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Development of Micro-Emulsion Gel Based Topical Delivery of Salicylic Acid and Neem Oil for the Management of Psoriasis

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ABSTRACT

Microemulsions (MEs) are clear, thermodynamically stable systems. They were used to solubilise drugs and to improve topical drug availability. Salicylic acid (SA) is a keratolytic agent used in topical products with antimicrobial actions. This study aimed to formulate an optimized SA micro emulsion gel for the slow, variable and incomplete oral drug absorption in patient suffering from psoriasis infection. The dispersion solubility of SA was studied in various oils, surfactants and co-surfactants and by constructing pseudo phase ternary diagram, micro emulsion area was identified. The optimized formulations of micro emulsion were subjected to thermodynamic stability tests. After stability study, stable formulation was characterized for droplet size, pH determination, centrifugation, % drug content in micro emulsion, zeta Potential and vesicle size measurement and then micro emulsion gel were prepared and characterized for spreadability, measurement of viscosity, drug content, *In-vitro* diffusion, *in-vitro* release data. Labrasol was selected as surfactant, plurol oleique as co surfactant and neem oil as oil component based on solubility study. The optimized formulation contained SA 0.05 (%w/w), labrasol (24%), plurol oleique (8 %) and neem oil (8%). The *in vitro* drug release from SA micro emulsion gel was found to be considerably higher in comparison to that of the pure drug. The *in-vitro* diffusion of micro emulsion gel was significantly good. Based on this study, it can be concluded the solubility and permeability of SA can be increased by formulating into micro emulsion gel.

Keywords: Salicylic Acid, Neem Oil, Micro-emulsion, *In-vitro* diffusion, Zeta potential, Stability, Labrasol

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INTRODUCTION

Psoriasis is a common, chronic, non-contagious, auto-immune disease that primarily affects the skin and seen in about 2-3% of population world-wide[1]. The word psora comes from Greek word which means to itch. Psoriasis, a term which has been in use since 133 AD, was originally grouped with leprosy until the 19th century. It has been suggested that biblical leprosy was in fact, the disorder known today as psoriasis[2]. It is mostly an inheritant disease, characterized by scaly, red and itchy plaques. The most commonly affected areas are the entire scalp and can also spread to the forehead, back of neck or behind ears, chest, arms, elbows, in the armpits, under the breasts, around the genitals, knees, legs, toenails and fingernails[3]. It affects males and females equally and also affects children, adult, older peoples and may occur at any age of life. It is more common in people between the ages of 15 and 35, According to National Psoriasis Foundation. Psoriasis is partly due to genetic and partly due to environmental factors[4]. Psoriasis can be categorized as mild, moderate and severe. Mild psoriasis leads to formation of rashes and when it becomes moderate the skin turns scaly. In severe conditions, the red patches may be present on skin surface and become itchy. This affects a person's professional and social life. The normal mechanism of body is to form new skin cells every month to replace the skin which is shed off. But, in psoriasis the new skin cells grow rapidly within days rather than weeks. This leads to accumulation of dead skin on the skin surface resulting in thick patches of red, dry and itchy skin[5]. Microemulsions (MEs) are clear, thermodynamically stable, optically isotropic systems. They are formed spontaneously upon mixing a suitable oil, water, and an amphiphile blend (surfactants either alone or in combination with a cosurfactant [6-9]. MEs offer advantages over traditional creams and lotions as topical drug delivery. They were used to solubilize drugs and to improve topical drug availability[10]. They are able to increase the rate and depth of moisturizing agents into skin. It has been suggested that MEs may dissolve the ordered structure of the stratum corneum lipids, leading to the loss of barrier properties of the skin[11]. Salicylic acid (SA) is a white fluffy crystalline powder, slightly soluble in

water (1 in 460 ml). It is used as keratolytic agent in topical products, and it has antimicrobial and fungicidal actions[12-14]. Antibacterial activity of SA was recorded by *Park et al*[15]and *Kupferwasser et al*[16] as adjuvant therapeutic agent in treatment of Staphylococcus aureus infections. SA is used topically in hyperkeratotic and scaling conditions to enhance the rate of loss of surface scales[17]. The side effects of SA include sensitivity, excessive drying, and irritation. Traditional formulations of SA in ointment bases have disadvantages of being greasy and irritant due to free crystals of the drug. In this study, SA was incorporated with different concentrations in an ME gel base. Characterization of the prepared systems was carried out by applying different evaluation tests to examine the effect of incorporation of the drug on the ME gel base.

MATERIALS AND METHODS

Salicylic acid was obtained as a gift sample from Macleods pharmaceuticals, Mumbai. Labrasol, Plurol Oleique was obtained from Gattefosse India Pvt. Ltd., Mumbai, India. Neem oil from Gogia Chemicals, Noida, India, All other surfactant and co surfactant were purchased from Hi Media and S. D. Fine Chem. Ltd., Mumbai. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Solubility studies

Solubility determination in the various oils, surfactants and co-surfactants for formulating micro emulsion drug delivery system. The solubility of the drug in different oils is an essential step for the micro emulsion formulation. So before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of micro emulsion drug delivery system. Drug powder of SA was added in excess to each of the oils, surfactants (S), cosurfactants (CoS) and then vortexed for mixing. After vortexing the samples were kept for 72 hours at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 5000 x g for 30 minutes to remove the undissolved drug[18, 19].

Pseudo-ternary phase diagram

In order to find out the concentration range of components for the existing range of microemulsion (ME), pseudo-ternary phase diagrams were constructed using water titration method (1,5) at ambient temperature. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of mixture of Labrasol® (LAB) and Neem Oil (PO), respectively. For each phase diagram at a specific S/CoS mixing ratio (Km), the ratios of oil to the mixture of S/CoS (Smix) were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Water was added drop by drop, under gentle agitation, to each oily mixture. Usually, after the clear mixture became turbid at a certain point (beginning of phase inversion area), the turbid mixture turned to be clear (beginning of O/W ME area) and, then, finally turbid once again (end of O/W ME area) with the continuous addition of water. The experiment was repeated for each S/CoS (1, 2 and 3). Based on these results, appropriate concentrations of oil, surfactant and cosurfactant were selected and used in the preparation of microemulsions (MEs) containing SA. During the titration, samples were stirred vigorously for a sufficient length of time for homogenization and the end product was visually monitored against a dark background by illuminating the samples with white light After being equilibrated at ambient temperature for 24 hours, the mixtures were assessed visually and determined as being ME, crude emulsions or ME gels. The stable MEs were also observed under polarizing light to conform their isotropic nature. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90°. Formation spontaneity of ME was evaluated by the addition of known amount of water at once to the known amount of oil, surfactant and secondary surfactant with controlled stirring. The ease of formation of clear ME was taken as the criteria of spontaneity.

Preparation of microemulsions

For further studies, from the constructed pseudotemary phase diagram, Neem oil (NO)/LAB/PO mixture with oil/Smix 0.250 was selected. Furthermore, six potential ME vehicles different from each other by water volume fraction, were selected and prepared at Km 3:1 and oil/Smix 0.250. The calculated amount of drug (0.05%w/w of SA) was added to the oily phase of ME and magnetically stirred until dissolved followed by addition of Smix in a fixed proportion to produce clear mixture. Then a defined proportion of water was added and stirred to produce clear ME of SA. Both, unloaded and drug-loaded MEs were prepared 48 h before investigations (we can reasonably assume that the drug distribution among the oil, water and surfactant micelles, attains the thermodynamic equilibrium) and stored at room temperature[18, 20] Table 1.

Table 1 Compositions of the selected microemulsion formulations

Components	Microemulsion					
	ME1	ME2	ME3	ME4	ME5	ME6
Neem oil (NO)	18	16	14	12	10	08
Labrasol (LAB)	54	48	42	36	30	24
Plurol Oleique	18	16	14	12	10	08
Water	10	20	30	40	50	60

Characterization of microemulsions

The physico-chemical parameters of prepared MEs were determined as follow[21-24].

Particle size and zeta potential measurements

The average droplet size and polydispersity index (PDI) of ME was measured by photon correlation spectroscopy (PCS) with in-built Zetasizer (Nano ZS, Malvern Instruments, UK) at 633 nm. Helium - neon gas laser having intensity of 4mW was the light source. The droplet size was calculated using Stokes-Einstein relationship by Zetasizer Software. Electrophoretic mobility (pm/s) was measured using small volume disposable zeta cell and converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation. All determinations were made in triplicate.

Determination of drug content

For determination of drug content, about 1 g of the formulation was weighed in a 10-ml volumetric flask and

dissolved in methanol; it was diluted appropriately and analyzed by the UV spectroscopy method.

pH measurement

pH of the micro emulsion was measured using (Digital pH meter MK).The electrode was rinsed with deionized water and blot was dried with a soft, clean paper. Then the electrode was dipped into the test solution. Then pH was recorded when the reading was stable after insertion of the electrode into the solution.

Conductivity measurement

The electric conductivity of ME was measured with a conductivity meter (Equip-Tronics, EQ - 664, Mumbai, India) equipped with inbuilt magnetic stirrer. This was done by using a conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as a conductor. The electrode was calibrated before using it for sample determination using 0.1 N KC1 (7.456 gm of KC1 / 1000 ml distilled water) having conductivity 0015.39 mS/cm.

Percent transmittance measurement

The percent transmittance of the formulation was measured using a colorimeter (Digital Colorimeter, D-801, Photocon, India) at 570-590 nm.

Viscosity measurement

The viscosity of ME was measured using a Brookfield Viscometer LVDV IIIU (Brookfield Engineering LABS, Stoughton, USA) with spindle SC 18 at 100 rpm using interval of 30 seconds. The calibration of viscometer was verified using viscosity Fluid standard-General purpose silicone fluids - having viscosity 5000 cp. All aspects of testing were controlled using Rheocalc Software.

Stability studies

The stability of ME formulations was studied through various parameters such as clarity, phase separation observation, droplet size determinations, viscosity measurements, conductivity measurements, pH and zeta potential determinations for 6 months at room temperature. In order to estimate metastable systems, the selected ME vehicles were centrifuged (Remi Lab, Mumbai) at 3000 x g for 30 minutes. Beside the physicochemical properties, the chemical stability of the investigated drug in the vehicle plays a major role. Therefore the drug content was analysed at defined time intervals during the observation period of 6 months. During the observation period the formulations were stored at room temperature in tubes to simulate patient usage conditions[25, 26]. During stability studies the samples were taken at fixed time intervals.

Formulation of microemulsion based gel of salicylic acid

Various gelling agents namely hydroxypropyl methylcellulose (Methocel K4M) and non-benzene grade carbopol polymers (Carbopol 97IP NF, Carbopol 974P NF and Carbopol980P NF) were evaluated for their ability to gel optimized SA-MEs at different concentrations. The suitable gelling agent was selected on the basis of compatibility with MEs structure, feel and ease of spreadability. Carbopol 97IP NF was selected as the gel matrix to prepare MBG. Carbopol 97 IP NF was slowly mixed with water. The oily phase was obtained by mixing appropriate concentrations of oil, surfactant and co-surfactant. Carbopol97 IP NF was entirely swelled in the water and its pH was adjusted by adding 50%w/w triethanolamine (TEA). MBG was obtained by mixing the swelled gel in water with the oily phase Table 2.

Table 2 Composition of microemulsion based gel containing salicylic acid

Components	Content
Salicylic acid	0.05 (%w/w)
Carbopol 971P NF (% w/w)	2
Neem oil (% w/w)	8
Labrasol (% w/w)	24
Water (g)	Quantity sufficient to produce 100 g of gel

Characterization of microemulsion based gel of salicylic acid

Determination of pH

The pH of the 10% (w/w) gel was determined using digital pH meter (HI 98107, Hanna Instruments, India.), standardized using pH 4 and 7 buffers before use.

Determination of spreadability

The spreadability of the gel was determined using the following technique: 0:5 gm gel was placed within a circle of 1cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 gm was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted and the mean diameter was taken by repeating the experiment three times.

Determination of drug content

For determination of drug content, about 1 gm of the gel was weighed in a 100-ml volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed by UV. Drug content was calculated by linear regression analysis of the calibration curve.

In-vitro diffusion study

An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Nanoemulsion gel equivalent to 5mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer.

RESULT AND DISCUSSION

The preliminary study showed that SA is colorless acicular crystals, sweetish acrid taste and odorless powder. It is freely soluble in ethanol, methanol and 0.1 N HCl soluble in chloroform, slightly soluble in water. The partition coefficient of SA was found to be 1.95±0.20. From the FT-IR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak Fig. 1, 2. On the basis of above study it was concluded that the solubility in the oils, surfactants and co surfactants like Labrasol, Plurol Oleique, neem oil was found to be soluble for the microemulsion preparation of SA. The λ_{max} of the SA was found to be 251 nm on UV spectrophotometer (Shimadzu 1700, Japan), which complies with the official literature. The melting point was in the range of 157-159^{0}C which is in compliance with the standard value of 158.6 °C as per Indian Pharmacopoeia. Compatibility of the drug with excipients was determined by differential scanning calorimetry (DSC) analysis. This study was carried out to

detect any change on chemical constitution of the drug after combination with the excipients in the ration (1:1) Fig. 3, 4. In formulation of ME system which will be used as drug delivery 'system suitable and optimum oil, surfactant and co-surfactant need to be chosen. So the solubility of SA in various media was analyzed in order to screen components of ME. From these results, neem oil, caprylocaproyl macrogol glycerides (LAB) and polyglyceryl-6-dioleate (plurol oleique) were subsequently used as the oil phase, surfactant and co-surfactant for the formulation of MEs containing SA in this study. Different physicochemical properties of the selected oils were studied and were found to be favorable for microemulsion drug delivery system. The vesicle size analysis of the optimized formulation ME6 was done using particle size analyzer. The mean vesicle size was found to be 16.9±0.3 nm. Zeta potential of the optimized formulation ME6 was determined using particle size analyzer. Zeta potential of optimized formulation was found to be -36.99±2.03 mV. Drug content is most important in microemulsion formulation and the data found are satisfactory. It was found to be 98.56± 2.45to 99.98±1.87% which shows the good capacity of formulation to hold the drug Table 3, 4. The formulation ME6 having appropriate physicochemical parameters, higher permeation parameters and a good potential to improve photo stability of SA was considered as optimized formulation. The microemulsion based gel (MBG) of the optimized formulation was developed using a suitable polymer capable of modifying the rheological behavior. Four different carbopol gel base prepared for optimization (0.5%, 1.0%, 1.5% and 2%) and evaluated for pH, spreadability, viscosity measurements and *in vitro* drug release studies. The viscosity of MBG containing 0.5%, 1.0%, 1.5% and 2% w/w Carbopol 97IP NF were 0.7 $\times 10^3$ cp, 1.8 $\times 10^3$ cp, 3.l x 10^3 cp and 6.5 x 10^3 cp, respectively. MBG containing 0.5% Carbopol 97IP NF had a relatively high fluidity. However 2% w/w Carbopol 97IP NF resulted in most appropriate fluidity for topical administration. So 2% w/w Carbopol 97IP NF as the optimum gel matrix was added to ME6 in order to obtain a MBG containing SA. The SA content of the MBG was found to 98.97 ± 0.053% w/w of the theoretical value (0.05% w/w). Gel spreadability is animportant parameter. Application of the formulation on inflamed part would be more comfortable if the base spreads easily exhibiting maximum slip and drag. When a weighed quantity of gel was placed in between two glass plates of known weight, it spreaded uniformly to produce a circle, the diameter of which is related to its spreadability; the larger the diameter, the better the spreadability. The data in table 5 indicate that the diameter of MBG was 7.2 ± 0.01 cm. While the diameter of the marketed gel was found to be 5.8 ± 0.02 cm indicating that the spreadability of MBG is better than that of conventional gel. This is because of the loose gel matrix nature of MBG formulation due to the presence of oil globules rather than the conventional gel matrix. *In vitro* drug release study of optimized formulation was carried out using modified franz diffusion cell. The optimized formulationME6 showed the maximum 67.3% drug release in 48 hrs Table 6.

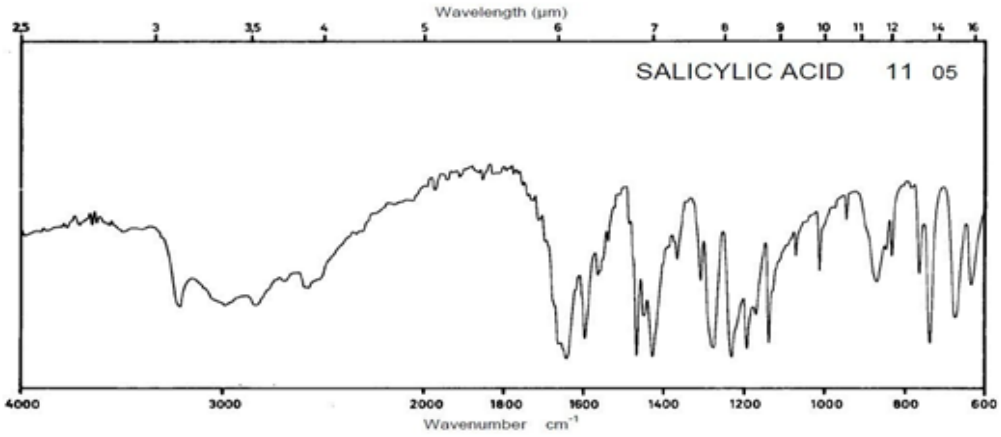

Fig. 1 Infrared spectrum of drug sample

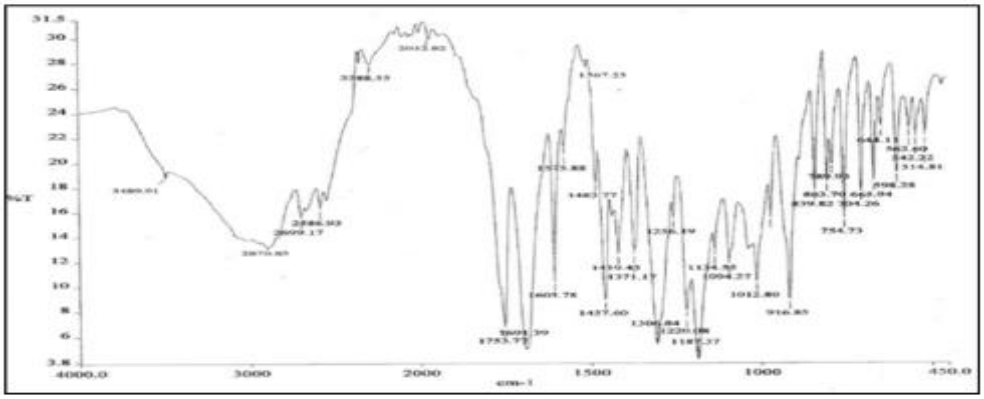

Fig. 2 Infrared spectrum of drug sample and excipients

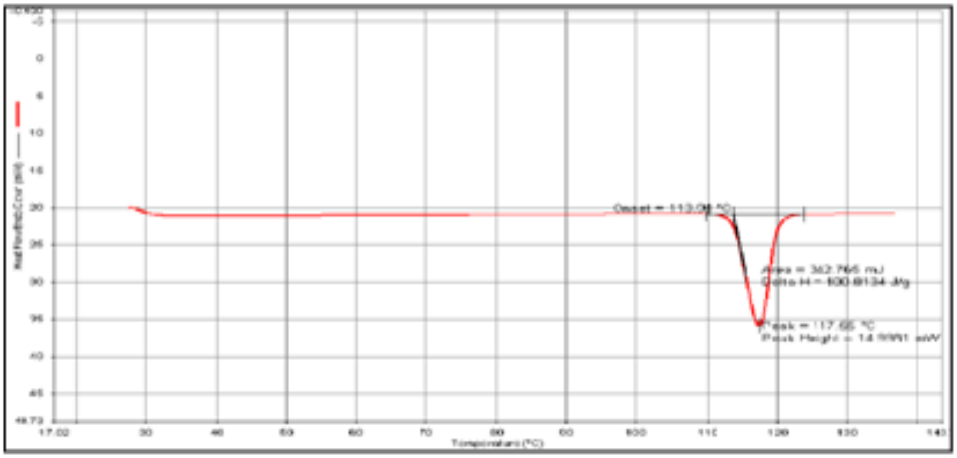

Fig. 3 DSC Thermo gram of drug sample

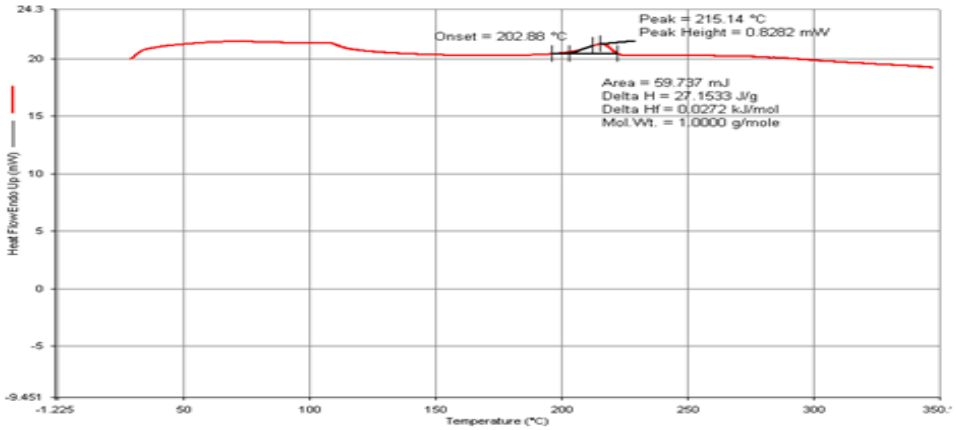

Fig. 4 DSC Thermo gram of drug sample and excipients

Table 3 Physicochemical parameters of salicylic acid loaded microemulsion

F. Code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Drug content (%w/w)	Transmittance (%)
ME1	72.3±0.4	0.241±0.03	-24.56±2.35	99.12±2.28	98.00±0.63
ME2	68.5±0.3	0.138±0.05	-29.69±1.75	99.28±2.20	98.17±0.35
ME3	62.2±0.4	0.243±0.02	-34.42±2.04	99.78±1.67	98.67±0.52
ME4	44.5±0.7	0.267±0.01	-31.15±2.33	98.56± 2.45	98.35±0.58
ME5	28.3±0.5	0.186±0.02	-39.42±2.31	99.46±1.85	98.83±0.41
ME6	16.9±0.3	0.122±0.01	-36.99±2.03	99.98±1.87	99.14±0.52

Table 4 Physicochemical parameters of salicylic acid loaded microemulsion

F. Code	pH	Conductivity (pS/cm)	Viscosity (cp)
ME1	6.38±0.08	2.9±0.1	125± 0.09
ME2	6.55±0.07	13.9±0.8	116±0.02
ME3	6.65±0.03	31.5±0.6	96±0.08
ME4	6.70±0.01	67.8±0.5	77±0.01
ME5	6.41±0.04	128.5±0.3	82±0.02
ME6	6.89±0.02	205.7±0.5	40±0.05

Table 5 Physicochemical characterization of microemulsion based gel (mean SEM, n=3)

Parameters	MBG
Drug content (% w/w)	98.97 ± 0.053
Spreadability (cm)	7.2 ±0.01
pH	6.2 ±0.03
Viscosity (cp)	6.5 x 10^3± 0.2x 10^3

Table 6 In-vitro drug diffusion of microemulsion

Time (Hr)	In-vitro drug diffusion	
	Pure Drug	ME6
1	41.63	19.3
2	46.27	22.1
4	48.83	26.4
6	51.85	31.9
8	57.14	45.7
10	65.75	49.8
12	79.77	55.6
18	86.75	59.3
24	99.12	62.6
48		67.3

CONCLUSIONS

This study demonstrates that a microemulsion formulation can be used to improve solubility, bioavailability of SA and overcome the difficulties associated with its use in the clinic. On analysing saturation solubility study result and pseudo ternary phase diagram, the oil, surfactant and co surfactant were selected. All the prepared formulations exhibited the microemulsion properties. The optimized formulation was evaluated for zeta potential, globule size analysis and stability study. The results suggest that the optimized formulation was stable and produced microemulsion. The in-vitro diffusion study of the formulation was higher as compared to pure drug, indicating that the prepared formulation is having higher solubility and permeability. Thus it can be concluded that microemulsion formulation can be used as a one of the formulation technique to enhance the bioavailability of the poorly soluble and permeable drugs. The present work aimed at developing a successful new topical formulation for the delivery of SA was able to increase the efficacy of the currently available commercial products for the topical treatment of psoriasis. In this study, we got success in the development and evaluation of microemulsion. Based on higher drug release and lower surfactants concentration, higher solubility as well as higher bioavailability without variable absorption has been optimized as microemulsion formulation of SA as oil, surfactants and co-surfactants. The above study leaves a future scope for refining technology which can further be used for preparation of various other micro/nano systems in pharmaceutical products.

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