ORIGINAL ARTICLE

Formulation and Evaluation of Trolamine Salicylate Microemulsion

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Abstract

Aim: The aim of this study was to formulate and perform optimization, characterization, and *in-vitro* evaluation of microemulsion containing trolamine salicylate (TMS) an anti-inflammatory agent for topical application. **Materials and Methods:** Microemulsion formulations of TMS were prepared from optimized microemulsion and effects of formulation variables such as solubility in different oils, surfactants and co-surfactants were assessed. Oleic acid was selected as oil phase, tween-80, and ethanol as surfactant and cosurfactant, respectively. From the ternary phase diagrams of surfactant (Tween 80)/cosurfactant (Ethanol) 1:1 ratio and oil (oleic acid), TMS-loaded microemulsion formulation A6* was selected on the basis of clarity. **Results and Discussion:** The microemulsion formulation A6* was found to be optically clear, transparent, and elegant in appearance, when compared to the other microemulsion formulations with pH values of 5.3–6.5 were showing suitability for topical preparations. The transmission electronic microscopy image of A6* showed that globules were spherical in shape, smooth surface, and indicated the existence of an isotropic dispersion of spherical droplets, leading to the assumption of inverse micelles because of the proportion of the constituents. Particle size of 297 nm indicates that there is a chance that the number of vesicles can interact with a fixed area of stratum corneum, thereby increasing the efficiency in percutaneous uptake. During *Ex-vivo* permeability study, the flux values and permeability coefficient of TMS Microemulsion (A-6*) were found to be 6.518 $\mu g/cm^{2}\,h$ and 3.259 $cm.h^{-1}$. The highest cumulative drug release for formulation A*6 was 95.048 ± 0.032% in 8 h. **Conclusion:** Microemulsion has low interfacial tension and allows excellent contact with skin surface, with the vehicle filling even wrinkles and microscopic gaps. This enhances the vehicle skin drug transfer. They have been used to improve the bioavailability of various poorly soluble drugs including non-steroidal anti-inflammatory drugs. The formulation was in nano range and hence the penetrability of the drug can be increased. Since this type of formulations can be easily developed and prepared; therefore, they can be of great help for the drugs that have less permeation across the skin. Among the distinctive formulations, A6* showed promising results, with respect to drug entrapment and percentage drug release.

Key words: Isotropic dispersion, microemulsion, oleic acid, trolamine salicylate

INTRODUCTION

Topical products are important classes of drug delivery systems and their use in therapy is becoming more widespread. Now a day, there are many studies in the area of drug penetration into the skin. They can incorporate both hydrophilic and lipophilic drugs and enhance their permeation. Since the formation of microemulsion requires high surfactant concentration, the skin irritation aspect must be considered mainly when they are intended to be applied for a more extended period. Topical delivery can be defined as the application of a drug or drug-containing formulation for the treatment of dermal, local soft tissue, and joint disorders. It provides sustained and controlled delivery of the drug, reduces systemic toxicity of the drug due to direct access to the target site, and provides ample surface area and a convenient route of administration of the drug. It is a painless administration and has improved patient acceptance.

Drugs should penetrate skin layers to ensure effective drug concentrations following topical administration. Types of the formulations as well as the physicochemical characteristics of

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drug molecules are effective parameters in the topical delivery of drugs. In topical administration, the entering of drugs to the systemic circulation is prevented or minimized. Thus, the systemic adverse effects of drugs may be avoided. Besides, topical preparations have better patient compliance due to their non-invasiveness and, they can be self-administered.[1]

The greatest challenge for dermal penetration is the tough horny layer, that is, stratum corneum (SC), the uppermost layer of the skin, which is the rate-limiting step for epidermal drug transport. The physicochemical factors of a drug like log pKa, solubility, and molecular mass also play an essential role in the selection of components for the topical delivery vehicle. For acidic and unstable drugs like, special consideration has to be made on the excipient selection for a topical vehicle which will not only mask the irritation potential of a drug due to an acidic group but also provide for effective topical delivery and maintenance of chemical integrity. Microemulsions were first introduced by Hoar and Schulman in 1943.[2] Microemulsions, which are optically isotropic, thermodynamically stable, low viscosity homogenous single-phase systems of water, oil, surfactant, and/or co-surfactant, have been studied as drug delivery systems because of their capacity to solubilize poorly water-soluble drugs as well as their enhancement of topical and systemic availability. It helps to solubilize the lipophilic drug moiety and it shows rapid and efficient penetration to the skin. Hence, it is beneficial for topical drug delivery.

MATERIALS AND METHODS

Materials

Oleic Acid was purchased from S. D. fine chemicals, Mumbai. Tween 80 was purchased from Qualigens chemicals, Mumbai. Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, and isopropyl alcohol were purchased from Loba Chemie (Mumbai, India). Ethanol was purchased from Changshu Yangyuan Chemical (China), and a gift sample of Trolamine Salicylate (TMS) was procured from Triveni Chemicals, Vapi, Gujrat.

Method of preparation

Screening of excipients[3,4]

To find out suitable excipients, the solubility of TMS was determined in various oils, surfactants, cosurfactants, and their mixtures. The presence of surfactant and cosurfactant in the system makes the interfacial tension very low. Therefore, microemulsion forms spontaneously, with an average droplet diameter of 10–200 nm or less. Analysis of drug was carried out using ultraviolet (UV) spectrophotometer at 236 nm.

Selection of oil phase

Solubility of TMS in various oils (Olive oil, soyabean oil, cotton seed oil and Oleic acid) was determined by dissolving an excess amount of TMS in 2 ml of each of the selected oils in 5-ml capacity stoppard vials separately and mixed using a vortex mixer. The mixture vials were then kept at 37 ± 10°C in an isothermal shaker for 72 h to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 min. The ssupernatant was taken and filtered through a 0.45 –µm membrane filter. The concentration of TMS was determined by UV spectrophotometer at λmax 236 nm with suitable dilution with phosphate buffered saline (PBS) pH 6.5. Appropriately diluted solutions of oils in PBS pH 6.5 were taken as blank. The solubility of TMS was determined.[5]

Selection of surfactant

The surfactant chosen must have lower interfacial tension and provide a flexible film that can deform round small droplets. The surfactant was selected on the basis of solubility. The phase diagram was developed by aqueous titration method.

Selection of co-surfactant

The cosurfactant plays an important role in reducing the interfacial film. The cosurfactant was selected on the basis of solubility. The phase diagram was developed by aqueous titration method. From the solubility data, Oleic acid (HLB Value 1.0) showed higher solubilizing capacity compare to other vehicles and hence was selected as oil phase. Tween-80 (HLB value 15) was selected as a surfactant due to its higher solubilizing capacity, biocompatibility, and ethanol was selected as cosurfactant due to its higher solubility, ability to form clear, and stable microemulsion formulation.

Construction of pseudo-ternary phase diagrams

Ternary phase diagram of microemulsion was prepared by Sigma plot version 11.0 software to obtained the microemulsion zone in which at any point, microemulsion can be prepared. To find out the concentration range of components for the existing range of microemulsions, pseudoternary phase diagrams containing different ratios of oil and surfactant: Cosurfactant was constructed using water titration method at ambient temperature (25°C). The appropriate microemulsion components were selected, ternary pseudo phase diagram was constructed to define the extent and nature of the microemulsion regions. To produce such diagrams, a large number of samples of different composition must be prepared. The microemulsion region is initially delineated by its isotropic nature and low viscosity.

Two dimensional ternary phase diagram was prepared using a constant ratio of surfactant to cosurfactant. Oleic acid (HLB value 1.0) was screened as the oil phase. Tween 80 (HLB value 15.0) was selected as surfactant and ethanol was selected as cosurfactant. Distilled water was used as an aqueous phase. Three phase diagrams were prepared with the 1:1, 2:1, and 3:1 weight ratios of Tween 80 to ethanol, respectively. For

each phase diagram at a specific surfactant/cosurfactant weight ratio, the ratios of oil to the mixture of surfactant and cosurfactant were varied as 9:1, 8:2 7:3, 6:4, 5:5, 4:6, 2:8, and 1:9. Water was added drop-wise to each oily mixture with continuous stirring at 37°C until the mixture became clear at a certain point. The end point of the titration was the point where the solution becomes cloudy or turbid. The quantity of the aqueous phase required to make the mixture turbid was noted. The percentages of the different incorporated pseudo phases were then calculated. Preliminary trial batches of microemulsion presented in Table 1 (Oil phase 10–90% in each batch).

The results of preliminary batches suggest that batch code A, B, and C group giving clear solution after titration with water and remain clear on dilution with water indicates formation of clear microemulsion hence selected for further evaluation study. Phase diagrams were prepared after calculating the percentage of each phase required forming microemulsion and then the medicated microemulsion were formulated. The pseudo-ternary phase diagrams with various weight ratios of Tween 80: ethanol are presented in Tables 2 (oleic acid as oil phase) and Figures 1 and 2.

Evaluation of microemulsion[6,7]

Optical transparency

Microemulsion was diluted for 50–100 times. Optical clarity was assessed visually as well as spectrophotometrically by measuring percentage of light transmitted at a wavelength of 400–800 nm by UV/Visible spectrophotometer.

Table 1: Preliminary batches of microemulsion

S. no.	Batch code	Surfactant: Cosurfactant ratio (Tween 80: ethanol)	Observation
1.	A	1:1	Clear
2.	B	2:1	Clear
3.	C	3:1	Clear

Table 2: Formulation of microemulsion (A) tween 80: Ethanol (1:1)

A (1:1)	S mix: Oil	Water (ml)	Oil %	S mix %	Water %
A1	9: 1	5	6.66	60	33.33
A2	8:2	4	14.28	47.14	38.57
A3	7:3	2	21.42	58.33	14.28
A4	6:4	0.3	38.83	58.25	2.91
A5	5:5	0.2	49.01	49.01	1.96
A6*	4:6	0.3	58.25	38.83	2.91
A7	3:7	0.1	69.30	29.12	0.99
A8	2:8	0.07	79.44	19.86	0.69
A9	1:9	0.04	86.53	9.61	0.398

*S mix: Mixture of surfactant and co-surfactant

pH Determination

The pH of the microemulsion was determined using digital pH meter (Equiptronics, 11E, India) to confirm that developed microemulsion is in accordance with that of human skin rendering them more acceptable. The excipients used in the formulation decide the pH final preparation and rout of administration. The microemulsion formulations had appropriate observed pH values varying from 5.3 to 6.5 for topical application desired pH for skin.

Drug content determination

1 ml of microemulsion formulations was transferred into a beaker containing 10 ml ethanol. The content of the beaker was stirred for 30 min and then kept for 24 h. After 24 h, the content of beaker was transferred into centrifuge tube and centrifuged at the 3000 rpm for 10 min. Supernatant was separated and filtered. Then, 0.1 ml of the supernatant was diluted appropriately with PBS pH 6.5 and assayed spectrophotometrically for drug content [Table 3].

$$Drug\ content\ (\%) = \frac{Actual\ amount\ of\ drug}{Theoretical\ amount\ of\ drug} \times 100$$

Viscosity measurements

The rheological properties play an important role in stability. Change in the rheological characteristics helps in determining the microemulsion region and its separation from another region. Rheological characterization is important to measure and control

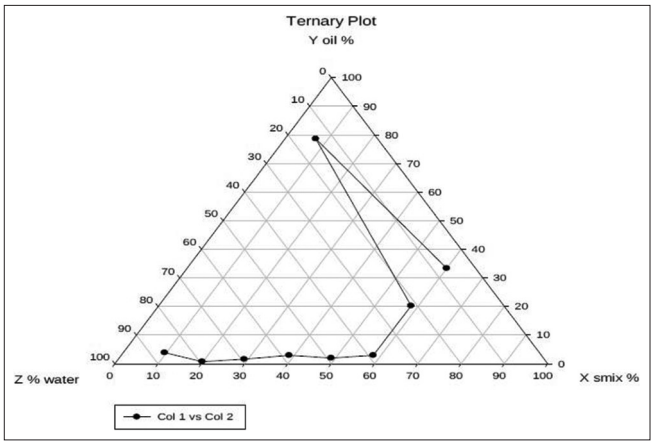

Figure 1: Ternary phase diagram of surfactant (Tween 80)/ Cosurfactant (ethanol) 1:1 Ratio and Oil (oleic acid)

Table 3: Characterization of microemulsion (A6*)

S. No.	Parameters	Results
1.	Visual observation	Clear
2.	pH	5.3–6.5±0.58
3.	Drug content	96.45±0.036
4.	Viscosity (cps)	2042±0.58cps

the flow properties to ensure product quality and effectiveness of the production. It helps in selection of dermatological formulation that will progress to clinical efficacy.

It can be determined by brookfield digital viscometer (DV-E model). The sample holder taken for the viscosity measurement was filled with the samples and then inserted into a flow jacket mounted on the viscometer. The samples adaptor (spindle no. 2) rotated at an optimum speed was used to measure the viscosity of the preparation. The measurement was made over the whole range of speed settings from 10 rpm to 100 rpm with 30 s. The formulation had the lowest viscosity value (2042 ± 0.54 cps) among the microemulsion formulations studied indicate high stable of microemulsion [Table 3].

Particle size and size distribution

Particle size and size distribution measurement of microemulsion were carried out by dynamic light scattering using (Zetasizer 3000, Malvern Instruments, Malvern, UK) particle size analyzer. Samples were placed in square glass cuvettes and droplet size analysis was carried out at temperature 25°C. The droplet size was found of microemulsion range that is 90.25 nm [Figure 3] and influenced by the concentration of the formulation. The effect of Smix (mixture of surfactant and cosurfactant) concentration on droplet size distribution of microemulsion was investigated. Table 4 is showing result of particle size distribution (nm) s intensity (%) of formulation. and graphical representation is shown in Figure 4.[8]

Zeta potential of the microemulsion

The zeta potential is an indication of the stability of the colloidal systems and indicates charge present on the colloidal systems. Highly positive or highly negative surface charge on oil globules indicates higher stability because of the anticipated surface repulsion between similar charged globules hence inhibiting aggregation of the colloidal oil globules. Zeta potential of formulations was determined using Malvern Zetasizer. Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment. The measurement was based on electrophoretic mobility (μm/s) of the particles, which was converted to the zeta potential by in built software of mastersizer 2000. The results of particle size and zeta potential measurement of the formulations are shown in Table 4.

Aggregation of colloidal oil globules is inhibited by negative surface charge on oil globules indicating higher degree of stability. The microemulsion having zeta potential –29.8 mV exhibits highly negative surface charge.[9]

Table 4: Particle size and zeta potential of microemulsion

S. No.	Parameters	Results
1.	Particle size	297 nm
2.	Zeta potential	–29.8 (mV)

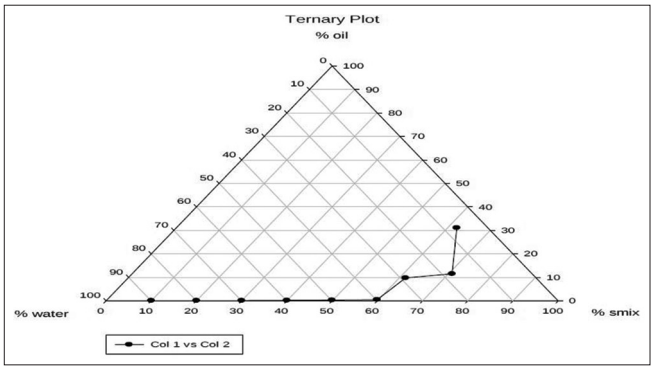

Figure 2: Ternary phase diagram of surfactant (Tween 80)/ Cosurfactant (Ethanol) 3:1 ratio and oil (oleic acid)

Transmission electronic microscopy (TEM)

TEM was the most important technique for the study of microstructures of microemulsions because it directly produces images at high resolution and it can capture any coexistent structure and microstructural transitions. TEM

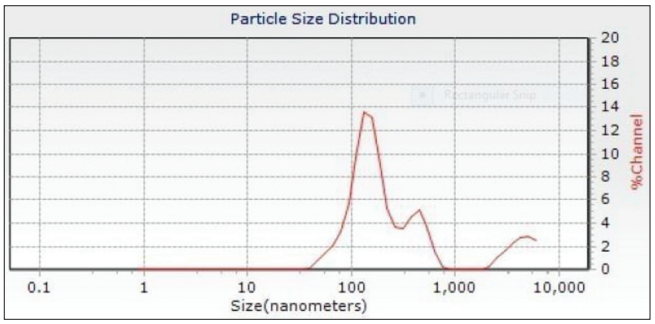

Figure 3: Droplet size (nm) versus intensity (%) of prepared microemulsion

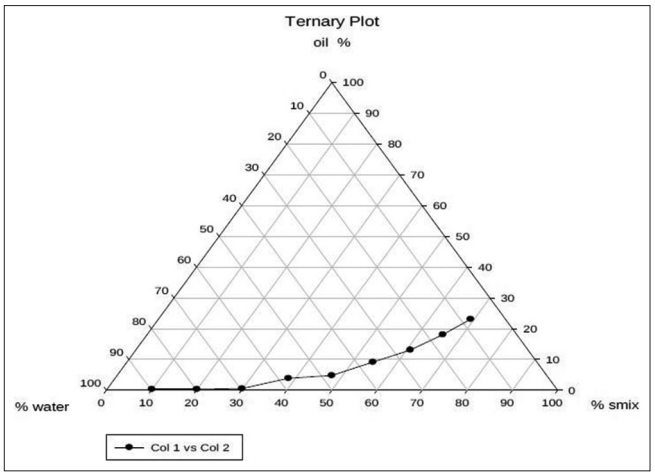

Figure 4: Ternary phase diagram of surfactant (Tween 80)/ Cosurfactant (Ethanol) 2:1Ratio and Oil (oleic acid)

determination is one of the studies conducted to confirm the particle size obtained by the laser scattering spectroscopy. In the TEM image, the microemulsion appeared dark and the surroundings were bright. Transmission electron microscopic image of TMS microemulsion shows the size of oil droplets. The micrograph exhibits, the droplets size of the sample was in the range of microemulsion as shown in Figure 5.

Ex-vivo permeability study

Skin permeation study was performed using Franz diffusion cell with an effective diffusion area of 2.899 cm^2. The experiment was carried out using cellulose membrane and. A circular piece of egg membrane about 3 cm diameters was sandwiched between donor and receptor compartments. In the donor compartment, the formulation was placed and the receptor compartment was filled with phosphate buffer pH 6.8, kept at constant temperature of 37 ± 0.5°C and stirred. At appropriate intervals (1, 2, 3, 4, 5, 6, 7, and 8 h), 3 ml aliquots were withdrawn and replaced with an equal volume of fresh receptor solution. Samples were analyzed by UV Spectrophotometer (Shimadzu-1800, Japan) at 236 nm using phosphate buffer (pH 6.8) as blank.

In vitro drug release study of tms loaded microemulsion

In vitro release of TMS-loaded microemulsions was done in dialysis method. The diffusion medium was taken as 200 ml of PBS pH 6.5. One end of the test tube was covered by egg membrane and other end of test tube was attached with burette stand. Moreover, that arrangement is fitted in such a manner that egg membrane directly touches to beaker that contained phosphate buffer saline (pH 6.5). Beaker that contains 200 ml PBS pH 6.5 solutions was placed on magnetic stirrer and stirred at 120 RPM at 37°C ± 0.5°C. The microemulsion formulation was kept in that test tube covered by egg membrane. The test tube was kept in the diffusion medium so as the formulation directly in contact with the phosphate buffer saline (pH 6.5). The drug samples were withdrawn at 1 h time interval from diffusion medium and maintain sink condition. Analyzed sample by a Shimadzu 1800 double beam UV visible spectrophotometer at 236 nm using phosphate buffer saline (pH 6.5)[10] and cumulative % drug release of TMS-loaded microemulsion graphical representation is shown in Figure 6.

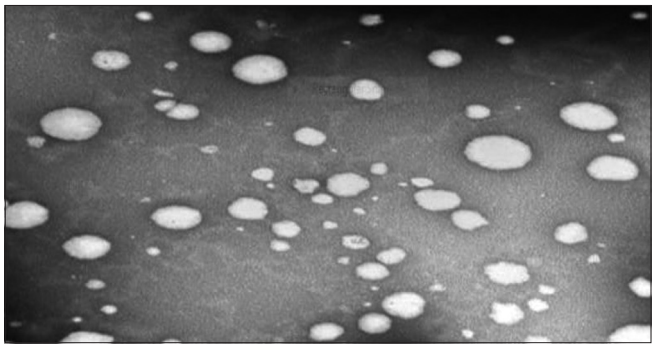

Figure 5: Transmission electronic microscopy image of trolamine salicylate microemulsion (×8000)

RESULTS AND DISCUSSION

Microemulsion was prepared by water titration method using oleic acid as oil phase, tween-80 as surfactant and ethanol as cosurfactant different oils, surfactants, and cosurfactants were screened to select ideal components of microemulsions with good solubility and excellent skin penetration of TMS. The solubility of TMS was highest in oleic acid followed by cotton seed oil, olive oil, and Soy bean oil.

The use of oleic acid is advantageous because it increases skin permeability by two mechanistic scenarios of the enhancer; (a) lipid fluidization, and (b) lipid phase separation, oleic acid is a model skin permeation enhancer, oleic acid facilitates penetration into the skin by disrupting the fluidity of the SC. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin.

Non-ionic surfactant was selected because they are generally less toxic, produce less skin irritation. The HLB value of the O/W type of microemulsion was (9–12). HLB value of Tween-80 (15), the required HLB for O/W type of emulsion for oleic acid is 17. The hydrophilic non-ionic surfactant, Tween-80, was chosen to formulate these microemulsion systems to provide a better permeation profile. The different cosurfactants such as PEG 400, ethanol, and propylene glycol were used.

In microemulsion, the cosurfactant lowers the interfacial tension of the surfactant film, resulting in a more flexibility and dynamic layer system. The thermodynamic driving force for the release reflects shows the relative activities of the drug in different phase. Since drug can be release from the internal phase to external phase and then from external phase to the skin, the relative activities may monitor the skin permeation flux.

In addition, the surfactant and cosurfactant may exist in each phase. Hence, TMS can partly solubilize in external phase. The depletion of TMS may be from the external phase because of the permeation in to the skin can be supplemented by the

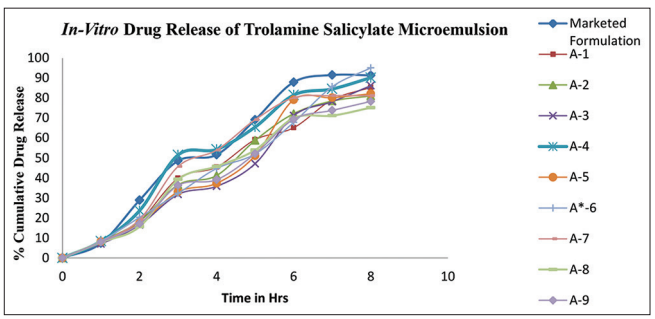

Figure 6: Cumulative % drug release of trolamine salicylate loaded microemulsion and marketed formulation

release of TMS from the internal phase. However, the oily mixtures of oleic acid, tween-80, and ethanol led to increase in drug solubility. After extensive screening for physical characteristics and appearance, final ratios of surfactants and cosurfactants were decided. The data of selection of surfactants and cosurfactants are given in Table 1.

The microemulsion A6* was subjected to the study of Optical transparency and found to be optically clear, transparent, and elegant in appearance when compared to the other microemulsion formulations. The pH values of A6* were (5.3–6.5 ± 0.58). Microemulsion was found to be suitable for topical as well as transdermal application because the pH of skin in the range 5.5–6.5. The microemulsion formulations were appropriate observed pH values, drug content, and viscosity and as shown in Table 3.

The morphology of A6* formulation was characterized using Transmission Electron Microscopy [Figure 5]. The TEM image of optimized best formulation showed that globules were spherical in shape and had smooth surface. The results TEM further indicated the existence of an isotropic dispersion of spherical droplets, leading to the assumption of inverse micelles because of the proportion of the constituents.

The results of particle size and zeta potential measurement of the formulation were evaluated. The particle size (nm) of the optimized formulation was found to be in the range of 297 nm shown in Table 4. Particle size is very small, and indicates that there is a chance that the number of vesicles that can interact with a fixed area of SC to increase, thereby increasing the efficiency in percutaneous uptake. The microemulsion having zeta potential –29.8 mV exhibits highly negative surface charge which indicates stability of the colloidal systems and charge present on the colloidal systems.

During *ex-vivo* permeability study, the flux values of TMS Microemulsion (A-6*) and marketed formulation were found to be 6.518 and 4.651 µg/cm^2 h, respectively. The permeability coefficient of TMS microemulsion (A-6*) and marketed formulation were found to be 3.259 and 2.491 cm.h-1. *In-vitro* release of TMS-loaded microemulsions was done in dialysis method. The amount of TMS permeated through egg membrane over 8 h period was plotted against the function of time [Figure 6], the cumulative % drug release for various TMS microemulsion formulations through the egg membrane was determined. TMS microemulsion formulation (A-6*) was showed that the highest the cumulative % drug release (95.048 ± 0.042% in 8 h) is given in Figure 6.

The microemulsion provides high perfection than marketed formulation. It was found that when the drug solubility in microemulsion formulation increased, the uptake through the cellulose membrane and cumulative amount of drug release also increases. It also shows from TEM that smaller the particle size bigger the surface area and hence better the drug release. Oleic acid has permeation enhancing capabilities and has significant influence on penetration of TMS. Ethanol also disrupts membrane lipids and so it improves the permeation of polar drugs.

CONCLUSION

In this study, new o/w microemulsion system containing TMS was studied for topical application. Different microemulsion formulations were selected using three ternary phase diagrams. Microemulsions could increase topical delivery of TMS by 95.048 ± 0.042%. The best formulation A6* consists of 4:6 (surfactant: co-surfactant) ratio. The result of TMS A6* formulation suggests that microemulsion was a promising vehicle for topical application of polar drugs having poor transdermal transport. Hence, microemulsion formulation containing TMS could be promising formulation as an alternative dosage form for effective pain management and anti-inflammatory therapy.

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