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Formulation and Evaluation of Tocopheryl Polyethylene Glycol (TPGS) Stabilised Nanoemulsion of Curcumin for Topical Application

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ABSTRACT

Introduction: Poorly water-soluble curcumin have limited penetration through stratum corneum after its topical administration and therefore advanced formulation is warranted.

Objectives: The present study aimed to formulate and evaluate a TPGS stabilised nanoemulsion based topical gel of curcumin.

Methods: The nanoemulsion was formulated using Sefsol-218:TPGS (1:1), Tween 60 and Solutol HS 15 as the oil phase, surfactant and cosurfactant respectively based on the results of solubility studies and miscibility studies using aqueous titration method followed by ultrasonication.

Results: The formulated nanoemulsion exhibited a pH of 5.86 ± 0.018, percentage transmittance of 99.31± 0.710, particle size and polydispersity index of 195.3 and 0.153 respectively and *in vitro* release of 90.1%, which was significantly higher ($p \leq 0.05$) as compared to that of drug suspension which exhibited a drug release of 43.9%. The results of *in vitro* skin permeation studies revealed that optimised nanoemulsion formulation exhibited a permeation of 78.7% which was significantly higher ($p \leq 0.05$) than the Curcumin suspension (37.61%) after 8 h study. The flux 143.7 (μg/cm^2/h) and permeability coefficient 9.58×10^{-2} of optimised nanoemulsion was significantly higher ($p \leq 0.05$) as compared to drug suspension which exhibits 65.85 μg/cm^2/h flux and 4.39×10^{-2} permeability coefficient. From drug retention studies it was observed that a significantly higher ($p \leq 0.05$) percentage of Curcumin was retained in the skin after nanoemulsion application (7.46%) in comparison to drug suspension (3.73%). Further, the results of the histopathological evaluation conducted using excised skin of rats confirmed that the curcumin loaded nanoemulsion was safe for topical application.

Conclusion: The results of the study established that the formulated curcumin loaded nanoemulsion could be used topically for the treatment of various skin infections.

Key Words: Curcumin, Nanoemulsion, Histopathological emulsion, Topical application

INTRODUCTION

Curcumin, a poorly water-soluble phytoconstituent, is limited superficially to stratum corneum after its topical administration.[1] Therefore, it necessitates the formulation of lipophilic carriers that release the drug in a controlled manner. Among the different lipophilic carriers investigated nanoemulsions have gained immense importance for the delivery of drugs. Reports in the literature suggest that nanoemulsions are a promising strategy for the delivery of drugs as they can penetrate through the subcutaneous barrier into the skin.[2,3] Nanoemulsions are thermodynamically stable formulations made by using oils, surfactants, cosurfactants and water.[4] As compared to the conventional formulations meant for topical administration nanoemulsions facilitate an increased amount of drug entrapment. In the present study, a nanoemulsion of curcumin for topical administration with enhanced skin retention has been formulated and evaluated.

MATERIALS AND METHODS

Chemicals and reagents

Curcumin was purchased from Sigma Aldrich Corporation(St. Louis, USA), Sefsol 218 was obtained as a gift sample from Nikko Chemicals(Tokyo, Japan), Labrasol, Labrafac, Capryol 90 were obtained as gift samples from gattefosse (Saint-

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Priest, France). Tween 80 was purchased from Central Drug House, New Delhi, India. PEG was purchased from Spectrochem Pvt Ltd Mumbai, India. Water was obtained from the Milli-Q-water purification system (Millipore, MA). All other chemicals and reagents were of analytical grade and procured from Merck (Mumbai, India) and S.D. Fine Chem. (Mumbai, India). The Ethical Clarence has been taken from the Institutional Ethical cum Research board with vide letter no. SGR/2019-51- 20

Formulation Development and Optimization

Solubility Studies

Assessment of solubility of the drug was done to select the most appropriate oil, surfactant and co-surfactant for the nanoemulsion formulation containing curcumin. For determination of the solubility of curcumin the oils (Arachis Oil, Castor oil, Cinnamon oil, Clove oil, Corn oil, Olive oil, TPGS: Sefsol-218 Oil (1:1), Sunflower oil and Tea tree oil), surfactants (Span 80, Tween 20, tween 60 and tween 80) and cosurfactants (Glycerol, Labrafac, Lauroglycol FCC, PEG 600 and Solutol HS 15) were taken in 5 ml stoppered vials and drug in excess quantities was put in the oils, surfactants and cosurfactants to produce supersaturated solutions and using a vortex mixer kept at 25±1°C in an incubator shaker mixed for 72h for the attainment of equilibrium, after which the samples were centrifuged for 15 min at 3000 rpm. The supernatant was collected after filtration through a 0.45µm membrane filter diluted with methanol if necessary and analysed by UV spectrophotometer at 420 nm. The experiment was done in triplicate. Miscibility studies were also performed to select the cosurfactants. For the conduct of the miscibility study, surfactant and co-surfactant were mixed in the ratio of 1:1 in 5ml vials with the help of a vortex mixer. These vials were then kept at 25±1°C in an incubator shaker for 72h for attaining equilibrium. After 72h the vials were kept overnight to ascertain the miscibility of the surfactants and co surfactants[5]. To confirm the miscibility, visual observations were done. The transparent or clear mixtures were considered for other studies.

Construction of Pseudoternary phase diagrams for formulation selection

For the formulation of nanoemulsions, an aqueous titration method was used and pseudo ternary phase diagrams were constructed with water, a mixture of surfactant and cosurfactant (Smix) as well as the oil phase. TheSmix was made in different ratios (1:1, 2:1, 3:1, 4:1, 5:1) by increasing the amount of surfactant added. Fourteen combinations of oil :Smix ratios (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 3:7, 1:2, 4:6, 7:3, 8:2, 9:1) were made. The aqueous phase was used for carrying out slow titration for all ratios of oil &Smix. The titrations were continued till a clear, transparent low viscosity nanoemulsion was obtained. The formulated nanoemulsions were kept undisturbed for 24 h to attain equilibrium and to eliminate metastable formulations.[6] From the phase diagrams constructed, different combinations of placebo formulations were selected based on the presence of the minimum quantity of oil that could solubilise the drug dose and the minimum Smix quantity. For drug-containing nanoemulsions, the same procedure was followed except for the incorporation of the drug.

Thermodynamic stability studies

These stability studies are a very integral part of nanoemulsion formulation development as they help in choosing the nanoemulsions which are stable and also help in eliminating the metastable and unstable formulations. The following thermodynamic studies were done for the drug-loaded nanoemulsions.[7]

Centrifugation study

In the centrifugation study, nanoemulsions were centrifuged at 25°C and 3000 rpm for 30 mins after which the presence of any physical instability, creaming or cracking them was Ssesses visually.

Heating cooling cycle

After passing the centrifugation study, the nanoemulsions were assessed for stability by performing the heating-cooling cycle. The temperature variation effect on nanoemulsion stability was checked by keeping the formulations between refrigerator temperature 4°C and 40°C for six cycles, with storage of not less than 48 hrs at each temperature.

Freeze-thaw cycle

Further stability was assessed by freeze-thaw cycle in which the nanoemulsions were kept at -21°C and +25°C with storage for not less than 48 hrs at each temperature.

Characterization of Nanoemulsion

The optimized telmisartan loaded oral nanoemulsions were assessed for :

Viscosity measurement

The nanoemulsion viscosity was evaluated by Brookfield Viscometer (Model DV-1 Prime) using spindle # 61 at 37± 0.5°C at 60 rpm. A two minutes wait time was used. The experiment was done in triplicate and the standard deviation ascertained.

pH

The pH of the nanoemulsions was checked using a digital pH meter. The experiment was done in triplicate and the standard deviation ascertained.

Percentage transmittance

For the percentage transmittance, a hundred times dilution was done for one ml of the formulation using the blank as distilled water and analyzed at 650nm by UV visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan Shimadzu). The observations were taken in triplicate and the standard deviation was calculated.[8]

Refractive index

Refractive indices of the nanoemulsions were evaluated using Abbe's refractometer. The observations were taken in triplicate and the standard deviation was calculated.

In vitro drug release and determination of release rate orders

Franz diffusion cell, with treated dialysis membrane mounted between the donor and receptor compartment, was used for the study.35 ml of acetate buffer pH 5.5:PEG 600 (6:4) was used as the release media which was maintained at 37±1°C with continuous stirring at 75 rpm. Drug loaded nanoemulsion (1 ml) was placed over the dialysis membrane in the donor compartment. Samples (3ml) were taken at 0.5,1, 1.5,2,2.5 3,3.5, 4, 5, 6, 7 and 8 hrs and analyzed by using UV spectrophotometer (Shimadzu, 1800, Japan)[9]. The experiments were done in triplicate.

To establish the kinetics of drug release of the formulation, data of *in vitro* drug release studies was plotted in different kinetic models: zero-order, First order, Higuchi model and Korsmeyer–Peppas model. The model which had the value of correlation coefficient near to 1 was confirmed to be the best fit model.[10]

In vitro skin permeation study and drug retention study of optimized nanoemulsion using excised rat skin

Excised rat skin was used for the study after cleaning. The skin was held between the compartments of the Franz diffusion cell. Phosphate buffer pH 7.4 with methanol (6:4) (35ml), kept at 37 ± 1 °C with stirring at 75 rpm was used as the media. The nanoemulsion(1ml) was placed over the skin in the donor compartment. Samples (5 ml) were taken at time intervals of 1,1.5, 2,2.5, 3,3.5, 4, 5, 6, 7 and 8 hrs and analyzed at 428 nm using a UV spectrophotometer (Shimadzu, 1800, Japan). A graph was plotted between the cumulative amount of nanoemulsion permeated through the Wistar rat skin (Q, µg/cm^2) as a function of time (hrs). From the slope and intercept of the straight line, drug flux (J_{ss}, µg/cm^2/h) was calculated. After dividing the flux with initial drug concentration (C_o) permeability coefficient (k_p) was obtained.

Further, the formulation remaining on the skin was removed and the skin after the cleaning was weighed, cut into small pieces followed by sonication with methanol for 15 mins for extracting curcumin. The solution so obtained was centrifuged, filtered and the drug content (µg/cm^2) was evaluated by using a UV spectrophotometer.

Histopathological Study

To ascertain whether the curcumin loaded nanoemulsion was suitable for topical application histopathological study was performed. Freshly excised abdominal rat skin was taken and after washing with phosphate-buffered saline pH 7.4 mounted in a franz diffusion cell with the epidermis facing the upper side. 1ml of the nanoemulsion was placed over the skin in the donor compartment. The receptor compartment was filled with Phosphate buffer pH 7.4 with methanol (6:4) and left for 24 hours under constant magnetic stirring. After 24 hours, the rat skins were removed; blot dried and sent for histopathological evaluation using eosin and haematoxylin staining. The results were compared with those obtained for normal rat skin without the application of the formulation.

Droplet size and zeta potential

Malvern Zetasizer (Malvern, Worcestershire, UK) was used to determine the average particle size (z-average) and polydispersity index (PDI) of the developed nanoemulsion, after dilution and filtration using a 0.22µm membrane filter. The observations were taken in triplicate for each nanoemulsion evaluated.

RESULTS

Formulation Development and Optimization

Solubility Studies

The solubility of curcumin was evaluated in different oils and the results are given in Figure 1. Among the different oils evaluated, curcumin exhibited maximum solubility (8.01±0.026 mg/ml) in a combination of TPGS: Sefsol-218 Oil (1:1) and the combination was therefore chosen as the oily phase for the formulation of nanoemulsion.

The solubility of curcumin in various surfactants was evaluated as per the given procedure. Curcumin exhibited the highest solubility in Tween 60. Based on the results of miscibility and solubility studies, Sefsol-18:TPGS (1:1), Tween 60 and Solutol HS 15were selected as oil, surfactant and cosurfactant respectively.

Construction of Pseudoternary phase diagrams for formulation selection

Placebo nanoemulsions were formulated using the chosen oil, surfactant and cosurfactants. Pseudo ternary phase diagrams were constructed for each Smix ratio prepared to identify the nanoemulsion region.

When Smix 1:0 was used (Figure 4), a reduced nanoemulsion area was observed with macroemulsions comprising most of the region in the constructed pseudo ternary phase diagrams. As the cosurfactant amount was increased from 0 to 1 (Smix 1:1), the nanoemulsion region was observed to increase. An increase in the surfactant concentration from 1:1 to 4:1in Smix increased the nanoemulsion area. The maximum nanoemulsion area was obtained with Smix 4:1.

Thermodynamic stability studies

The placebo formulations selected from pseudo ternary phase diagrams were subjected to thermodynamic studies. In those placebo nanoemulsions which remained stable, the drug was added and then the drug-loaded formulations prepared were again subjected to thermodynamic stability studies. The formulations which exhibited turbidity and phase separation were discarded as they failed the tests for physical stability. Table 1 gives the compositions of stable drug-loaded nanoemulsion formulations which were taken up for further evaluation studies.

Table 1: Composition of drug-loaded nanoemulsions that passed thermodynamic stability studies

Formulation Code	Sefsol (ml)	Tween60: Solutol HS 15 (Smix) (ml)	Distilled water (ml)	Drug (mg)	Volume of nanoemulsion (ml)
A1	0.500	4.500	5.000	15	10
R8	0.500	4.000	5.500	15	10
H1	0.5	4.5	5.000	15	10

Characterization of nanoemulsion formulations

Viscosity

The nanoemulsions exhibited viscosity in the range 17cp to 20cp.

pH

The nanoemulsions had a pH between 5 to 6, which was similar to the skinpH.

Percentage Transmittance

The percentage transmittances of all the developed formulations were found to be near 100% which confirmed the clarity and transparency of the optimized formulation.[8]

Refractive index

The refractive index of nanoemulsions was close to that of sefsol-218:TPGS (1:1). Since no significant difference ($p<0.05$) was observed in the refractive index value of placebo as well as drug-loaded nanoemulsions, the absence of any interaction between excipient and drug was confirmed.

In vitro drug release and determination of release rate orders

Thestudy was performed for comparing the curcumin release from stable formulations (A1, R8 and H1) with that of drug suspension. The results are given in Figure 1.

In vitro release of formulation A1 showed maximum release (90.10%) which was significantly higher ($p< 0.05$) than the formulations R8 (82.9%), H1(61.60 %) and curcumin suspension (43.90%) after 8h of study.

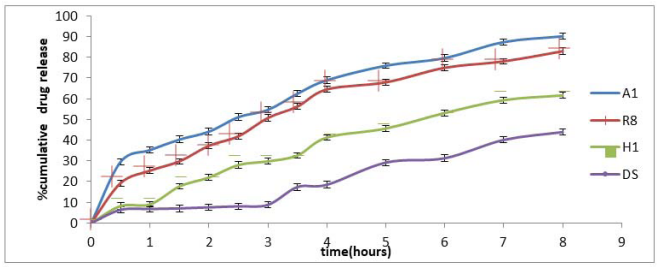

Figure 1: Comparison of in vitro release profile of selected nanoemulsion formulations with drug suspension.

Based on the observations of viscosity, refractive index, pH, percentage transmittance, and *in vitro* release studies, nanoemulsion A1 was chosen as an optimized formulation and further assessed for various parameters. Kinetic analysis of the *in vitro* data of optimized nanoemulsion (A1) done revealed that the correlation coefficient (R^2) for the Higuchi model was near to unity. It was confirmed that the release of curcumin from nanoemulsion obeyed the Higuchi model.

In vitro skin permeation and drug retention study using excised rat skin

The results of the permeation studies revealed that nanoemulsion A1 exhibited an *in-vitro* permeation of 78.7% which was significantly higher ($p<0.05$) in comparison to that of the suspension of the drug which exhibited a permeation of 37.61%. The results are given in figure 2.

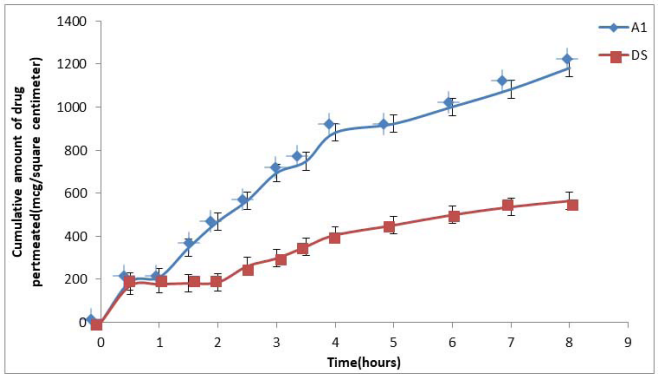

Figure 2: Cumulative amount of drug permeated versus time for optimized nanoemulsion formulation (A1) and drug suspension (DS).

The results of flux (J_{ss}), as well as permeability coefficient (kp) of formulation (A1) and suspension of drug, are given in Table 2.

Table 2: Determination of flux (J_{ss}) and permeability coefficient (k_p) of the optimized nanoemulsion (A1) and drug suspension

S. No.	Formulations	Flux (µg/cm²/h) ± SD (n=3)	Permeability coefficient (k_p) ± SD (n=3)
1	Nanoemulsion (A1)	143.7±0.125	9.58×10^{-2}±0.13
2	Drug suspension	65.85±0.129	4.39×10^{-2}±0.16

The drug retention (mg) and % drug retained in the skin were determined and the results are given in Figures 3 and 4.

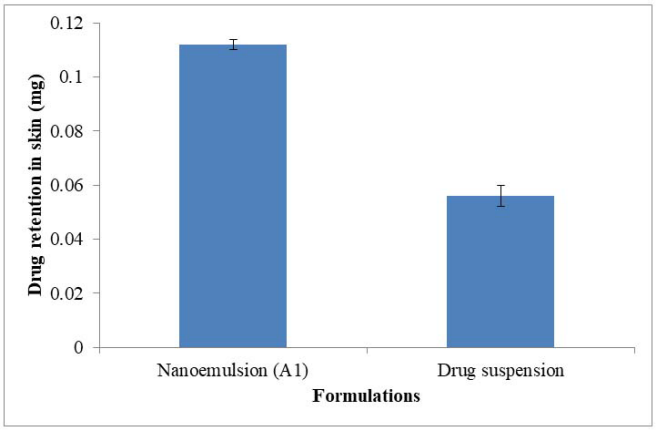

Figure 3: Comparison of formulations for drug retention in skin layers.

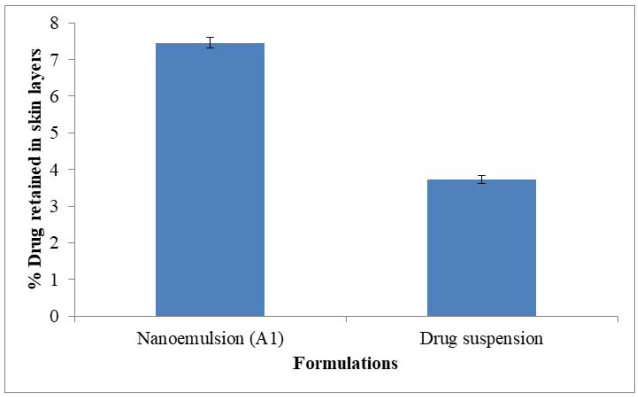

Figure 4: Comparison of formulations for percentage drug retained in skin layers.

Histopathological Study

The histopathological evaluation of the curcumin loaded nanoemulsion treated skin samples showed normal skin histology with the well-defined epidermis, dermis, subcutaneous tissue; similar to that of untreated skin samples. The epidermis revealed a normal stratified squamous keratinized epithelium, with no significant and remarkable evidence of necrosis or destructive changes. Thus the results of histopathological evaluation confirmed that the curcumin loaded nanoemulsion was safe as no changes were observed in the skin after application of the formulation as compared to the control skin (Figure 5).

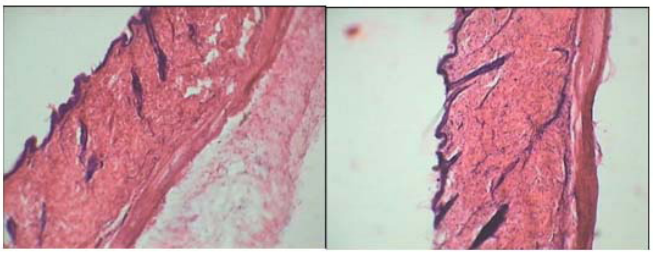

Figure 5: Photomicrographs showing histopathological sections; 40 x magnification (hematoxylin and eosin-stained) of normal untreated rat skin (Left), rat skin treated with curcumin loaded nanoemulsion (Right).

Droplet size and zeta potential

The average particle size and polydispersity index of all the formulations was in the nano range with formulation A1 exhibiting a particle size of195.3and polydispersity index of 0.153 respectively. Zeta potential for formulation A1 was -17.51± 0.121 which was between -30mV to +30mVand confirmed the stability of the nanoemulsion.

DISCUSSION

For the selection of oil for the formulation of nanoemulsion, the most important factor to be taken into consideration is

the drug solubility in the selected oil. For the drug to be and remain in a solubilised form in the nanoemulsion, the oil solubility of the drug is a very important consideration (Bali et al., 2010). Among the different oils evaluated, curcumin exhibited maximum solubility (8.01±0.026 mg/ml) in a combination of TPGS: Sefsol-218 Oil (1:1) and the combination was therefore chosen as the oil phase for the formulation of nanoemulsion.[9,10]

Surfactants and cosurfactants have a very important role to play in the formulation of nanoemulsions. They are reported to decrease the free energy required for the formulation of emulsion and thereby improve the thermodynamic stability of the nanoemulsion.[11] They also form a flexible lipophilic film around the globules and thereby prevent their coalescence. Among the different cosurfactants evaluated curcumin exhibited maximum solubility in Tween 60 and was therefore chosen for further studies.

Cosurfactants are also a very important component of the nanoemulsion formulation. Although they are beneficial when used at a proper concentration, using them at high concentrations makes the system unstable with increased globule size.[12] Among the different cosurfactants evaluated curcumin exhibited maximum solubility in Solutol HS 15 and was therefore chosen for further studies. Based on the results of miscibility and solubility studies, Sefsol-18:TPGS (1:1), Tween 60 and Solutol HS 15were chosen for the nanoemulsion formulation.

For each Smix ratio, Pseudoternary phase diagrams were prepared to identify the nanoemulsion region. When Smix 1:0 was used (Figure 4), a reduced nanoemulsion area was observed with macroemulsions comprising most of the region in the constructed pseudo ternary phase diagrams. The observations suggested the inability of the surfactant alone to solubilise the oil, because of which the interfacial tension did not decrease to low levels sufficiently which in turn led to the production of a non-homogeneous solution.[15,16] As the cosurfactant amount was enhanced from 0 to 1 (Smix 1:1), an enhancement in the nanoemulsion region was seen, which could be attributed to the higher and better penetration of the oil phase in the hydrophilic portion of the surfactant monomers which in turn caused a reduction in the interfacial tension which in turn enhanced the fluidity of the interface with an increase in the system entropy. An increase in the surfactant concentration from 1:1 to 4:1 in Smix increased the nanoemulsion area. The maximum nanoemulsion area was obtained with Smix 4:1. This was attributed to increased solubilisation of oil, decreased interfacial tension which resulted in increased fluidity and formulation of a flexible film around the oil globules of the dispersed phase and enhanced entropy of the formulated system.[12,13]

The drug-loaded formulations prepared were subjected to thermodynamic stability studies. The formulations which exhibited turbidity and phase separation were discarded as they failed the tests for physical stability. Two reasons namely Ostwald ripening and temperature quenching occurring due to segregation of oily phase as well as distribution of small size droplets supported by alteration in curvature free energy were attributed for instability in the system.[14]The nanoemulsions that passed the studies were chosen for further studies.

The viscosities of various formulations were optimum. The viscosity, if optimum of nanoemulsion meant for topical administration, aids in its easy application on the skin. Undesirable effects which can cause skin irritancy can result in case the pH of the topical formulation is not similar to that of the skin. All three nanoemulsion formulations had a pH between 5-6. The percentage transmittance values of various nanoemulsions were found to be close to 100%. Higher percentage transmittance indicated a more clear and transparent nanoemulsion.

The formulations exhibited a refractive index close to that of sefsol -218:TPGS (1:1). The absence of any interaction between excipient and drug was confirmed due to the absence of any significant difference between the placebo and drug-loaded nanoemulsions. *In vitro* release of formulation A1 showed maximum release (90.10%) which was significantly higher ($p< 0.05$) than the formulations R8 (82.9%), H1(61.60 %) and curcumin suspension (43.90%) after 8h of study. The enhanced drug release from the nanoemulsion in comparison to the curcumin suspension could be attributed to the nanosize of the developed formulation, which in turn resulted in increased release of the drug from the nanoemulsion.[14,15]

From the results of *in vitro* skin permeation studies, maximum permeation of Curcumin (78.7%) was detected for the formulation A1 which was significantly higher ($p\leq0.05$) than the Curcumin suspension (37.61%) after 8 h study. The flux 143.7 ($\mu g/cm^2/h$) and permeability coefficient 9.58×10^{-2} of A1 nanoemulsion were significantly higher ($p\leq0.05$) as compared to drug suspension which exhibited 65.85 $\mu g/cm^2/h$ flux and 4.39×10^{-2} permeability coefficient. The reason could be attributed to the nanosize of the formulation.[16]

From the above results, a significantly higher ($p\leq0.05$) amount of Curcumin was localized into the skin after application of nanoemulsion formulation (7.46) in comparison to drug suspension (3.73). The higher retention of the nanoemulsion formulation (A1) in the skin could be attributed to the greater extraction efficiency of skin lipids.[15,16] The results of the study confirmed that the formulation was safe for topical application, which could be attributed to the use of excipients that were in GRAS (Generally regarded as Safe) category.

Droplet size analysis is a critical factor influencing the stability and absorption of nanoemulsion. The smaller the

droplet size, the larger the interfacial surface which leads to greater absorption. The average particle size and polydispersity index of all the formulations was in the nano range with formulation A1 exhibiting a particle size of195.3 and polydispersity index of 0.153 respectively.[17,18] Since the stability of nanoemulsions is directly related to the surface charge, [16] determination of zeta potential is a very important characterization parameter for nanoemulsions. Instability in nanoemulsions occurs when the electrostatic repulsive forces decrease. Zeta potential for formulation A1 was -17.51± 0.121 indicating that the nanoemulsion was stable.

CONCLUSION

The curcumin loaded nanoemulsion was successfully formulated with satisfactory pH, viscosity, refractive index, *in-vitro* drug release and *in-vitro* permeation. The formulation has the potential for topical application for the treatment of various skin diseases.

Conflict of interest: None

The Ethical Clarence has been taken from the Institutional Ethical cum Research board with vide letter no. SGR/2019-51-20.

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