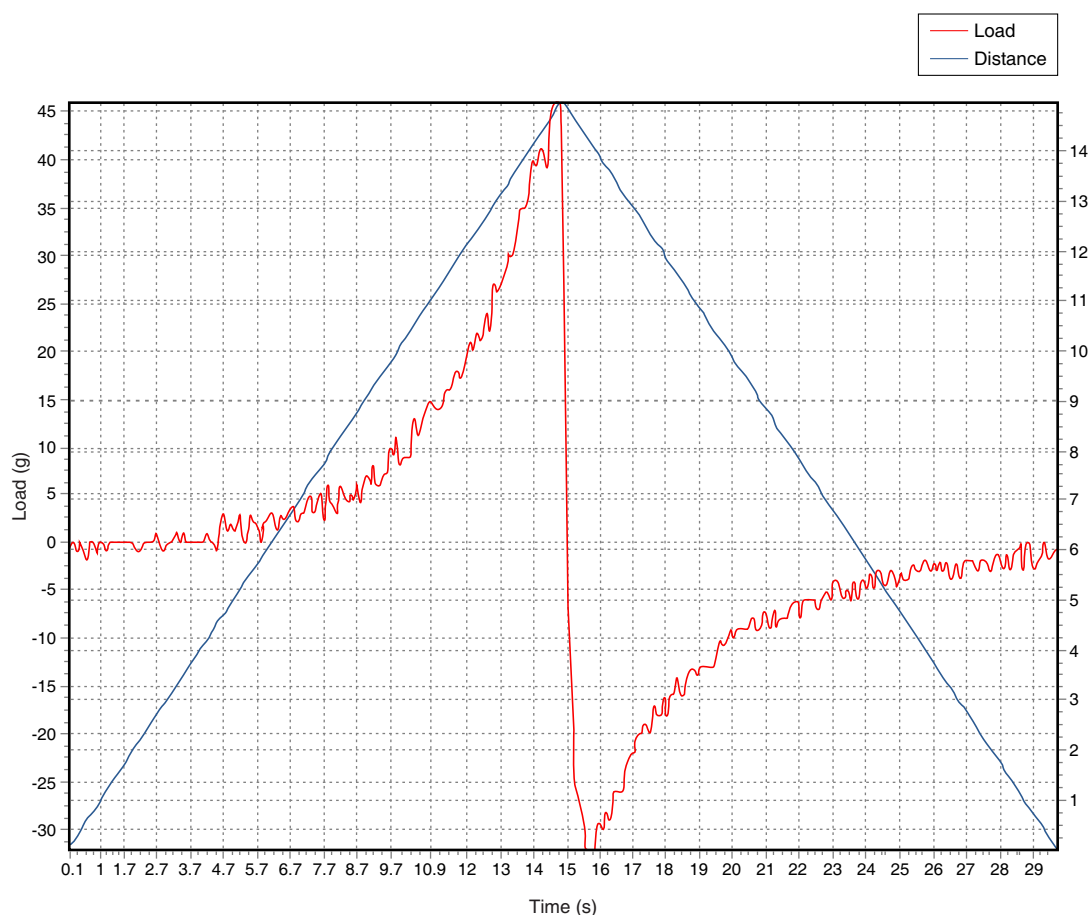


XRD patterns of : (c) Blank ME

Fig. (5). XRD patterns of : (a) EPE; (b) EPE-ME; (c) Blank ME.

Table 7. Physical properties of EPE-MEBC.

Evaluation Parameter	Result		
Colour	White		
State	Semi-solid		
Texture	Smooth		
Homogeneity	Homogenous		
Drug content	98.67%		
pH	6.7		
Rheological properties:	Non-newtonian behavior		
1. Spreadability	Hardness	Adhesive Force	Adhesiveness
	46gm	32gm	1.40mJ
2. Viscosity	21.68P (Ideal Character)		
Tube extrudability	0.23gm		
% Drug release	81.95%		

**Fig. (6).** Texture curve of MEBC.

of pure drug and EPE-ME did not show crystalline peaks of the pure drug; instead, EPE-ME showed a different pattern of XRD, which resembles to that of blank ME. Thus, it can be established that the EPE is completely incorporated in ME and is present in amorphous form.

3.7. Evaluation of EPE-loaded Microemulsion Based Cream

In this study, EPE-MEBC was found to be homogenous, smooth in texture with appropriate pH and spreadability, indicates durability and stability of the formulation in Table 7.

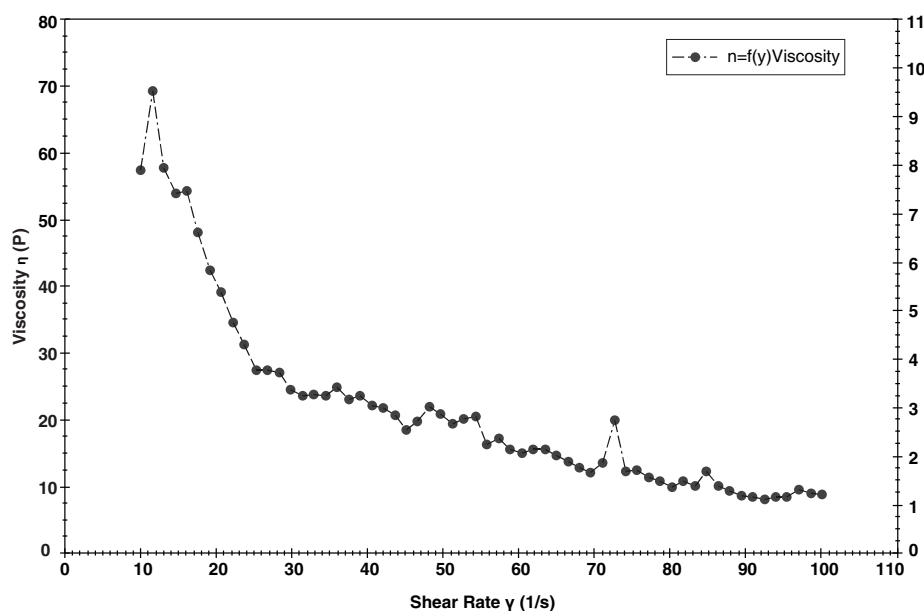


Fig. (7). Graph of viscosity vs shear rate.

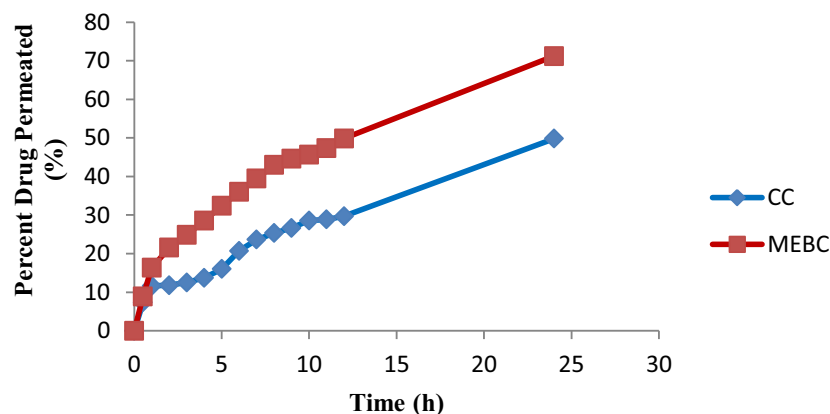


Fig. (8). Comparison of *ex vivo* permeation profiles of MEBC and conventional cream of EPE through rat skin.

The ability of a semisolid formulation to spread on the skin plays a vital role in administration and thus the efficacy of formulation in the topical therapy was determined by texture analysis. Texture analysis, revealed that the EPE-MEBC possessed fairly good strength, ease of spreading and adequate cohesiveness; which are essential for application and retaining of formulation on the skin. Further, uniformity of texture curve plotted to employ TexturePro CT V1.8 Build 31 software (Fig. 6), confirmed the smoothness of EPE-MEBC and absence of any grittiness or lumps. It reflects the ideal value for the skin spreadability for EPE-MEBC. These results support the notion that the cream has significant spreadability.

Generally, topical creams have high viscosity at low shear rates and vice versa which means topical

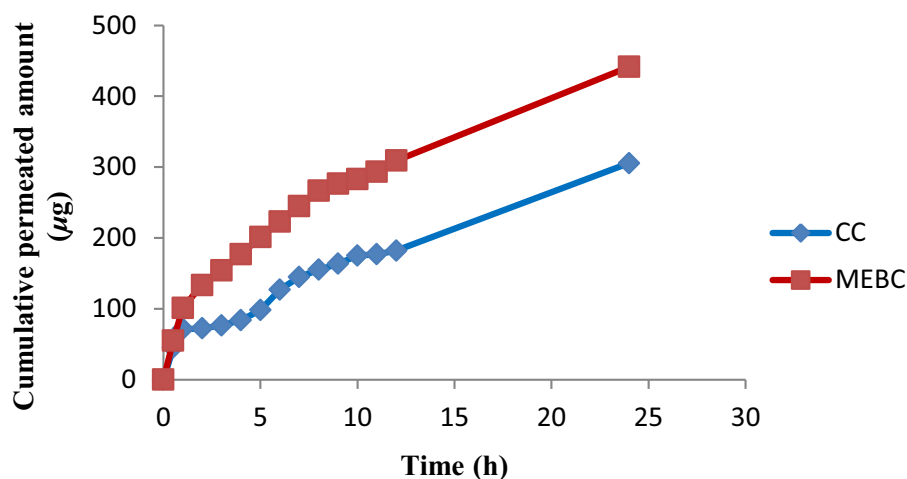
creams are non-newtonian in nature. Therefore, it is essentially advisable that estimation of viscosity should be performed at shear rates that are equivalent to the situation trying to simulate [40]. MEBC shows the ideal characteristic of viscosity and viscosity decreases with the increase in shear rate, which is depicted in Fig. (7).

3.8. *Ex-vivo* Drug Permeation and Skin Retention Studies

To study the influence of formulation ingredients on permeation of EPE from MEBC and CC were investigated by *ex-vivo* drug permeation and skin retention studies for a period of 24h (Table 8). The CC of EPE exhibited only 46.85% and EPE-MEBC showed 71.20% drug permeation at the end of 24h (Fig. 8) indicating that the MEBC resulted

Table 8. Comparison of permeation parameters of EPE-MEBC and conventional cream.

Parameters	EPE-MEBC	Conventional Cream
Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	9.8	4.2
Skin retention ($\mu\text{g}/\text{mg}$)	0.051	0.016
Permeability coefficient ($\text{cm}^2/\text{h}; \times 10^{-2}$)	0.016	0.0068
Enhancement ratio	2.33	-
S value	0.99	-

**Fig. (9).** *Ex vivo* permeation profile of EPE from MEBC and conventional cream using excised rat skin at the end of 24h employing a Franz diffusion assembly.

in considerable improvement in permeation of EPE.

The mean cumulative amount of EPE permeated during 24h for MEBC and CC were obtained $441.44\mu\text{g}$ and $305.37\mu\text{g}$ respectively (Fig. 9). The flux values after 24h for the EPE-MEBC and CC were found to be 140.59 and $97.25\text{mcg}/\text{cm}^2/\text{h}$ respectively. Comparison of cumulative permeation between EPE-MEBC and the CC demonstrated that EPE-ME enhanced drug permeation significantly by almost 2.33-fold than CC.

According to ME formulation, smaller the particles are the bigger their surface, which leads to an increase release. Besides, at the first time point that EPE was detected (the lag time of each formulation) ranged from 0.68 h and 0.17 h for CC and MEBC respectively, indicating that the drug permeation rate significantly gets affected by the composition of formulation. As the contribution of IPM in enhancing EPE permeation through the skin by disrupting the fluidity of SC and could increase the flux (J_{ss}) in skin, simultaneously increases permeation coefficient [41, 42]. Surfac-

tants in the composition might be responsible for enhanced permeation from ME. The non-ionic surfactants reportedly emulsify sebum, thereby enhancing the thermodynamic coefficient of drug, allowing it to penetrate into cells more effectively [43]. The surfactants used may exert effects on lymphatic transport. Tween 80 was chosen as cosurfactant in the ME, promotes drug entry into the lymph and is in fact regarded as an ideal excipient for lymphotropic vehicles. Moreover, the close contact with skin might contribute to the direct release of EPE from water droplets into the skin without the transfer of the drug in the continuous phase. Also, water content acts as a reservoir of drug, from which drug gets partitioned into the oil phase and released into the skin.

The results of EPE 'percentage permeated', 'percentage deposited' and 'percentage remained on the skin, for both formulations were evaluated at the end of 24h studies which are illustrated in Fig. (10). The deposition potential of EPE-MEBC was found to be 3.35-fold more than the CC. These observations confirmed the earlier findings

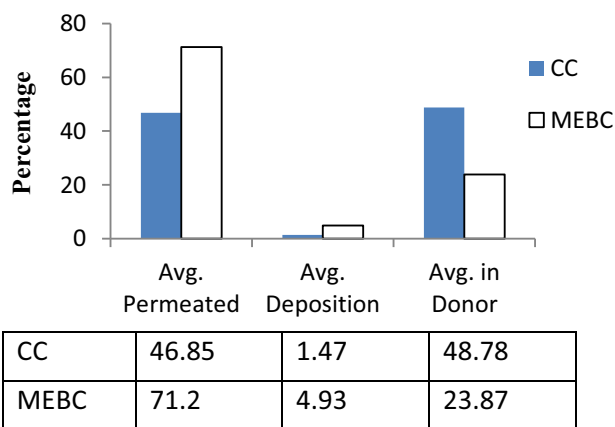


Fig. (10). *Ex vivo* deposition and permeation of EPE from MEBC and CC using excised rat skin at the end of 24 h employing a Franz diffusion assembly.

3.9. *In-vitro* Release Study

The effectiveness of any nanoformulation is described by the rate of drug release. Therefore, *in-vitro* release study was performed to compare the release rate of EPE from MEBC and CC (Fig. 11), a significant increase in the percentage of drug release was achieved in case of MEBC. But EPE-MEBC depicted slightly lower drug release of 81.95%, compared to EPE-ME which may be attributed to slow diffusion of drug through cream base. Still, there was no significant difference in drug release of ME and MEBC. It shows that microemulsion incorporated into cream base retained intact in their structure because if ME was struc-

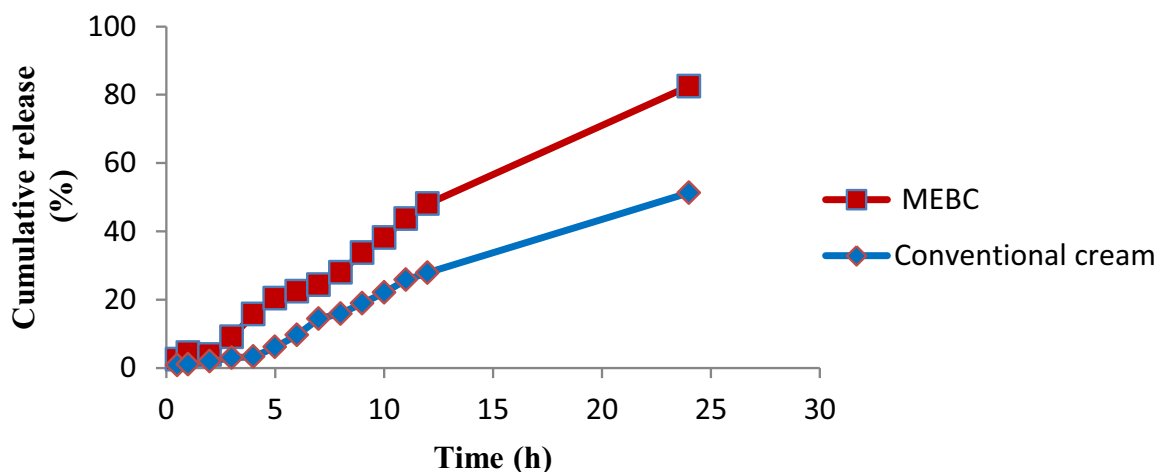


Fig. (11). *In-vitro* release profile of EPE from MEBC and conventional cream using dialysis bag method at the end of 24 h.

about ME being deposited in the skin, thus acting as a depot to give sustained release by retaining within close vicinity of the target. But the average amount of EPE present in the donor compartment of MEBC was approximately 23.87% whereas 48.78% for CC, indicates that highest permeation for MEBC, which can be attributed to its high flux value.

Although, it might attempt to establish the mechanism of EPE permeation through skin from MEBC, we believe that while EPE getting transported across the skin, probably the ionized drug in aqueous droplets dispersed in the oil phase get carried through the skin by carrier effect of penetrating oil. This was one of the reasons to employ w/o ME approach for topical delivery of EPE as it improves transdermal delivery.

turally modified it would have led to the burst release.

EPE-MEBC exhibited no change in drug release, phase separation and no drug precipitation or colour change when it was subjected to stability study at 30±2°C/ 65±5% RH and 40±2°C/ 75±5% RH for 3 months. The organoleptic features like consistency, firmness and physical appearance were also observed and no significant change was found in these characters.

CONCLUSION

The ME based cream containing EPE wherein the drug is incorporated in stable water-in-oil microemulsion has been successfully formulated using mild agitation. Results from comparative stud-

ies with conventional cream in respect of *in-vitro* release and *ex-vivo* drug permeation and skin retention studies have led to the understanding about ME based cream shows faster onset of action with sustain drug release pattern.

However, from the present investigations we can conclude that the role of water-in-oil microemulsion is effective in transdermal delivery of EPE for the treatment of muscle spasticity. For many other water soluble drugs which are intended to exhibit sustain action by topical route, such developed technology platform is proposed to be used. It is further suggested that by focusing on the composition of ME; release, permeation and hence pharmacodynamic activity can be modulated.

LIST OF ABBREVIATIONS

EPE	=	Eperisone Hydrochloride
SC	=	Stratum Corneum
ME	=	Microemulsion
o/w ME	=	oil-in-water Microemulsion
w/o ME	=	water-in-oil Microemulsion
Cap 90	=	Capryol 90
IPM	=	Isopropyl Myristate
SD	=	Sprague Dawley
RP-HPLC	=	Reversed Phase High Performance Liquid Chromatography
Smix	=	Blend of Surfactant and Co-surfactant
OO	=	Olive Oil
EPE-ME	=	Eperisone Hydrochloride Loaded Microemulsion
%T	=	% Transmittance
RI	=	Refractive Index
EPE-MEBC	=	Eperisone Hydrochloride Loaded Microemulsion Based Cream
CC	=	Conventional Cream
PBS	=	Phosphate Buffer Solution
S Value	=	Selectivity Index

PC	=	Flux/Saturated Solubility
CSA	=	Cumulative Amount in Skin/Saturated Solubility
HLB	=	Hydrophile Lipophile Balance
ST60	=	Span 60:Tween 60
ST80	=	Span 80:Tween 80
ST85	=	Span 80:Tween 85
PDI	=	Polydispersity Index
H/C	=	Heating Cooling Cycle
F/T	=	Freeze Thaw Cycle
Cent	=	Centrifugation Cycle
DSC	=	Differential Scanning Calorimetry
XRD	=	X-ray Diffraction

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ex-vivo drug Permeation and Skin Retention Studies was approved by the Institutional Animal Ethics Committee of CPCSEA, Ministry of cultures, Government of India with the Protocol No. BVCP/IAEC/01/2018.

HUMAN AND ANIMAL RIGHTS

No humans were used in the experiments. All the reported experiments on animals were in accordance with the guidelines complied by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Ministry of cultures, Government of India).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the Science - Hub at,

- <https://www.ncbi.nlm.nih.gov/pubmed/24492583> (Reference number - 24).

- <https://www.ncbi.nlm.nih.gov/pubmed/11587484> (Reference number - 4).
- <https://www.ncbi.nlm.nih.gov/pubmed/16843424> (Reference number - 9).
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3283952/> (Reference number - 43).

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

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