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Der Pharmacia Sinica, 2015, 6(2): 69-79

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Enhanced permeability of Cyclosporine from a transdermally applied nanoemulgel

Mahesh Begur[1], Vasanth Kumar Pai[1], D. V. Gowda[2], Atul Srivastava*[2], H. V. Raghundan[2], Chetan G. Shinde[2] and Manusri N. [2]

[1]*Dept. of Industrial Chemistry, Kuvempu University, Shankaraghatta, BR project, Shiomoga, Karnataka, India*
[2]*Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagara, Mysore, India*

ABSTRACT

The objective of this study was to investigate the potential of nanoemulgel for transdermal delivery of Cyclosporine. Different nanoemulsion components (oil, surfactant, and cosurfactant) were selected on the basis of solubility and emulsification ability. Pseudoternary phase diagrams were constructed using titration method to figure out the concentration range of components. Guar gum was added as gel matrix to convert nanoemulsion into nanoemulgel. Drug loaded nanoemulsions and nanoemulgels were characterized for particle size, transmission electron microscopy, viscosity, pH, rheology, spreadability, drug content, *in vitro* skin permeation using rat abdominal skin and stability studies. Nanoemulgel containing 20% oleic acid as oil, 65% Tween 80, and Transcutol P as surfactant cosurfactant mixture, 15% water, 2% drug, and 0.5% Guar gum was concluded as optimized formulation (BF5). The drug content of the optimized formulation was found to be 99.5 %. The flux value through rat skin was found to be 0.078 mg/cm^2/h. The ex vivo permeation profile of optimized formulation was compared with nanoemulsion and marketed formulation. Nanoemulgel showed significantly higher ($P < 0.05$) cumulative amount of drug permeated and flux along with lower lag time and skin retention than marketed formulation. Thus, the study substantiated that nanoemulgel formulation can be used as a feasible alternative to conventional formulations of cyclosporine with advanced permeation characteristics for transdermal application.

Keywords: Nanoemulsion; Emul-gel; Ternary Phase Diagram; Transdermal potential; Prolonged action

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INTRODUCTION

The potent immunosuppressive agent, Cyclosporine (CSA) is a neutral, lipophilic cyclic undecapeptide (M.W. 1203 Daltons), with very low water solubility [1]. CSA has been recommended for treatment of various kinds of immune related disorders of the skin such as psoriasis, atopic dermatitis and alopecia areata among others. However, long-term systemic administration of CSA causes serious adverse effects including renal dysfunction, chronic nephrotoxicity and hypertension. The overwhelming systemic toxicity concerns have limited the use of CSA in the clinic [2].

Previous attempts by other investigators to deliver CSA topically have met with only limited success. The poor topical delivery of CSA could be due to a number of factors, including the high molecular weight of CSA and the lack of a balanced partition coefficient (log P ocatnol /water =2.92). These physicochemical properties make CSA a very difficult and challenging drug model for transdermal delivery [3, 4].

It is therefore desirable to develop a novel transdermal vehicle system that does not necessitate the use of chemical enhancers to facilitate drug permeation through the skin. One of the most promising techniques for enhancement of

transdermal permeation of drug is the nanoemulsion. In prior studies, nanoemulsion as carrier system has been exploited for transdermal delivery of various drugs [5, 6].

Nanoemulsions are thermodynamically stable, transparent, or translucent dispersion of two immiscible liquids, such as oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having the droplet size of less than 100 nm [7].

Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties in vitro, as well as in vivo. Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions and gels. However, the low viscosity of nanoemulsion constrains its application in transdermal delivery due to cumbersome use [8].

In the past decade, scientists and industrial researchers have taken increasing interests in the field of pharmaceutical semisolid dosage forms especially nanoemulgels, primarily due to their homogenous behavior and jelly-like consistency. Nanoemulgel, which also known as the formation of nanoemulsion based hydrogel is the addition of nanoemulsion system into hydrogel matrix. Usually, hydrogel encounter a limitation of unable to transport lipophilic drugs. Therefore, solubilization of lipophillic drug into the oily phase of emulsion which later added into gel base is necessary to enhance limitation of hydrogel besides promoting better stability and drug release [9].

In the present work an attempt was made to enhance the permeation of CSA through the skin by incorporating the drug into an oil phase (drug carrier) and dispersing this oil phase as nano sized globules into aqueous gel phase using homogenizers for effective delivery of drug through the skin.

MATERIALS AND METHODS

2.1 Materials

Cyclosporine was kindly supplied by PerkinElmer (Waltham, MA, USA). Oleic acid, glycerol triacetate (Triacetin), olive oil and diethylene glycol monoethyl ether (Transcutol P), were purchased from E-Merck (Darmstadt, Germany). Polyoxy-35-castor oil (Cremophor EL) Tween 20, Span20 and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used in the study were of analytical reagent grade.

2.2 Methods

2.2.1 Solubility studies of Cyclosporine in different components

The important parameter for screening of components is the solubility of Cyclosporine (CSA) in oils, surfactants and cosurfactants. The solubility of CSA in different oils, surfactants, and cosurfactants was determined by taking excess amount of CSA in 2 mL of each of the selected oils (Triacetin, oleic acid and olive oil), surfactants (Tween 20, Tween 80, Span 20 and cremophore EL), and cosurfactants (Transcutol P, PEG 200, PEG 400 and propylene glycol) in 5 mL capacity stoppered vials. The vials were continuously stirred in an isothermal shaker for 48 hours at 37 ± 0.5^0C to attain equilibrium. The equilibrated samples were centrifuged at 5000 rpm for 10 min. The supernatant was filtered through 0.45µm membrane filter. The concentration of CSA in each oils, surfactants, and cosurfactants was analyzed using High Performance Liquid chromatography.

2.2.2 Pseudoternary Phase Diagram Study

The phase diagram was developed using aqueous phase titration method [10]. Oleic acid was used as the oil phase, Tween 80 and Transcutol P was selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactants and cosurfactants [S_{mix}] were mixed in different weight ratios (1:0, 1:1 and 2:1) to determine the optimum ratio which can result in maximum nanoemulsion area. For each phase diagram, oil and specific S_{mix} were mixed well in different ratios from 1:9 to 9:1 in different vials. The ratio of oil to surfactant varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The mixtures were titrated with the aqueous phase, and visual observations were made for transparent and easily flowable oil-in-water (o/w) nanoemulsions. The physical state of the true nanoemulsion was marked on a pseudoternary phase diagrams with one axis representing the aqueous phase, and the other representing a mixture of surfactant and cosurfactant at fixed weight ratios (S_{mix} ratios).

2.2.3 Thermodynamic stability tests

Selected formulations were centrifuged at 3000 rpm for 25 min. Formulations which did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperature (4°C) and 45°C, with storage at each temperature for not less than 48 h, were undertaken. The formulations, which were found to be stable at these temperatures, were subjected to freeze-thaw cycle test. Three freeze-thaw cycles were carried out for the formulation between -21°C and 25°C. The formulations that survived thermodynamic stability tests were carried out for characterization.

2.2.4 Formulation of Cyclosporine loaded nanoemulsion

From each pseudoternary phase diagram constructed, different formulas were selected from the nanoemulsion region. The preparation of CSA-loaded nanoemulsion (2% w/w) was performed by dissolving CSA to mixtures of oil, surfactant cosurfactant mixture with varying ratio. An appropriate amount of aqueous phase was added to the mixture in a drop wise manner and stirred for 5min to obtain CSA loaded nanoemulsion. The prepared formulations were subjected to different thermodynamic stability tests.

2.2.5 Optimization of Nanoemulsion

2.2.5.1 Transmission electron microscopy (TEM)

The morphology and microstructure of drug loaded nanoemulsion was studied using TEM (Morgagni 268D SEI, USA) operating at 200 KV and of a 0.18 nm capable of point to point resolution. Nanoemulsion formulations were diluted with water (1:10). A drop of nanoemulsion was deposited on the holey film grid, stained by 1% aqueous solution of phosphotungestic acid and observed after drying.

2.2.5.2 Micromeritics of Nanoemulsion

Globule size distribution and polydispersity index (PDI) of the nanoemulsions was performed by Photon correlation spectroscopy (PCS) known as dynamic light scattering using a zeta-sizer 3000(Malvern Instruments, Malvern, UK). Samples were prepared or diluted with dust-free ultra pure water and light scattering was measured at 25°C at a scattering angle of 90° [11].

2.2.5.3 Viscosity determination of Nanoemulsion

The viscosity of the formulations was determined without any dilution using Brookfield viscometer (Brookfield DV-II+ Pro viscometer) at 25 ± 0.5°C.

2.2.6 Formulation of Nanoemulgel

Nanoemulgel of CSA was formulated using guar gum (0.5 %) selected as a gel matrix base. First, the optimized quantity of guar gum was dispersed in purified water and kept for 24 hours for complete swelling. The oily phase was obtained by mixing accurately weighed quantity of oleic acid, tween 80, Transcutol P and CSA. The oil phase was kept under stirring until the oil phase becomes a clear solution. The total amount of the oil phase was accurately weighed and was transferred slowly to the formed gel. The stirring was continued at 1100 rpm to ensure the oil phase is dispersed into fine globules and the drug is distributed uniformly throughout the gel or aqueous phase. The addition of oil phase containing CSA is to be done very carefully since a small quantity of oil phase left over may vary the final dose of the formulation. The stirring is continued for 15 min to ensure the uniformity of the formulation.

2.2.7 Physical characterization of Nanoemulgel

The prepared nanoemulgels were characterized for pH measurement, Viscosity measurements and rheological properties, consistency, spreadability, drug content, content uniformity, *In vitro* drug Diffusion studies, *In vitro* skin permeation studies and stability studies.

2.2.7.1 Measurement of pH

The measurement of pH is essential for two reasons. One is the pH of human skin ranges from 5-7 and if the formulations pH is too acidic or too basic, it may cause damage to the skin or may cause severe irritation. Another reason is that extreme conditions of pH may degrade the CSA. The CSA may undergo acid or alkaline hydrolysis. The pH of the formulations was measured with digital pH meter at ambient temperature.

2.2.7.2 Viscosity measurements and rheological properties

The viscosity of the emulgel was measured using a Brookfield viscometer (Brookfield DV-II+ Pro viscometer) with spindle 04. Since gel is a semi solid having higher consistencies than common polymeric solutions or emulsions, a cone or plate setup is used rather than the spindle setup for the measurement of viscosity. The cone / plate setup was connected to temperature controlling water bath to maintain the desired temperature. A sample of around 200mg was placed on the plate and the setup was raised to touch the rotatable probe. The test was started and viscosity of sample was measured at various shear stresses.

2.2.7.3 Spreadability

The spreadability of nanoemulgels was determined by measuring the spreading diameter of nanoemulgel between the two glass slides after 1 min. A weight of 350 mg of nanoemulgel was placed within a circle of diameter 1cm which is marked initially on the glass slide over which another glass slide was placed. The increase in diameter as a result of weights added leading to spreading of gel was measured. It was calculated by using the following formula,

$$S = \frac{m.l}{t} \quad (1)$$

Where S = spreadability; m = weight placed to the upper slides; l = length of upper slide and; t= time taken in seconds.

2.2.7.4 Drug content

To a 200ml clean, dry volumetric flask approximately 2g of nanoemulgel weighed and care was taken so that the gel does not stick to the walls of the volumetric flask. Around 120 ml of diluent was added and the flask was shaken well to break the lumps of gel. The mixture was sonicated for 50 min with occasional shaking. The samples were cooled to room temperature, was made up to the volume with diluent and mixed well to ensure the uniformity. The sample was filtered through 0.45µm filter and loaded into the HPLC for further analysis. The samples were prepared in duplicate. The assay was calculated by the formula:

$$\% \text{ Assay} = \frac{sampl\ area}{stand\ area} * \frac{\text{stand wt}}{dilution} * std\ dil * sample\ dil * \frac{\text{volume}}{wt\ of\ gel} * 1000 * \frac{\text{potency}}{100} \quad (2)$$

2.2.7.5 *Ex vivo skin* permeation studies (Rat abdominal skin)

The rat abdominal skin was placed in supine position and the hair on the abdomen was trimmed off without damaging the skin. A superficial V cut was made at the bottom of the abdomen and the skin was cut slowly by separating the skin from subcutaneous fat tissue. The skin was placed in physiological saline for around 15min. Then the extra subcutaneous fat is removed with forceps. The skin was then placed on the diffusion cell assembly, where stratum corneum side was facing the donor compartment and dermal side was facing the receiver compartment. The receptor compartment consisted of 30 mL phosphate buffer of pH 7.4 as receptor fluid agitated at 100 rpm and maintained at 37 ± 0.5° C throughout the experiments. The prepared formulation was applied onto the membrane in donor compartment. An aliquot of 2mL sample was withdrawn at suitable time intervals (0.5, 1, 2, 3, 6, 12, and 24 h), filtered through 0.45-µm membrane filter and analyzed for drug content. Cumulative CSA permeated through the skin was calculated for each formulation.

2.2.7.6 Permeation data analysis

The cumulative amount of CSA permeated through the skin per unit area was plotted as a function of time for each formulation. The rate of drug permeation (flux) at steady state (J_{ss}) through skin was determined from the slope of the linear portion of plotted curve. The lag time (T_{lag}) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa.

2.2.8 Mathematical model fitting of various formulations

The obtained release data were fit into various kinetic models to know which kinetic model will best fit the obtained drug release profile. The parameters like "n" the diffusion exponent "k" the release rate constant and "R" regression co-efficient were determined to know the release mechanisms. The various kinetic models used were Zero order, First order, Higuchi model and Peppas model.

2.2.9 Stability studies

The optimized nanoemulgel formulation was selected for stability studies. The formulation was packed into collapsible tubes and sealed. The stability studies were carried out for 30 days by maintaining stability conditions as per ICH guidelines. Samples were withdrawn on 30th day and were analyzed for the drug content and preservative content. The drug release and diffusion studies were also carried out.

RESULTS AND DISCUSSION

3.1 Solubility studies of Cyclosporine in different components

The most important criterion for the screening of excipients (oils, surfactants, and cosurfactants) is the solubility of poorly soluble drug. The solubility of CSA in various oils, surfactants and cosurfactants were investigated (Table 1). Among the selected oils that were screened, CSA exhibited maximum solubility in the oleic acid (22.50 ± 0.76 mg/mL) and oleic acid was selected as an oil phase. Oleic acid has been reported as a powerful penetration enhancer for transdermal delivery, as it enhances the fluidity of the intercellular lipid barriers by developing separate domains and induces highly permeable pathways [12].

Out of various surfactants and cosurfactants screened, Tween 80 (6.19 ±0.05mg/mL) and Transcutol P (5.87±0.12 mg/mL) respectively, showed highest solubility of CSA. Therefore, Tween80 and Transcutol P were selected as surfactant and cosurfactant, respectively, for the phase study. Moreover, tween 80 is a nonionic surfactant which is

non-toxic when compared with ionic surfactants and has appropriate blend of low and high hydrophilic lipophillic balance (HLB=15), which can result in a stable nanoemulsion [13].

Table 1: Solubility of cyclosporine in oils, surfactants and co-surfactants

Components	Solubility mean (mg/ml) ±S.D*	Components	Solubility mean (mg/ml) ±S.D*
Oleic acid	22.50 ± 0.76	Tween 80	6.19 ± 0.05
Labrafac	5.32 ± 0.05	Cremophore EL	1.21 ± 0.22
Triacetin	1.34 ± 0.12	Span 20	0.83 ± 0.12
Olive oil	12.87 ± 0.15	Transcutol	5.87 ± 0.12
Capmul MCM	3.56 ± 0.21	PEG 200	2.22 ± 0.32
Soyabean oil	2.34 ± 0.11	PEG 400	2.56 ± 0.23
Tween 20	1.33 ± 0.11	Propylene glycol	1.28 ± 0.10

*n= 3 *Standard deviation*

Table 2: Composition of selected nanoemulsion formulations

Formulation code	Components (%w/w)		
	Oil	Tween 80: Transcutol (1:1)	Water
F1	10	75	15
F2	10	65	25
F3	10	55	35
F4	20	75	5
F5	20	65	15
F6	20	55	25
F7	30	65	5
F8	30	55	15

Table 3: Droplet Size, Polydispersity index, and Viscosity of the Nanoemulsion Formulations

Formulation code	Droplet size (nm) (Mean ± S.D.)	PDI	Viscosity (mPa.S) (Mean± S.D.)
F1	124.2 ± 1.22	0.262	23.2 ± 2.16
F2	121.6 ± 2.37	0.301	20.1 ± 1.39
F3	120.5 ± 1.36	0.279	18.9 ± 1.61
F4	136.2 ± 3.21	0.225	28.6 ± 2.39
F5	135.6 ± 4.21	0.313	25.5 ± 4.21
F6	133.2 ± 4.68	0.246	23.6 ± 3.12
F7	147.3 ± 5.26	0.195	35.6 ± 2.45
F8	146.6 ± 5.18	0.232	31.2 ± 1.62

*n= 3 *Standard deviation*

Table No 4: Physical characterization of CSA loaded Nanoemulgel

Formulation code	pH	Viscosity (m.PaS)	Spreadability ($gcmS^{-1}$)	Drug Content (%)
BF1	6.7 ± 0.44	12156.3 ± 0.32	5.9 ± 0.66	97.3 ± 0.34
BF2	6.4 ± 0.32	13145.6 ± 0.38	5.8 ± 0.42	98.2 ± 0.67
BF3	6.3 ± 0.84	13956.3 ± 0.57	6.0 ± 0.11	96.8 ± 0.96
BF4	6.5 ± 0.66	14454.8 ± 0.43	6.0 ± 0.53	97.3 ± 0.83
BF5	6.4 ± 0.43	15789.3 ± 0.86	5.9 ± 0.58	99.5 ± 0.62
BF6	6.3 ± 0.32	15432.4 ± 0.31	5.7 ± 0.15	98.7 ± 0.43
BF7	6.4 ± 0.78	16956.4 ± 0.63	5.9 ± 0.39	97.6 ± 0.21
BF8	6.5 ± 0.65	15657.8 ± 0.44	6.0 ± 0.53	97.9 ± 0.33

*n= 3 *Standard deviation*

Table No 5: Permeation parameters of various nanoemulgel formulations

Formulation code	CADP (mg/cm^2)	Drug Flux ($mg/cm^2/h$)	Lag time (h)	Drug retained (mg)
BF1	0.84 ± 0.05	0.051 ± 0.21	0.51 ± 0.12	1.36 ± 0.45
BF2	0.89± 0.03	0.059 ± 0.16	0.49 ± 0.23	1.19 ± 0.13
BF3	0.95 ± 0.06	0.062 ± 0.38	0.48 ± 0.36	0.94 ± 0.25
BF4	1.15 ± 0.04	0.069 ± 0.42	0.46 ± 0.17	1.42 ± 0.36
BF5	1.38 ± 0.03	0.078 ± 0.11	0.42 ± 0.33	0.78 ± 0.49
BF6	1.35 ± 0.07	0.076 ± 0.23	0.45 ± 0.29	1.26 ± 0.26
BF7	1.29 ± 0.04	0.058 ± 0.34	0.51 ± 0.43	1.21 ± 0.38
BF8	1.26 ± 0.05	0.045 ± 0.49	0.55 ± 0.18	1.15 ± 0.65

*n= 3 *Standard deviation*
CADP: Cumulative amount of drug permeated

Table No 6: Comparative permeation parameters of different formulations

Formulation code	CADP (mg/cm^2)	Drug Flux (mg/cm^2/h)	Lag time (h)	Drug retained (mg)	E_{pen}	LAE
Drug Solution	0.84 ± 0.05	0.041 ± 0.31	2.96 ± 0.12	1.46 ± 0.05	1	2.28
Plain drug gel	0.91± 0.15	0.057 ± 0.26	2.79 ± 0.23	1.29 ± 0.13	1.16	1.46
Marketed formulation	1.12 ± 0.21	0.062 ± 0.32	1.18 ± 0.36	0.97± 0.24	1.28	1.12
F5	1.51 ± 0.08	0.079 ± 0.12	1.46 ± 0.17	0.56 ± 0.12	1.49	0.12
BF5	1.38 ± 0.03	0.078 ± 0.11	0.42 ± 0.33	0.78 ± 0.29	1.39	0.22

*n= 3 *Standard deviation*
$E_{pen;}$ Enhancement ratio; LAE: Local accumulation efficiency

Table No 7: Permeation kinetics of different formulations

Formulation code	Zero order	Peppas	Higuchi	First Order
BF1	0.9368	0.9844	0.9940	0.9863
BF2	0.9250	0.9837	0.9924	0.9767
BF3	0.9275	0.9821	0.9925	0.9805
BF4	0.9355	0.9746	0.9904	0.9835
BF5	0.9273	0.9711	0.9905	0.9802
BF6	0.9273	0.9757	0.9902	0.9806
BF7	0.9316	0.9690	0.9900	0.9816
BF8	0.9293	0.9730	0.9909	0.9807

Table No 8: Stability analysis data for formulation BF5

Formulation	Stability condition	Days	Drug content (%)	pH	Transparency
BF5	25°C/60% RH	0	100.01± 0.5	6.54 ± 0.6	+
		15	97.99± 0.2	6.61 ± 0.3	+
		30	97.99± 0.3	6.63 ± 0.2	+
		45	97.98 ± 0.1	6.68 ± 0.4	+
		60	97.97 ± 0.4	6.67 ± 0.1	+
BF5	40°C/75%RH	0	100.3± 0.5	6.64 ± 0.6	+
		15	97.97± 0.1	6.65 ± 0.1	+
		30	97.96± 0.2	6.58 ± 0.4	+
		45	97.95 ± 0.3	6.67 ± 0.3	+
		60	97.95 ± 0.2	6.76 ± 0.3	+

Standard deviation n = 3; +: presence; -: absence.

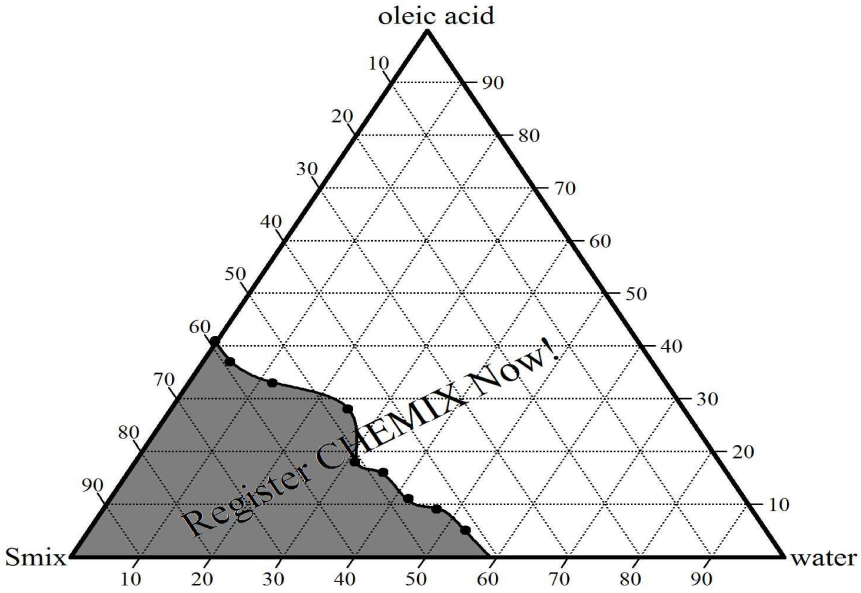

Figure 1. Pseudo-ternary phase diagrams of o/w nanoemulsion region of Oleic acid (oil phase), Tween 80 (surfactant), and Transcutol-HP (cosurfactant). Shaded part represents the nanoemulsion region

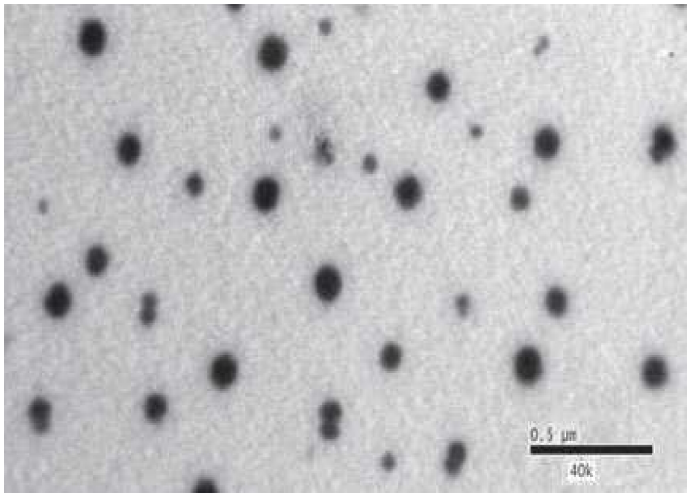

Figure 2. Transmission electron microscopy image of CSA nanoemulsion

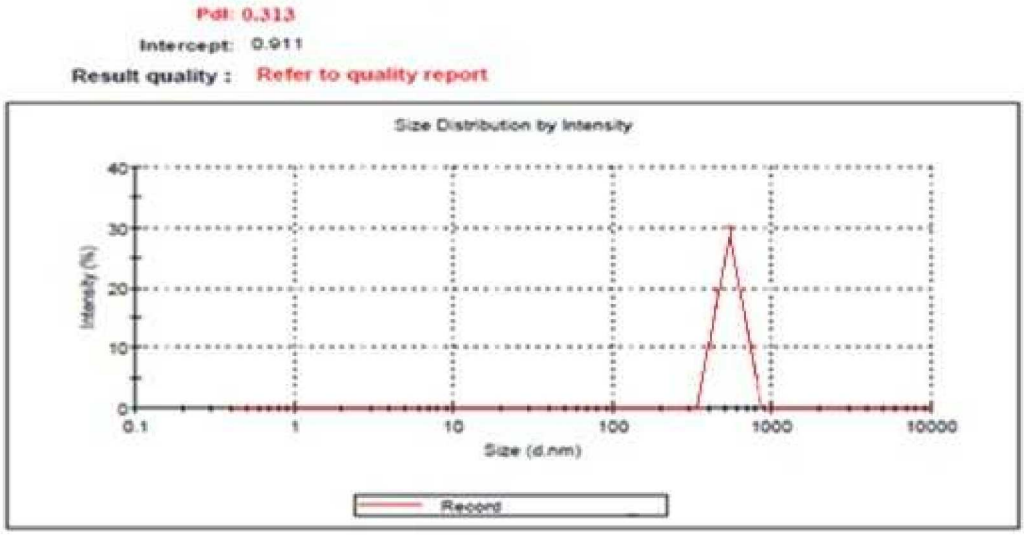

Figure 3. Size distribution of CSA nanoemulsion

3.2 Pseudoternary Phase Diagram Study

The pseudoternary phase diagram (Figure 1) was constructed to delineate the phase boundaries of different phases, with the shaded region highlighting the true o/w nanoemulsion region. The screening of surfactant and cosurfactants on the basis of solubility is difficult because all surfactant and cosurfactant cannot be solubilized in all type of oil phase. Surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of the nanoemulsion, provides a flexible film that can readily deform around droplets. So, in this work, we carried out the selection of surfactant and co surfactant based on formation of larger nanoemulsion region in the pseudo ternary phase diagram. Constructed pseudoternary phase diagrams are self explanatory about the presence of nanoemulsion region which assists easy selection of ingredients proportions for preparation of stable formulation. As surfactant to the cosurfactant ratio 1:0 was used, it showed significant nanoemulsion area. This showed that Tween 80 could be used alone without a cosurfactant, but higher concentration of surfactant can cause skin irritation [14]. Hence, it was decided to incorporate cosurfactant with the surfactant in the ratio 1:1. A large o/w nanoemulsion area was observed. The reason attributed to the condition may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers by decreasing oil phase size due to the use of cosurfactant. Another reason could be an increase in the entropy of the system. As we increased the surfactant concentration to 2:1, nanoemulsion region was decreased as compared to surfactant to cosurfactant mixture ratio of 1:1. Thus, 1:1 ratio of surfactant to co-surfactant was selected as optimized ratio which can be used further in the formulation of nanoemulsion from which different concentrations of components for formulation of nanoemulsion were pooled randomly.

3.3 Thermodynamic Stability Studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant, and water, making them stable and not subject to phase separation, creaming, or cracking. It is the thermostability that differentiates nano- or microemulsions from emulsions that have kinetic stability and eventually phase-separate. Thus, stability the formulations were tested for their thermodynamic stability by using centrifugation, a heating-cooling cycle, and a freeze-thaw cycle. Only formulations that survived the thermodynamic tests were selected for further study. The compositions of selected formulations are given in (Table 2).

3.4 Formulation of Cyclosporine loaded nanoemulsion

Exactly 2% wt/wt of CSA, which was kept constant in all the selected formulations, was added to mixtures of oil and Smix with varying ratios pooled from pseudoternary phase diagrams and then an appropriate amount of water was added to the mixture in a drop wise manner. The nanoemulsion containing CSA was obtained by stirring the mixture at ambient temperature.

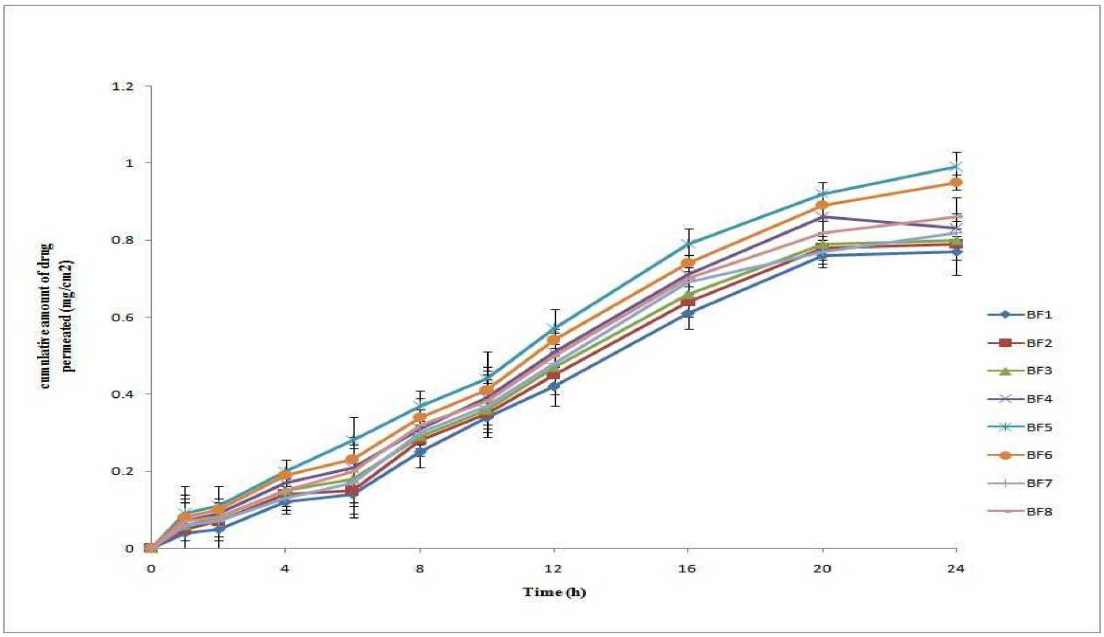

Figure 4. Permeation profile of various nanoemulgel formulations

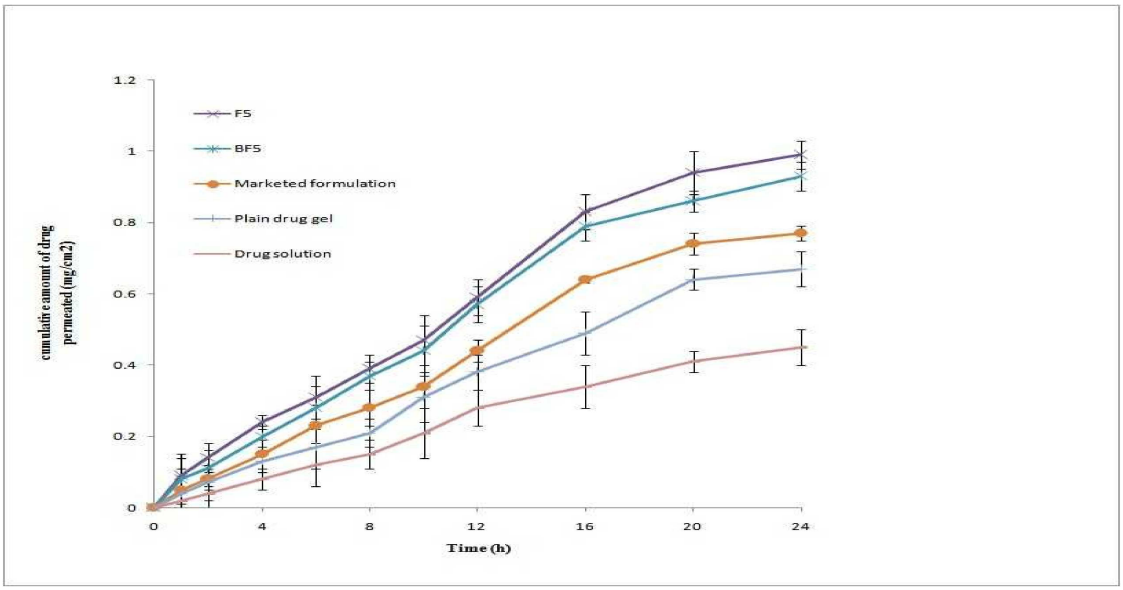

Figure 5. Comparison of Permeation profile of optimized nanoemulgel formulations (BF5) with marketed product and plain nanoemulsion

3.5 Optimization of Nanoemulsion

3.5.1 Transmission electron microscopy (TEM)

The transmission electron microscope showed a positive image of CSA nanoemulsion (F5) as shown in Figure 2. The figure revealed that, the shape of oil droplets of dispersed phase were found to be spherical and were of nanometer range.

3.5.2 Micromeritics of Nanoemulsion

The droplet size increased substantially with increased in the concentration of oil in the formulations. The droplet size of formulation F1, containing 10% of the oil content was found to be lowered significantly compared with other formulations. The average droplet size of all the formulation was in the nano range which is depicted from low polydispersity values. Polydispersity is a measure of particle homogeneity. The polydispersity indexes (PDI) of all the formulations were very low (Figure 3), indicating uniformity of droplet size within formulation. The PDI values of all the formulations were found in low range (0.195-0.301), indicating homogeneous dispersion of globules (Table 3).

3.5.3 Viscosity determination

The viscosity of nanoemulsion was found in the range of 18.9 ± 1.61 to 35.6 ± 2.45 mPas (Table 3). It was observed that all true nanoemulsions formulations (F1-F8) had low values of viscosity and not suitable for topical use. Hence nanoemulsion was incorporated into a gel matrix, resulting into nanoemulgel (BF1-BF8) having high viscosities. It was observed that as the concentration of surfactant and cosurfactant increases, viscosity of the formulation also gets increased.

3.6 Formulation of Nanoemulgel

The low viscosities of the prepared nanoemulsions obstructed their applicability for dermal use. Hence their viscosities were increased by incorporating nanoemulsions into a gel matrix of guar gum resulting into a nanoemulgels which were found to be consistent, uniform and highly viscous to be applied dermally. Guar gum was expected to offer good biophysical and sensorial benefits to the skin for an effective and efficient transdermal delivery. Guar gum is easily soluble in cold water and has high viscosity, even at low concentration. Hence it is widely used as thickener and stabilizer of suspension and emulsion [15]. Moreover, it is a naturally obtained polysaccharide and hence assigned as GRAS (generally recognized as safe) label. The polymer solution was added to the oil-S_{mix} under stirring till a transparent gel was formed. The formulation was stored at 4°C and was subjected to characterization.

3.7 Physical characterization of CSA loaded Nanoemulgel

3.7.1 Measurement of pH

The pH values of the prepared NEG formulations were found between 6.4 to7.0. This shows the pH value lies in the normal pH range of the skin which is considered acceptable to avoid the risk of irritation upon application to the skin. The results are shown in the Table 4.

3.7.2 Viscosity measurements and rheological properties

The performance of topical formulation is monitored by its rheological behavior, which governs its flowability, spreadability, and release of drug. The release of drug from the formulation is governed by its components and consistency of the formulation. It can be observed (Table 4) that increase in surfactant concentration leads to increase in viscosity of the nanoemulgel. Tween 80, used as surfactant here, was more soluble in the external aqueous phase. That is, because the concentration of water soluble surfactant in the system increased, the self-association of these amphiphilic molecules increased and formed different sizes and shapes of micellar aggregates [16]. As the concentration in external phase increased, the network will form between the surfactants molecules, micelles, and oil droplets. The denser the network, the closer the distance between the dispersed phases and the higher the viscosity.

3.7.3 Spreadability

Spreadability is an important parameter to be observed in topical formulations. It plays an important role in the patient compliance and help in uniform application of the gel to the skin as gels should spread easily upon application. Spreadability mainly depends on viscosity of the gel. Increase in viscosity leads to decrease in spreadability. The spreadability of all formulations was found to be in the range of 4.7± 0.28 to 5.2 ± 0.53 (Table 4). The large diameter signifies better spreadability.

3.7.4 Drug Content

The drug content of the nanoemulgel formulation was in the range of 96.8 ± 0.96 to 99.5± 0.62 (Table 4). The

results showed that the drug is distributed uniformly throughout the formulation and drug loss was minimum.

3.7.5 *Ex vivo* skin permeation studies

The *ex vivo* skin permeation study were performed to compare the drug releases from the nanoemulgel formulations (BF1-BF8) and conventional emulgel (CEG) (Figure 4). Nanoemulgel, in particular, was known to enhance permeation rates in deep skin layers and decrease lag time when compared to conventional formulations. It was well reported in previous works that nanoemulgel could perform as drug reservoir where drug was released from inner phase to outer phase and then further into the skin. The enhanced transdermal drug delivery might have resulted due to different mechanisms, which include the permeation enhancement of different components of nanoemulgel.

The cumulative amount of drug permeated (CADP), flux, lag time and skin retention were calculated for each formulation of nanoemulgel. The formulation BF5 showed the highest CADP (1.38 ±0.03 mg/cm^2) as compared to other formulations. Also a comparatively higher flux (0.078 ± 0.11) was observed for this formulation. In addition, lower lag time (0.51 h) and less skin retention (0.78 ± 0.49 mg/cm^2) of BF5 than the other formulations tested made it considerable for being selected as the optimized formulation (Table 5). In the present study, oleic acid (oil phase) was employed as an integral component which is widely known for increasing permeation. The results revealed that, as the oleic acid in the formulation was increased from 10%w/w to 20%w/w, the flux (rate of permeation) was increased. This The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized formulation BF5 was subjected to stability studies according to ICH guidelines by maintaining storage conditions at 25°C/60% RH and 40°C/75% RH for 30 days. There was no marked change in physical appearance assay, pH and drug content (Table 8). Thus, the nanoemulgel formulation could be beneficial in improving bioavailability and permeation of cyclosporine for transdermal delivery.

Results also indicated that in nanoemulgel formulations (BF4-BF6) as the surfactant cosurfactant mixture concentration was decreased from75% to55%, the skin permeation rate was increased twofold. The reason attributed to the situation could be increase in thermodynamic activity of drug in nanoemulgel at lower content of surfactant. It is well said that the thermodynamic activity of drug in the formulation is a sufficient driving force for the release and permeation of drug into the skin. The formulations (BF4-BF6) were also compared on the basis of concentration of aqueous phase, as water was the hydrophilic domain of nanoemulgel. When water content was increased, cumulative amount of drug permeated increased substantially.

3.7.6 Comparison of Permeation Studies of Marketed Formulation, Optimized Nanoemulgel, Nanoemulsion, and Plain Drug Gel and Drug Solution.

The nanoemulgel formulation had higher flux (0.078 ± 0.11 mg/cm^2/h) than conventional marketed formulation (1.12 ± 0.21 mg/cm^2/h), plain drug gel (0.91± 0.15mg/cm^2/h), and drug solution (0.84 ± 0.05 mg/cm^2/h) depicted in Table 6, Figure 5. The lower local accumulation efficiency (LAE) of nanoemulgel (Table 6) which was found to be 0, elucidated the lower retention of drug in the skin and confirmed that maximum amount of drug has been permeated through skin. The LAE for drug solution was found to be the highest, that is, 2.28, showing that drug has permeated through skin to a negligible extent and has been retained in skin only. In the present study, nanoemulgel was also compared with plain nanoemulsion. It was observed that the flux of nanoemulgel was lower than nanoemulsion, which may be due to higher viscosity of the formulation. When the flux of plain nanoemulsion (F5) was compared using unpaired student t-test, no significant ($P > 0.05$) difference was observed (Table 6). Though the nanoemulgel had lower flux, it can be favored over the nanoemulsion, due to prolonged effect and increased viscosity from view point of its applicability on skin.

3.8 Mathematical Model Fitting of Drug Release data

In vitro release studies of NEG formulations were fit into various kinetic models to determine the best fit model. The best fit model with the highest correlation coefficient values or regression coefficients (r^2) for the formulations are given in the Table 7. The results indicated that, the best fit model was found to be Higuchi model. The *in vitro* release data when fit into koresmeyer peppas equation the 'n' value was found to be 0.518 indicating non-fickian diffusion.

3.9 Stability Studies

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized formulation BF5 was subjected to stability studies according to ICH guidelines by maintaining storage conditions at 25°C/60% RH and 40°C/75% RH for 30 days. There was no marked change in physical appearance assay, pH and drug content (Table 8). Thus, the nanoemulgel formulation could be beneficial in improving bioavailability and permeation of cyclosporine for transdermal delivery.

CONCLUSION

The novel nanoemulgel of cyclosporine with suitable viscosity was successfully formulated for transdermal application. Nanoemulgel was formulated by addition of guar gum into nanoemulsion which resulted in increase in viscosity and had no significant influence on penetration of cyclosporine. The permeation rate of nanoemulgel was 2.0 times higher than that of drug solution.

The optimized formulation was compared with conventional marketed formulation and showed significant higher permeation profile which justifies the nanoemulgel system to be a promising surrogate carrier for transdermal delivery of cyclosporine.

Acknowledgements
The authors are thankful to JSS College of Pharmacy, JSS University, Mysore for providing lab facility and support.

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