International Journal of Drug Delivery 4 (2012) 184-197

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Topical nanoemulsion of turmeric oil for psoriasis: characterization, ex vivo and in vivo assessment.

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Abstract

Psoriasis is a chronic; T lymphocyte mediated autoimmune inflammatory disorder characterized by well-defined erythematous (reddish) plaques with large adherent silvery scales that affects the skin and other parts of the body. The essential oil in turmeric is anti-inflammatory and effective in treating chronic disorders like psoriasis that have inflammation as a root symptom. Nanoemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water, surfactant and co-surfactant with a droplet size usually in the range of 20–200 nm. Their long-term stability and ease of preparation (spontaneous emulsification) make it promising tool for drug delivery. The aim of this study was to obtain nanoemulsions of turmeric oil for psoriasis and to evaluate their physical stability, irritation potential and *in vivo* inflammatory activity. For the preparation of nanoemulsion titration method was used which was composed of 15% turmeric oil, 42 % Smix (1:1) and 43 % distilled water. The nanoemulsion was stable during the period of study and was found to be practically non-irritating in the organotypic HET-CAM model. The anti inflammatory activity of optimized nonoemulsion was carried out by carragennen induced paw edema and found to be 70.35 % inhibition.

Keywords: Psoriasis, turmeric oil, nanoemulsion, anti-inflammatory, physical stability.

Introduction

Psoriasis is a chronic; T lymphocyte mediated autoimmune inflammatory disorder characterized by well-defined erythematous (reddish) plaques with large adherent silvery scales that affects the skin, joints, and tendons in up to 2.5% of the population worldwide [1-3]. The main abnormality in psoriasis is an increased proliferation of the skin layers due to excessive division of the cell in the basal layers of the skin. Severe itching may associate with the plaques. Dryness of the skin and silvery scaling are characteristics of this condition. The large number of anti psoriasis medications currently available to treat psoriasis raises important questions about fetal safety if a woman with psoriasis becomes pregnant while taking these medications. Unfortunately, published information on the effects of these medications on the developing embryo or fetus is limited. The teratogenic risk in human pregnancy was found to be undetermined for 91.2% of drug treatments approved in the United States between 1980 and 2000 [4]. Because of this uncertainty in risk estimation, women with psoriasis may be advised to use less effective treatment if they become pregnant or avoid treatment altogether because of concerns that the treatment might cause birth defects in their children. So our main aim is to treat such disorder by using some nature source.

Many herbs have demonstrated anti-inflammatory activity. Turmeric (*Curcuma longa* belonging to the family of Zingiberaceae) is a rhizome, or fleshy root, that has long history in both Chinese and Ayurvedic (Indian) medicine as an anti-inflammatory agent. The essential oil in turmeric is anti-inflammatory and effective in treating chronic disorders like psoriasis that have inflammation as a root symptom. Turmeric oil has also demonstrated as a potent anti-inflammatory activity in a variety of experimental animal models [5]. Curcumin in turmeric oil [6] inhibits leukotriene formation, inhibits platelet aggregation and stabilizes neutrophilic lysosomal membranes, thus inhibiting inflammation at the cellular level [7]. Curcumin is reported to possess greater anti-inflammatory activity than ibuprofen [8]. Pharmacological treatments for psoriasis are generally based on antiproliferative, anti-inflammatory, or differentiation-modifying activity or some combination of these actions. Like most

antipsoriatic drugs, turmeric oil was found to inhibit the keratinocyte proliferation, suggesting its potential in suppressing psoriasis [9].

Turmeric oil consists of a unique set of sesquiterpenoids considered to have significant pharmacological activities. Antifungal, insect repellent, antibacterial, antimutagenic, and anticarcinogenic activities [10-12] of turmeric oil have been reported.

Nanoemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water, surfactant and co-surfactant with a droplet size usually in the range of 20–200 nm [13-16]. Their long-term stability, ease of preparation (spontaneous emulsification), and high solubilization of drug molecules make it promising tool for drug delivery. Recently, much attention has been focused on the colloidal drug delivery systems such as microemulsions, solid lipid nanoparticles and liposome for topical delivery of drugs because of low side effects, high bioavailability, good patient compliance, etc. [17-19].

Nanoemulsions are well characterized and are a promising drug delivery system with practical applications for pharmaceutical, cosmetic and chemical industry applications. They have been used in intravenous, oral and ocular drug administrations and have reduced drug side effects and improved the pharmacological effects of the drugs given [20-22].

The aim of this study was to obtain nanoemulsions of turmeric oil and to evaluate their physical stability, irritating potential and in vivo inflammatory activity.

Materials and methods

Materials

Turmeric oil was gifted from Lala Jagdish Prasad & company, Kanpur, India. Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monooleate (Tween 80), lecithin, labrasol, isopropyl alcohol were procured from S.D Fine Chemicals (Mumbai, India). Water was obtained from Milli Q water purification system (Millipore, MA). All chemicals and solvents were of analytical grade.

Preparation of nanoemulsion

For the preparation of nanoemulsion of turmeric oil the following surfactant and co-surfactant mixtures were evaluated to find out a stable nanoemulsion formulation: Tween 20/isopropyl alcohol, Tween 80/ isopropyl alcohol, Labrasol/ isopropyl alcohol and Lecithin/ isopropyl alcohol.

The phase diagram method was used with different combination of surfactant and co-surfactant having 1:1 ratio in all the combination because only with the help of surfactant we cannot reduce interfacial tension [23, 24], which can produce flexible film for the formulation of stable nanoemulsion. Here only 1:1 ratio of different type of surfactants and co-surfactant was taken so that surfactant concentration kept at its minimum concentration because high concentration of S_{mix} may cause irritation and toxicity to the tissues [25]. All emulsions were prepared by spontaneous saponification method (titration method).

Phase studies

For the formation of nanoemulsion, turmeric oil is used as oil phase, Tween 20, Tween 80, Lecithin and Labrasol used as a surfactant and Isopropyl alcohol used as a co-surfactant. The S_{mix} were chosen in fixed concentration (1:1) for each phase diagram. For each phase diagram, oil and specific S_{mix} were mixed well in different ratios. Sixteen different combinations of oil and S_{mix} (1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were made so that maximum ratio could be covered for the study to delineate the boundaries of the phases formed precisely in the phase diagrams [26]. Slow titration with aqueous phase was done for each weight ratio of oil and S_{mix} under moderate stirring, and visual observation was used for transparent and easily flowable nanoemulsion. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after being tilted to an angle of 90°. The physical state of nanoemulsion was marked on a pseudo three component phase diagram with one axis representing the aqueous phase, second representing oil, and the third representing a mixture of surfactant and co-surfactant at fixed weight ratio (S_{mix} ratio).

Selection of formulations

The pseudoternary phase diagrams which shows maximum nonaemulsion region was taken for further studies. From the phase diagrams, a number of nanoemulsions formulations were taken with different ratio of oil, S_{mix} and water. Selected formulations were subjected to various physical stability tests.

Thermodynamic stability testing of nanoemulsions

In order to find out the stable nanoemulsion and to discard the unstable or metastable nanoemulsions the placebo nanoemulsions were subjected to following thermodynamic stability studies.

Freeze thaw cycle: Nanoemulsions were kept in deep freezer (at -20 °C) for 24h. After 24h the nanoemulsions were removed and kept at room temperature. The thermodynamically stable nanoemulsions returned to their original form within 2-3 min. 2-3 such cycles were repeated.

Centrifugation studies: Nanoemulsions after freeze thaw cycle were subjected to centrifugation studies where they were made to undergo centrifugation for 30 min. at 5,000 rpm in a centrifuge. The stable formulations did not show any phase separation or turbidity [27].

Heating cooling cycle: Six cycles between refrigerator temperature (4 ^{0}C) and 40 ^{0}C with storage of 48 hours were performed. Those formulations which were stable at these temperature, subjected to further study.

Characterization of nanoemulsions

Globule size analysis The droplet size of the nanoemulsions was determined by photon correlation spectroscopy, which analyses the fluctuations in light scattering due to Brownian motion of the particles using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25℃ at a 90° angle [28].

Viscosity Viscosity of nanoemulsion was determined by using Brookfield LV rotational viscometer at 2.5, 5, 10 and 20 rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times. The viscosity values at 5 rpm were selected

Refractive index The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing one drop of the formulation on the slide in triplicate at 25℃.

pH Measurements The apparent pH of the formulations was measured by a pH meter (Mettler Toledo MP 220, Greifensee, Switzerland) in triplicate at 25℃.

Transmission Electron Microscopy (TEM) Morphology and structure of the nanoemulsion were studied using Morgagni 268D electron microscope (Fei Company, Netherlands) operating at 70 kV capable of point-to-point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. In order to perform transmission electron microscopy (TEM) observations, a drop of the nanoemulsion was suitably diluted with water and applied on a carbon-coated grid, then treated with a drop of 2% phosphotungstic acid and left for 30 s. The coated grid was dried and then taken on a slide and covered with a cover slip and observed under the microscope.

In vitro Irritant test in an organotypic model - HET-CAM

(Hen's Egg Test on the Chorioallantoic Membrane)

The HET-CAM test is routinely used to evaluate the potential eye irritation of raw materials but can in some cases be used to evaluate skin irritation, e.g. in the case of surfactants. Irritation causes alterations in the vascular system of the HET-CAM that result in membrane discoloration, haemorrhaging and increased perfusion. The method used in this manuscript is a modification of the method described by Luepke [29] and adapted by Mehling [30] that allows the immediate evaluation of irritation by solid or liquid substances in the hen's egg chorioallantoic membrane. Each substance was tested on three fertilised eggs that were incubated for 9 days prior to testing. The CAM (Chorioallantoic Membrane) was exposed to 300 μL of one of the following substances: (1) nanoemulsion (pH 6.54), (2) surfactant solution blend (5% sorbitan oleate, 5% PEG-30 castor oil and 90% water (pH 6.32), (3) Sodium lauryl sulphate (SLS) 10% w/w (positive control, pH 6.05) and (4) saline solution (negative control, pH 6.0). The CAM was rinsed with physiological saline solution after 30 seconds of exposure to each substance, and the intensity of the reactions (hyperaemia, haemorrhage and coagulation) was semi-quantitatively assessed on a scale of 0.5, 2 and 5 minutes after treatment; longer observation times give no additional important information. The numerical time-dependent scores for hyperaemia, haemorrhage and coagulation are summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21. The mean value of four tests makes possible an assessment by a classification scheme analogous to the Draize categories (Table 1).

In vivo anti-inflammatory effect on carrageenan-induced paw-edema in rats

Paw edema can be induced by murine carrageenan. Male Sprague-Dawley rats weighing 150-180 g were used for the experiments. The protocol to carry out animal studies was approved by the Institutional Animal Ethics Committee, Translam Institute of Pharmaceutical Education and Research, Meerut, U.P., India. The committee's guidelines were followed for the studies. All measurements were performed at 24±1℃ in an air-conditioned room. The animals were randomly divided into eight groups of six rats each for administration. The rats of the first control group were treated with normal saline [31]. The other seven experimental groups received different topical formulations of turmeric oil nanoemulsions. To induce local inflammation, 50 μ l of 1% carrageenan (w/v) in saline was injected into the plantar surface of the left hind paw of the rats at time zero, using a 27-gauge needle coupled to a 100 ml Hamilton syringe. In the first experiment, 60 minutes later, turmeric oil nanoemulsions were applied, nonocclusively, to the paws of the animals and spread gently. Animals were then housed in polypropylene cages with framed metal mesh on the floor to prevent absorption of applied products by sawdust. The animals were maintained without access to food and water during the experiment.

Measurements of foot volume were performed by the method described by [32] using water plethysmometer (LE 7500, Letica Scientific Instruments, Barcelona, Spain) before and 1, 2, 3, 4, and upto 24 hours after the injection of carrageenan into the planter region of the left hind paw. The degree of paw swelling was calculated as

$$\text{Swelling}(\%)=(v_t-v_0)/v_0 \quad X100 \text{----------(1)}$$

where V_t (ml) is the volume of the carrageenan-treated paw, V_o is that of the nontreated paw.

On the basis of equation (1), the percentage edema inhibition was calculated as

Inhibition (%)= 1-%swelling of thenanoemulsion treatrd group / swelling of the control group X100
(2)

Histopathology studies

Abdominal skin of Wistar rats was treated with the optimized turmeric oil nanoemulsion A3. After 24 h, the rats were killed and skin samples were taken from untreated (control) and treated areas. Each specimen was stored in 10% formalin solution in

phosphate buffer saline (pH 7.4). The specimens were cut into sections vertically. Each section was dehydrated using ethanol embedded in paraffin wax for fixing and stained with hematoxylin and eosin. These samples were then observed under light microscope (Motic, Japan) and compared with control samples [33].

Stability studies of optimized formulation

Stability studies on optimized nanoemulsion were performed by keeping the sample at 4 ± 0.5 ^{0}C, 25 ± 0.5 ^{0}C and 40 ± 0.5 ^{0}C. These studies were performed for the period of 3 months. The droplet size, viscosity, pH, refractive index and electrical conductivity were determined at 0, 1, 2 and 3 months.

Results and discussion

The most important criteria for selection of all the nanoemulsion components is that all the excipients should be pharmaceutically acceptable for topical application, depending upon the requirement and falling under the generally-regarded-as-safe category.

Screening criteria for oil selection

In this study only turmeric oil was selected for the formulation of nanoemulsion because this oil considered as a active substance for the treatment of psoriasis. There is no any drug used so solubility study was omitted.

Screening criteria for surfactants

The most critical problem related to the nanoemulsion-based systems is the toxicity of the components. Large amounts of surfactants may cause skin irritation when administered topical. So the proper selection of surfactants becomes necessary. It is, therefore, important to determine the surfactant concentration properly and use the minimum concentration in the formulation. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower CMCs. Also, o/w nanoemulsion dosage forms for topical use based on nonionic surfactants are likely to offer *in vivo* stability [34]. Therefore, proper selection of surfactants becomes a crucial factor. Another important criterion is the selection of surfactant with proper HLB value. Hydrophilic surfactant and co-surfactant are considered to prefer the interface and to lower the necessary energy to form the nanoemulsions, as a result improving the stability. For example, the required HLB value to form o/w nanoemulsion is greater than 10 [35]. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water.
After selection of oil phase, the main aim was to identify the surfactant that has the highest solubilization capacity for the oil. In the present study, four surfactants, namely, Tween 20, Tween 80, Lecithin, and Labrasol were chosen for screening. Nonionic surfactants were selected since they are known to be less affected by pH and changes in ionic strength, are generally regarded as safe, and are biocompatible. Ionic surfactants were excluded from the study due to toxicological concerns. Here, we have selected the surfactant giving the maximum nanoemulsion formulation in phase diagram.

Screening of co-surfactants

From literature we know that only surfactants are responsible for the formulation of nanoemulsion, the role of cosurfactant is to reduce further interfacial tension to make nanoemulsion more stable by providing flexible film. Isopropyl alcohol was selected as a co-surfactant because it is used for topical delivery and also act as a penetration enhancer.and also it comes under GRAS category.

Preparation of pseudo ternary phase diagram

Formulations were carefully observed so that the metastable systems were not selected, although the free energy required to form a nanoemulsion is very low and the formation is thermodynamically spontaneous [36]. On the basis of psedoternary phase diagram lecithin and isopropyl alcohol (1:1) was selected for further study because this combination of surfactant and co-surfactant able to produce maximum nanoemulsion area (Figure. 1). When co-surfactant was added with surfactant in equal amounts, a higher nanoemulsion region was observed, perhaps because of the further reduction of the interfacial tension and increased fluidity of the interface at S_{mix} 1:1. Further increase of Smix ratio was not studies because high surfactant may cause irritation.

Thermodynamic stability tests of nanoemulsion

In order to exclude the possibility of metastable formulations, stress testing is required. Some representative formulations were taken from the o/w nanoemulsion region of the phase diagram constructed at Smix 1:1 for lecithin and isopropyl alcohol, and were subjected to the thermodynamic stability tests such as heating cooling cycle, freeze thaw cycle, and centrifugation. Results of thermodynamically stable formulations were shown in (Table 2). Thermodynamic stability test confers long term stability to the nanoemulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability and will eventually phase-separate [37]. Thermodynamically stable formulations were selected for further studies.

Characterization of the Selected Nanoemulsions

The nanoemulsions were selected for further optimization which all ready passed stress test.
Globule size analysis The droplet size increased with increase in the concentration of the oil in the formulations (Table 3). However, the droplet size of all the formulations was in the nano range. The low polydispersibility values observed for all the formulations indicated uniformity of droplet size within each formulation.

The droplets in the nanoemulsion appear dark, and the surroundings are bright; a "positive" image was seen using TEM (Figure 2 and Figure 3). Some droplet sizes were measured using TEM, as it is capable of point-to-point resolution. The uniformity of particles size was investigated with the help of size distribution analysis as shown in (Figure 4 and Figure 5).

Viscosity Viscosity tends to increase with the oil content. As the oil content was increased from 5% w/w to 25% w/w, an increase in the viscosity of the formulations was observed (Table 3). The viscosity of formulation A2 was significantly lower than that of the other formulations ($p < 0.05$), which might be due to its lower oil content. Overall, very low viscosity of the formulations was observed, which is expected for nanoemulsions.

pH Measurements The apparent pH of all formulations were measured by pH meter in triplicate at 25 ± 1℃ and found to in between 5-6 (Table 3). This pH range is optimum for topical formulation.

Refractive index Refractive index is the net value of the components of nanoemulsion and indicates the isotropic nature of the formulation. The mean value of the refractive index for all the formulations was given in (Table 3). The lowest values of RI was seen in A2 formulation, might be due to a increase in water content, as water has a comparatively lower refractive index (the refractive index of water is 1.334).

In vitro Irritant test in an organotypic model - HET-CAM

(Hen's Egg Test on the Chorioallantoic Membrane)

Topical application products must have a low ocular/ mucous membrane and a low dermal irritation potential.

The irritation potential depends on the concentration of the substance as well as the chemical composition and the pH of the formulation [38].

The HET-CAM test can help evaluate the irritation potential of substances *in vitro* and *in vivo* [39]. The CAM showed no signs of irritation after application of either the nanoemulsion or the negative control substance, so the nanoemulsions were therefore considered practically non-irritating. The surfactant solution by itself caused mild hyperaemia, which suggests that the presence of turmeric oil in the nanoemulsion may have protected the chorioallantoic membrane from the irritating effects of the surfactant solution (Table 4). The pH values were the same for all samples tested to eliminate pH as a variable in the HET-CAM results.

The HET-CAM test showed that the nanoemulsions containing turmeric oil was essentially non-irritating.

In vivo anti-inflammatory effect on carrageenan-induced paw-edema in rats

The formulations A2 and A3 were chosen for the in vivo study due to its high stability and lack of irritation in the HET-CAM test. The anti-inflammatory effects of optimized formulations were compared with the control. The anti-inflammatory activity of optimized formulations of nanoemulsion was evaluated using the carrageenan-induced hind paw edema method using digital Plethysmometer. The rat's left footpad became edematous soon after injection of carrageenan and reached its peak at 3 h (75.2 %). Mean percent edema and % inhibition of inflammation of all the three groups were calculated which was shown in Figure 6. Inhibition of edema was found to be highest in the groups in which nanoemulsion A3 was applied. The nanoemulsions A2 and A3 inhibited edema ($P < 0.05$) 62.33% and 70.35 % respectively up to 24 h. Control formulation inhibited the edema 42.98 % up to 24 h. Based on the anti-inflammatory studies, it can be concluded that A3 formulation shows maximum inhibition of edema than the A2 formulation and the control.

Histopathology studies

The influence of turmeric oil nanoemulsion on anatomical structure of the rat skin was assessed with the help of histopathological studies. After observation of light power photomicrograph of control and treated skin (Figure 7), it was found that no significant different was seen in the shape and size of the tissue of rat skin.

Stability study

The formulation A3 was tested at three different storage temperatures: 4 ± 0.5℃, 25 ± 0.5℃ and 40 ± 0.5℃. Droplet size measurements are a good indicator of the formulation stability. A fast droplet size increase indicates low system stability. The droplet size for this formulation remained constant over 90 days for all temperature conditions (Figure 8). The nanoemulsions had polydispersity index values below 0.2 throughout the 90-day testing period, indicating the high fidelity of the system (low polydispersity), which may reflect the overall stability of this formulation and synthesis method. Polydispersity values near 1.0 are indicative of a polydisperse system. The long term stability of nanoemulsions was previously evaluated and was also verified by stability studies conducted over three months. The O/W nanoemulsion produced by low energy emulsification showed no difference in droplet size over the study period at both 25℃ and 4℃.

The long term stability of nanoemulsions was previously evaluated and was also verified by stability studies conducted over three months. The O/W nanoemulsion produced by low energy emulsification showed no difference in droplet size over the study period at both 25℃ and 4℃ [40]. The O/W nanoemulsion demonstrated high physical stability, corroborating our results for temperatures of 4 ± 0.5℃ and 25 ± 0.5℃. Low-energy emulsification is better at producing stable nanoemulsions than its higher energy counterpart. When nanoemulsions were prepared using a high pressure homogeniser, the droplet size was initially around 56 nm; however, the particles slightly increased in size after 30 days at either 25 or 4℃. The low-energy emulsification method used in our study showed high stability with respect to the droplet size and polydispersity index.

The viscosity and RI were also determined at 4^0C, 25^0C and 40^0C. These parameters were determined at 0, 1, 2 and 3 months. It was found that viscosity and RI were slightly increased

in time at all temperatures (Figure 9 and Figure 10). These parameters were compared for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple

Table 1: Classification of cumulative scores in the chorioallantoic membrane test (According Luepke 1985) [29]

Cumulative Score	Irritation assessment
0-0.9	Practically none
1-4.9	Slight
5-8.9	Moderate
9-21	Strong

Table 2: Composition of selected nanoemulsion

Oil used: Turmeric oil, Surfactant used: Lecithin, Cosurfactant used: Isopropyl Alcohol, External phase: Distilled water								
S_{mix} Ratio	Formulation code	Oil (%)	Smix (%)	Water (%)	H/C	Cent	Freeze	Result
1:1	A1	5	33	62	√	√	X	Failed
	A2	10	38	52	√	√	√	Passed
	A3	15	42	43	√	√	√	Passed
	A4	20	47	33	√	√	√	Passed
	A5	25	50	25	√	X	√	Failed

Table 3: Characteristics of the turmeric oil nanoemulsions

Formulation Code	Mean Globule Size (nm)	Poly dispersivity	Viscosity (mP)	pH	Refractive Index	Electrical Conductivity (µs/cm)
A2	31.52	0.157	38.44± 1.17	5.9 ± 0.03	1.431 ± 0.011	121
A3	56.05	0.192	41.41 ± 1.32	6.1 ± 0.02	1.463 ± 0.013	116

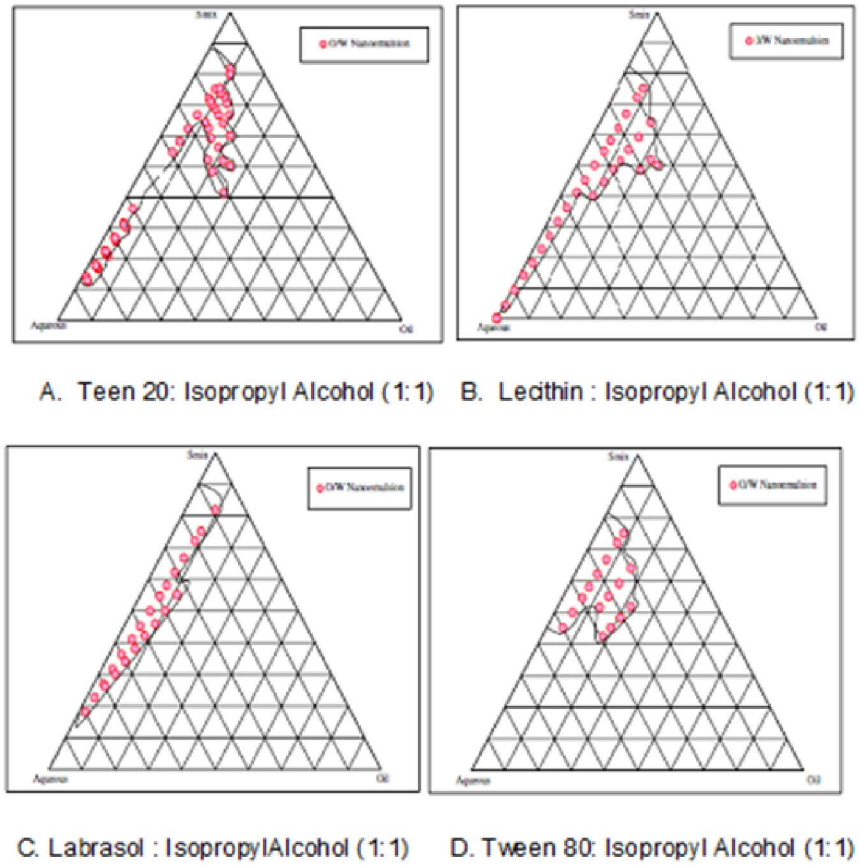

Figure: 1 Pseudoternary phase diagram indicating o/w nanoemulsion region using Turmeric oil with different surfactant

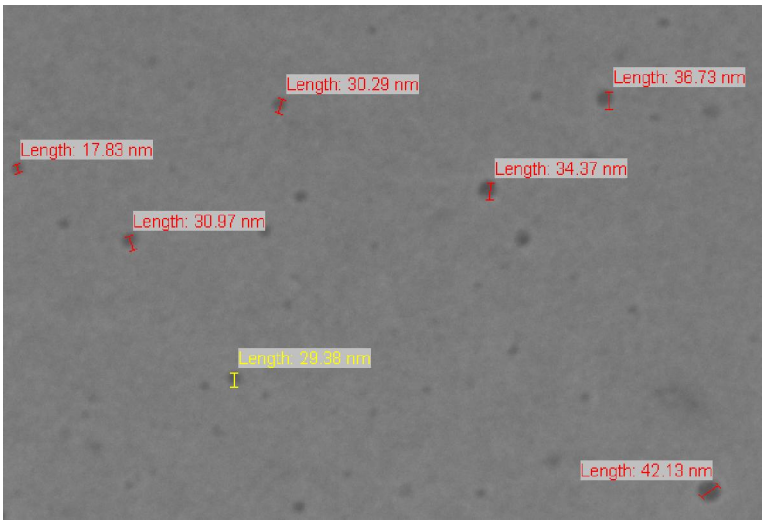

Figure 2: TEM photograph of particle size of nanoemulsion (A2)

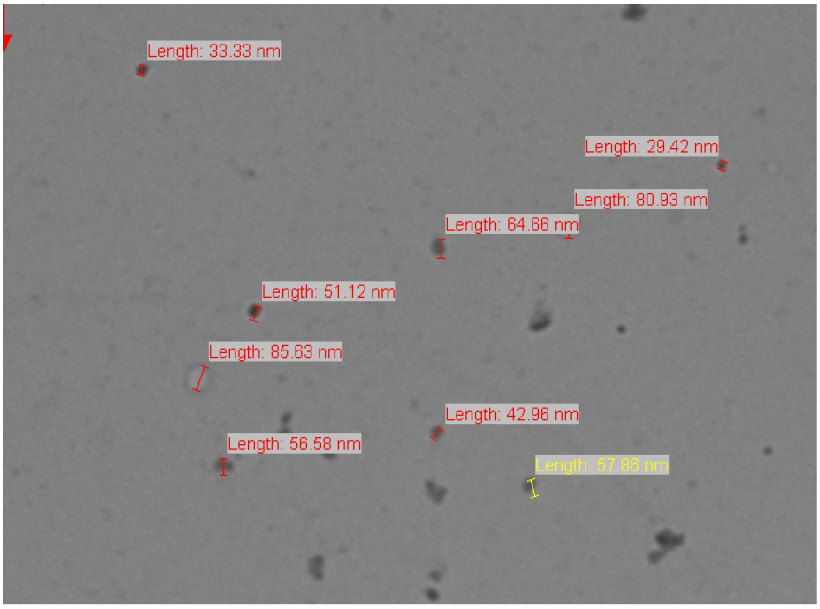

Figure 3: TEM photograph of particle size of nanoemulsion (A3)

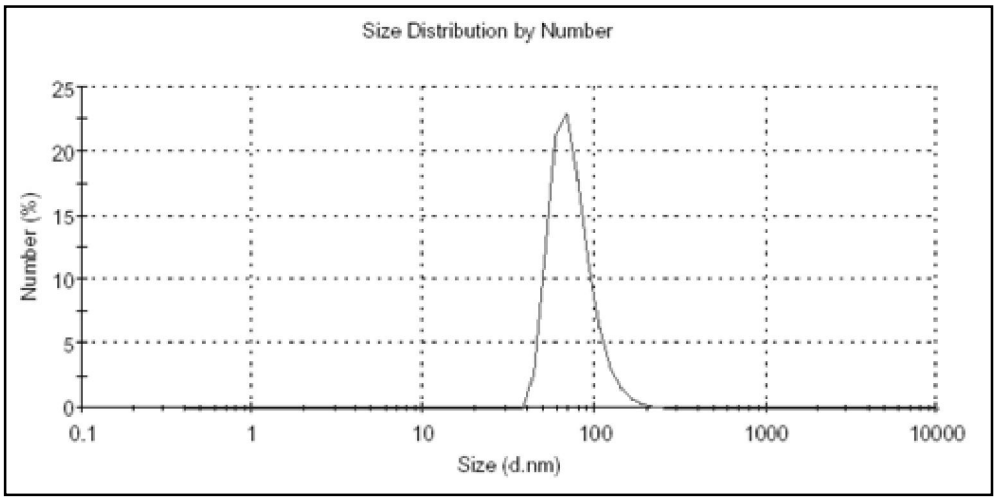

Figure 4: Droplet size and size distribution of nanoemulsion formulation (A2).

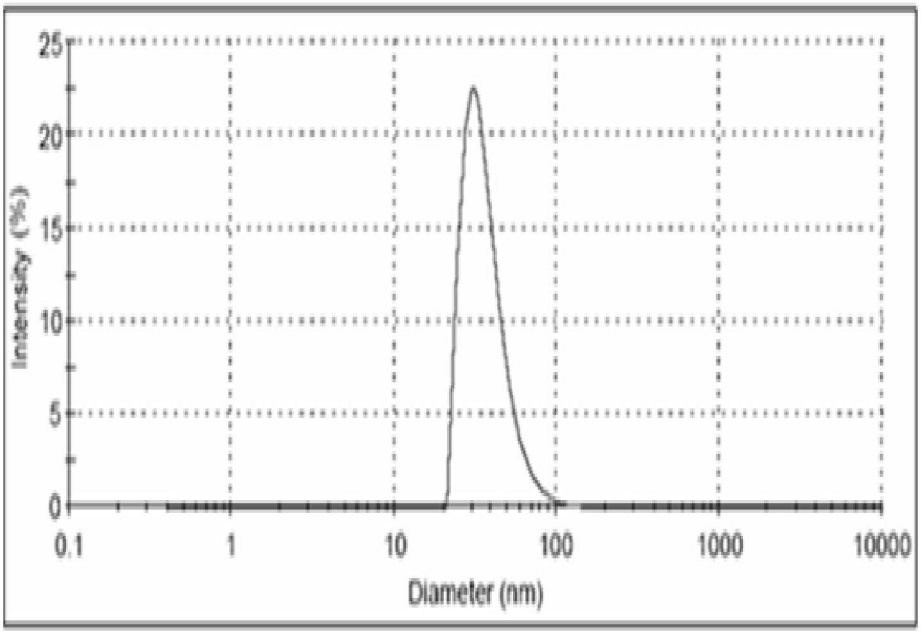

Figure 5: Droplet size and size distribution of nanoemulsion formulation (A3).

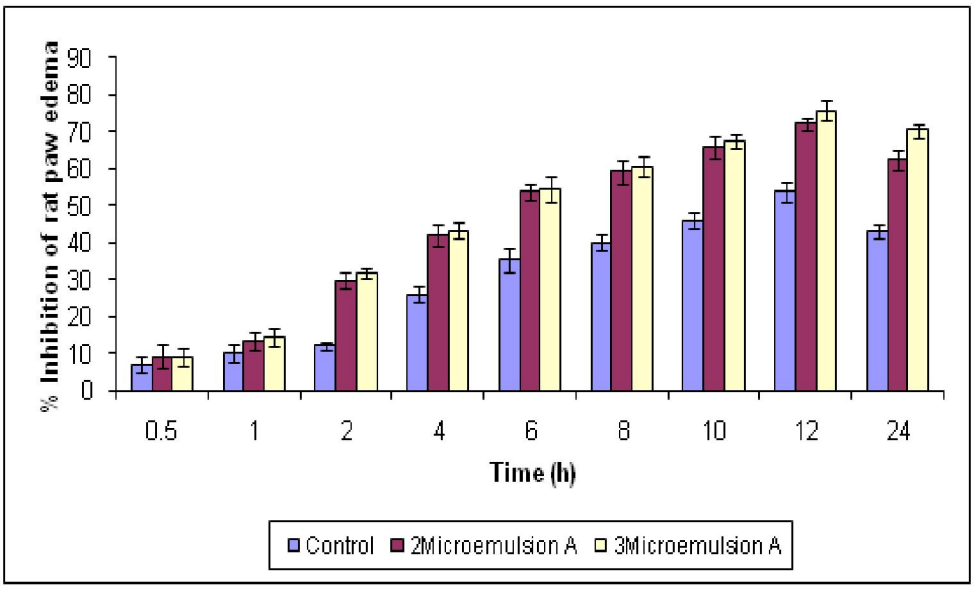

Figure 6: Comparison of anti-inflammatory activity of turmeric oil nanoemulsions A2 and A3 with control

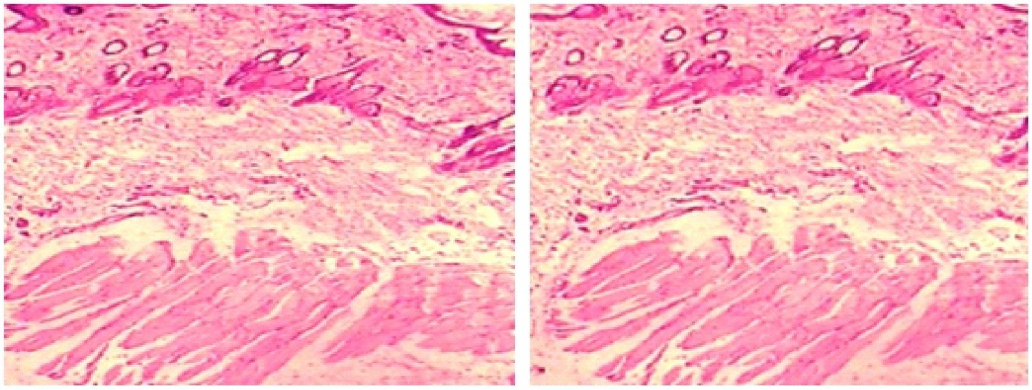

Figure 7: Light power photomicrograph of (a) Control skin (b) Treated skin.

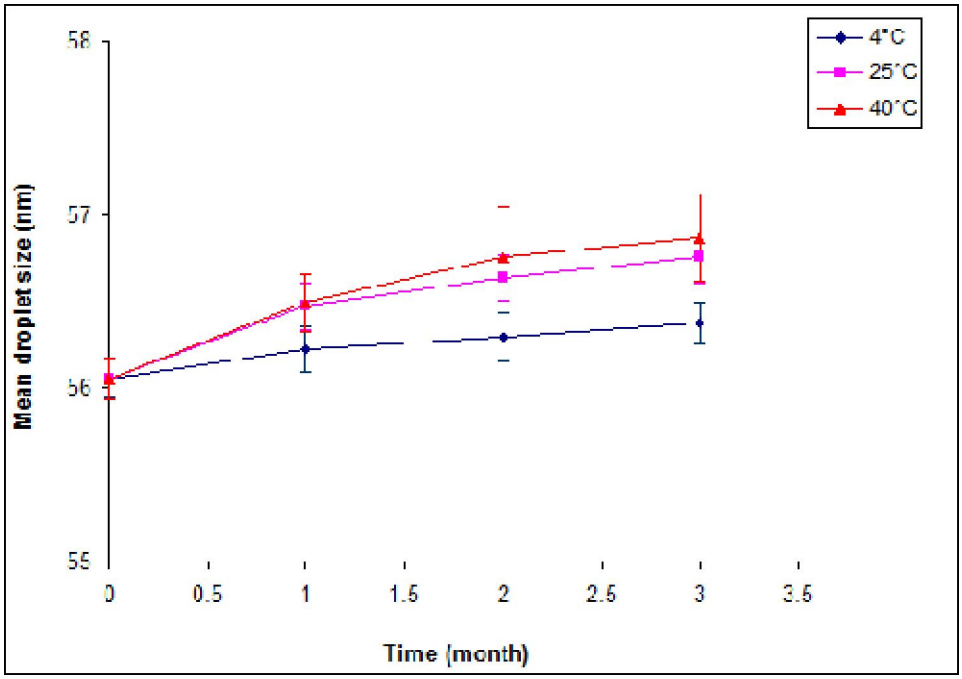

Figure 8: Nanoemulsion mean droplet size under different storage conditions during a 90-day stability test.

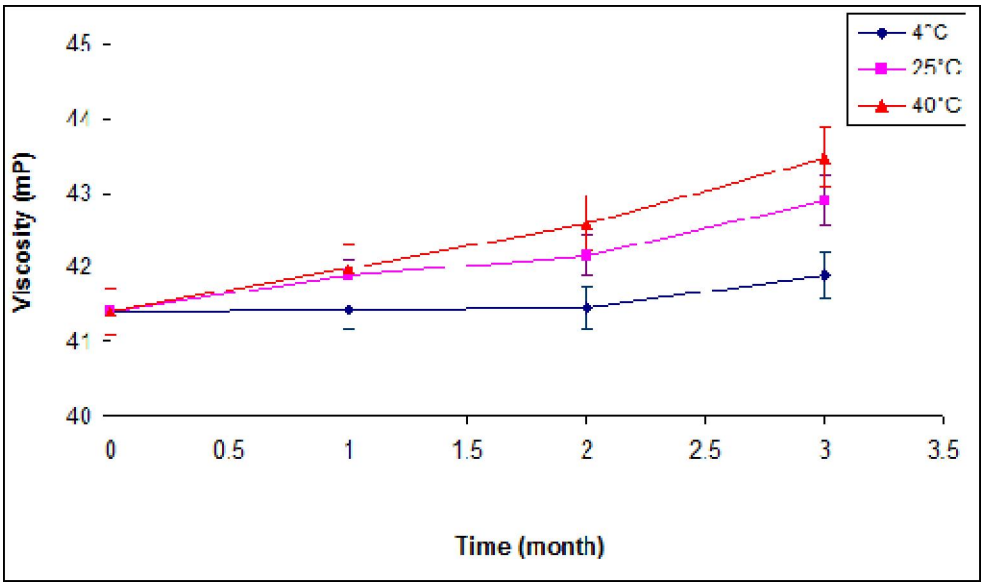

Figure 9: Viscosity of nanoemulsions over time under different storage conditions.

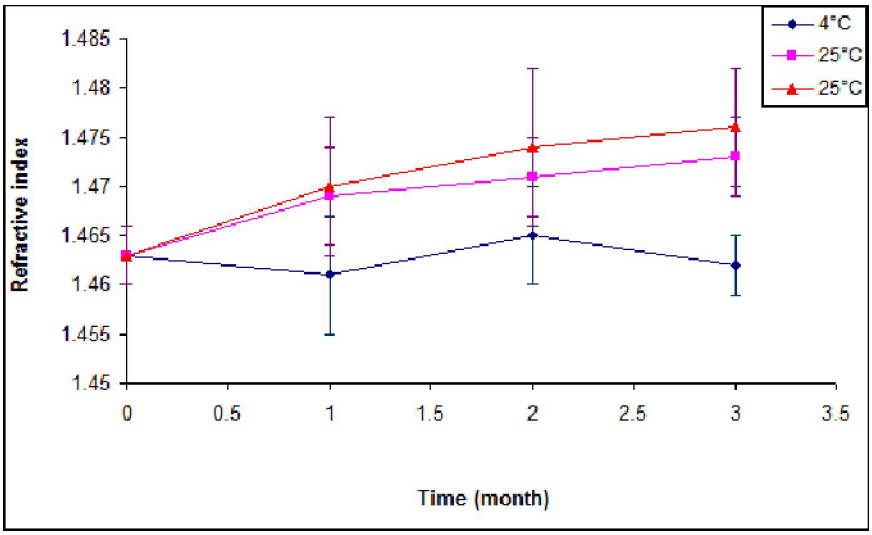

Figure 10: Refrective index of nanoemulsions over time under different storage conditions.

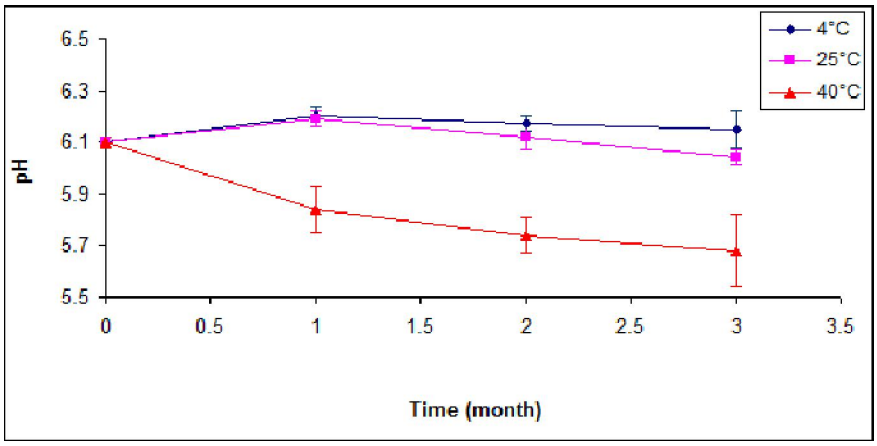

Figure11: pH of nanoemulsions over time under different storage conditions.

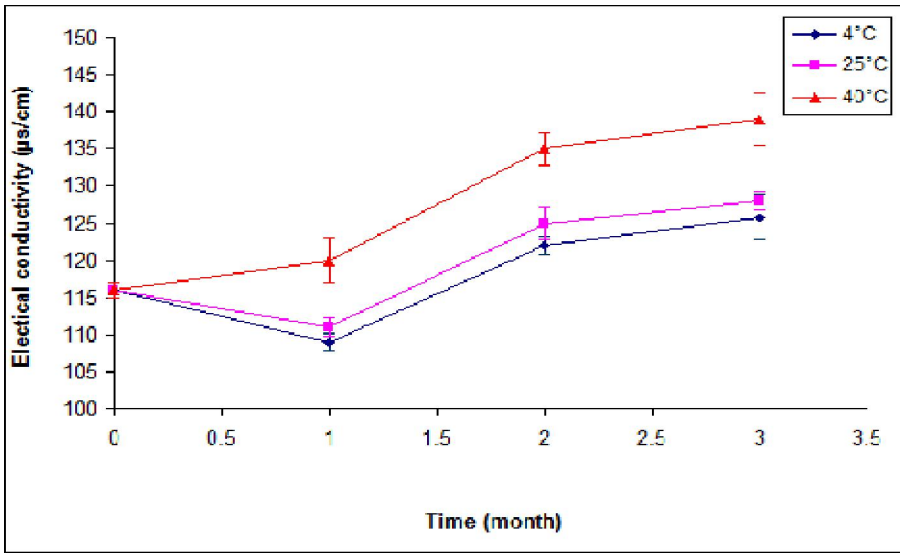

Figure 12: Electrical conductivity of nanoemulsions over time under different storage conditions.

comparisons test using GraphPad Instat software (GraphPad Software Inc., CA, USA). The changes in these parameters were not statistically significant ($P \geq 0.05$). Monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Emulsions produced with vegetable oils may experience a decrease in pH due to the hydrolysis of fatty acid esters into free fatty acid degradation products [41].

The nanoemulsions had stable pH values for almost all conditions tested (Figure 11). Only at a temperature of 40 ± 0.5℃ and 90 days of incubation was there a statistically significant decrease in the pH of the nanoemulsion. The high temperature might have destabilised the nanoemulsion by hydrolysis, but it did not affect the overall quality of the nanoemulsions because the pH values remained around pH 6.0, which is an acceptable, non-skin irritating pH value. The nanoemulsion showed changes in electrical conductivity at all storage conditions (Figure 12). Changes in the electrical conductivity can indicate nanoemulsion instability and may influence the nanoemulsion droplet size. In these studies, changes in electrical conductivity did not affect the nanoemulsion droplet size (Fig 8). It is difficult to assess the emulsion stability solely by electrical conductivity because the relationship between an increase in electrical conductivity and emulsion instability is not linear [42]. Thus, we could not conclusively determine the nanoemulsion's stability by this parameter. However, because the particle size and the pH value did not significantly change across different conditions, we considered our nanoemulsion to be stable.

Summary and Conclusion

For the preparation of nanoemulsion titration method was used which was composed of 15%, turmeric oil, 42 % S_{mix} (1:1) and 43 % distilled water. The nanoemulsion was stable during the period of study and was found to be practically non-irritating in the organotypic HET-CAM model. The non-irritation of the optimized formulation was also confirmed by histopathological study using rat skin. The anti inflammatory activity of optimized nonoemulsion was carried out by carragennen induced paw edema and found to be 70.35 % inhibition. This nanoemulsion could serve as an alternative treatment for skin diseases such as atopic dermatitis and psoriasis.

Authors' contributions

MS Ali carried out the conception and design, participated in the sequence alignment and drafted the manuscript. MS Alam carried out the analysis and interpretation of data and revising it critically for important intellectual content. F Imam participated in animal studies. MR Siddiqui have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

Conflict of Interest

There is no conflict of interest

Acknowledgement

The author's are thankful to Lala Jagdish Prasad & company, Kanpur, India for providing turmeric oil as a gift sample.

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