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ORIGINAL

Investigation of effect of non-ionic surfactant on preparation of griseofulvin non-aqueous nanoemulsion

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Abstract Generally emulsions are water-in-oil or oil-in-water type, but emulsions may contain polar liquid as one of the phase. Non-aqueous emulsions are useful in many situations where presence of water is not desirable, and formulation of active ingredients which undergo hydrolysis or oxidation in the presence of water. The study was to design a stable non-aqueous nanoemulsion (NANE) using cosmetically approved ingredients as a vehicle for the water-sensitive active ingredients. Non-aqueous nanoemulsion was designed to increase the dermal penetration and permeation and study solubility and dermal bioavailability of griseofulvin. For better compliance, the NANEs will be incorporated in cosmetics or personal care products. A non-aqueous system was obtained with glycerin and olive oil stabilized by glycerol monosterate with co-surfactant. It was observed that emulsification behavior is completely unpredictable and conventional theories of emulsification and HLB system cannot be applied here. An optimized NANE was obtained through implementation of pseudo-ternary phase diagram. Pseudo-ternary phase diagram was constructed using surfactant and co-surfactant ratio (1:1, 2:1, 3:1, 4:1) and nanoemulsion region was determined and further characterized for pH, rheology, globule size analysis, zeta potential and stability. Stability studies (agitation, centrifugation, freeze thaw cycle, accelerated stability) were carried out at 5, 25 and 40 °C. Cream was stable at 5 and 25. Results proved that NANE can be used as vehicle for the poorly water-soluble drug, suspension vehicles and oleogels.

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Introduction

The microemulsions can be used to deliver drugs via several routes and their composition and structure enable them to incorporate greater amount of drug than other drug delivery systems [1]. Microemulsions are comparatively thermodynamically stable systems and gained the wide acceptance because of their enhanced drug solubilization, thermodynamic stability, and ease of manufacture [2]. The non-aqueous nanoemulsion (NANE) useful for drug delivery and principally overcomes the problem of slow and incomplete dissolution of poorly water-soluble drugs with water unstable and/or unsavory drug [3–6].

Emulsion is one of the most convenient and advantageous formulations in which one of the liquid phases is water; however, emulsion can be formulated without an aqueous phase to produce anhydrous, non-aqueous or oil-in-oil emulsions/microemulsion [6]. Such systems can replace conventional emulsions where the presence of water has to be avoided [7–12]. Such systems can reduce the inherent limitations and facilitate the formation of solubilized phases from which absorption may occur. Unfortunately, the major difficulty in formulating NANE arises from the lack of appropriate data on surfactant action in relevant non-aqueous media, or indeed, the dearth of suitable surfactant designed for such specialized system [13].

Oil-in-polyhydroxylic solvent microemulsion of poorly water-soluble antifungal drug was designed and developed using olive oil, glycerin and glycerol monosterate to improve the stability and elegancy of NANE formulation [14].

The use of pseudo-ternary phase diagram is required to map the optimal composition range for excipients; this technique is mainly used to map the microemulsion areas [15, 16]. Pseudo-ternary phase diagrams can be used to show the influence of changes in the volume fractions of the different phases on the phase behavior of the system [17].

On the other hand, from the pharmacological point of view, surfactants with low critical micelle concentration (CMC) value have more stable micelles [18].

Surfactants with a high CMC value may dissociate into monomers, their content may precipitate in the blood [19], and they are not suitable for drug delivery. Often the use of co-surfactants is required for the optimal formation of a nano and microemulsion and co-surfactant is often the second surfactant but may also refer to a low-molecular weight amphiphile, such as an alcohol [19]. Co-surfactants increase the flexibility of the surfactant film around the nanoemulsion droplet. The role of the co-surfactant is to overcome the repulsive forces of similar phases and fluidity of the oil and water to increase the permeability of two phases to form a microemulsion [20]. Short- and medium-chain alcohols, such as butanol, pentanol, ethanol, isopropanol, or propylene glycol, are commonly added as co-surfactants.

Nanoemulsions are characterized using dynamic light scattering, polarized light microscopy, electrical conductivity, and rheology [21]. Dynamic light scattering is used to measure nanoscale particles of liquid mediums such as nanoemulsions.

In the present work, the formulation of NANE using olive oil and glycerin is discussed and use of pseudo-ternary phase diagram explored to map the optimal composition range for three excipients and can be used to show the influence of changes in the volume fractions of the different phases on the phase behavior of the system [21]. The precise coexistence/pseudo-ternary curve measurement of a ternary nanoemulsion, consisting of olive oil, GMS, and glycerin, at the critical volume fraction and a constant molar ratio was determined.

Methods

Materials

Analytical grade materials were used for this study. Griseofulvin, Glycerin (Loba Chemical, Mumbai, India), Olive oil (Poona Chemical Laboratory, Pune, India), and Glycerol monostearate (Research Lab Fine Chemical Industry, Mumbai, India) were purchased. Methanol, chloroform, distilled water, phosphate buffer pH (7.4) were also used throughout the study. All other chemicals and reagent were of analytical grade and were used without further purification.

Construction of pseudo-ternary phase diagram

To investigate concentration range of components for the existing boundary of NANE, pseudo-ternary phase diagram was constructed using the titration method.

Titration method

The phase diagram was prepared with the 1:1, 2:1, 3:1, 4:1 weight ratio of Glycerol monostearate/Ethanol as surfactant–co-surfactant mixture. The oil phase and the surfactant mixture (w/w) were mixed with ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. In the resultant mixtures, glycerine was added dropwise till the first sign of turbidity appeared and then clear solution after equilibrium was observed to identify the end point. After equilibrium was reached, the mixtures were checked visually for phase clarity and flowability. The resultant emulsion with a clear or bluish appearance, exhibiting good stability (being stable after centrifugation for 10 min at 2000 rpm) and flow ability was defined as a nanoemulsion [22]. Ternary phase diagrams were constructed using Chemix School 9.0 Software. The compositions are expressed in Table 1.

Formulation of nonaqueous nanoemulsion (NANE)

A wide range of oils and surfactants (Table 1) were screened for the formulation of NANE. The NANE contains lower amount of surfactant having better stability, so the optimized formula of NANE loaded with Griseofulvin 0.5 % (w/w) in 70 % of olive oil, required quantity of different Smix (GMS and ethanol) ratio was added to oil phase and mixture was make up to 100 % (w/w) with slow addition of glycerin with continuous stirring using Ultraturrex homogenizer (Unique Biological, India) (5000–6000 rpm) [23, 24] with gentle heating. All the samples were stirred for 24 h at 5000 rpm using Ultraturrex homogenizer. After phase preparation, nanoemulsion area was identified by visual inspection (a nanoemulsion is optically clear and transparent) and polarized light microscopy (Labomed, India). In each system, samples of the NANE area were separated, and the o/g area of the pseudo-ternary phase diagram was plotted using Chemix School (3) software [25].

Globule size analysis

Globule size analysis of NANE was carried out through Beckman coulter counter (Malvern size Analyzer,

Table 1 Effect of type of surfactant and their combinations on emulsification of o/g and g/o non aqueous nanoemulsion (NANE)

Type of surfactant	Appearance of non aqueous nanoemulsion	Stability of NANE	Type of NANE
Tween 20	M	+	–
Tween 40	M	+	–
Tween 60	M	+	–
Tween 80	M	+	–
Span 20	O	++	o/g
Span 40	O	++	o/g
Span 60	O	++	o/g
Span 80	O	+++	o/g
Span 85	M	+++	o/g
SLS	C	–	–
GMS	C	+++	g/o
Tween 80 + Span 80	M	+	o/g
Tween 80 + Span 40	O	–	
Tween 60 + Span 80	O	–	
Tween 60 + Span 40	O	–	
Tween 60 + Span 20	–	#	
Tween 80 + Span 20	–	#	
Tween 20 + Span 20	–	#	

Emulsification/stable: no phase separation for at least 7 days

NO emulsification, – phase separation, + phase separation within 1 week, ++ stable up to 3 weeks, +++ stable, *C* cream like consistency, *O* opaque, *M* milky, *o/g* mineral oil-in-glycerin type of non aqueous nanoemulsion (NANE), *g/o* glycerin-in-mineral oil type of non aqueous nanoemulsion (NANE)

Germany) based on the laser diffraction phenomenon. During a laser diffraction experiment, particles are illuminated in a collimated laser beam, causing the light to be scattered in a variety of directions. Larger globules brought a high intensity of scattering at low angles to the beam and smaller particles, and create a low-intensity signal at far wider angles. This angular scattering was measured with specially designed detectors and particle size distribution is resolute [26–28]. The polydispersity index (PI) of NANE gives an indication of width of the size distribution of particle population in the nanoemulsion.

Stability study

The chemical and physical stability of the NANE was subjected to stability study (Remi Corp., Mumbai). The thermodynamic stability of NANE was resolute with centrifugation at 3500 rpm and 25 ± 1 °C for 30 min. Non-aqueous nanoemulsion was examined for changes in color, viscosity and drug content for the period of 3 months [29–32].

Agitation test

Accurately weighed 5 gm of NANE was placed in reciprocating shaker (Remi Corp., Mumbai) at 60 cycles/min for 24 h at room temperature. After stipulated period cream was observed for any signs of phase separation [29].

Centrifugation test

Accurately weighed 5 g of NANE was centrifuged (Remi Corp, Mumabi) at 3500 rpm for 30 min. After stipulated period, cream was observed for any signs of phase separation [30].

Freeze–thaw cycles

The NANE was kept at −10 and 25 °C for 48 h and observed for phase separation and viscosity (Brookfield Rheometer R/S, Germany) after three freeze–thaw cycles' separation [31].

Drug content

The Griseofulvin NANE was evaluated for its drug content using methanol as blank at 291 nm [31, 32]. 0.25 g of NANE was dissolved in 50 mL of methanol, and sonicated for 30 min. The sample was extracted with methanol and analyzed at 291 nm using UV–Visible Spectrophotometer (Shimedazu Corp., Japan).

Results and discussion

Screening of surfactant

Conventional emulsion shows better stability when having the optimum HLB with hydrophilic and lipophilic groups [4]. Initially, a combination of surfactants was screened using hydrophilic and hydrophobic surfactants in 1:1 ratio. But, it was observed that single surfactant shows more stability in formulation of NANE than the combination of surfactants (Table 1). All surfactants produce stable nanoemulsion, viz. Tween 20, 40, 60 and 80 and able to formulate a NANE having milky appearance at 10 % surfactant concentration but phase separation occurred within a week. Hydrophobic surfactants were found to be more efficient; Span 20 and 40 produced emulsions which were stable up to 3 weeks. Span 80 and 85 produced NANE at even lower surfactant concentrations which were stable for a period more than 4 weeks. So these formulations were considered as stable for formulating NANE of griseofulvin.

Critical micelle concentration

Critical micelle concentration is not a point concentration, but a range below CMC surface active agent is preferentially adsorbed mainly at air–water interface as monomer [5]. When surfactant monomer encounters the polyhydroxylic environment, polyhydroxylic molecule rejects the water-insoluble hydrocarbon tail of amphiphiles. The tail directs away from the polar solvent, while the hydrophilic head is attached by polar solvent molecule by electrostatic attraction force. As the concentration increases in the interface, the bulk phase becomes saturated with monomer. As the concentration of surfactant in solution increases, the surface tension decreases. The CMC was measured and 2×10^{-4} was found satisfactory.

Pseudo-ternary phase diagram

The chemix school (3) pseudo-ternary phase diagram was used for the selection of optimized batch on basis of emulsification region. The phases were identified by visual inspection. A nanoemulsion is optically clear and transparent and the samples with transparent appearance were separated for further investigations.

Pseudo-ternary phase diagrams were constructed using olive oil, GMS (surfactant) and ethanol (co-surfactant) mass ratio (Figs. 1, 2, 3, 4). It was observed that the surfactant alone was ineffective in reducing the g/o interfacial tension enough to provide a NANE with desirable properties.

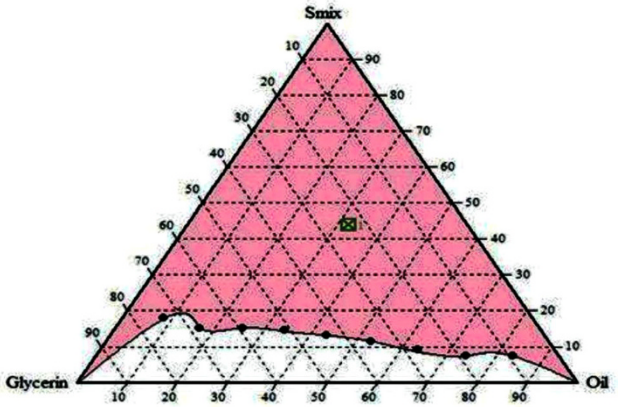

Fig. 1 Pseudo-ternary phase diagram of the griseofulvin non-aqueous nanoemulsion (NANE) region with combination of co-surfactant (ethanol) and surfactant (GMS), i.e., Smix; (a) Smix ratio 1:1

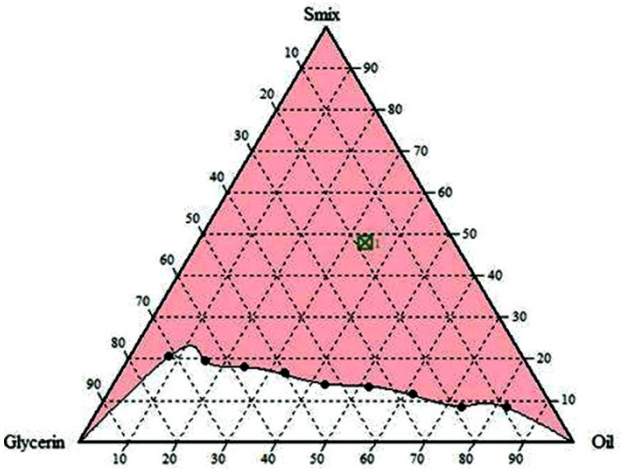

Fig. 2 Pseudo-ternary phase diagram of the griseofulvin non-aqueous nanoemulsion (NANE) region with combination of co-surfactant (ethanol) and surfactant (GMS), i.e., Smix; (b) Smix ratio 2:1

Effect of surfactant and co-surfactant ratio on NANE

A large NANE was obtained through the surfactant-rich apex, maximum concentration of oil that could be solubilized and increased amount of co-surfactant with respect to surfactant. In Smix 1:1 ratio maximum amount of oil that could be solubilized was 15 % (w/w). This might be due to the incorporation of co-surfactant and resembles for enhanced penetration of the oil phase in the hydrophobic zone of the surfactant monomer, which in turn reduced the interfacial tension and increased the flexibility and fluidity of the interface, ultimately leading to increased entropy of the system.

When co-surfactant ratio reaches up to 2:1, the total area of NANE decreased. Therefore, as the surfactant ratio increased, and nanoemulsion area decreased. In contrast

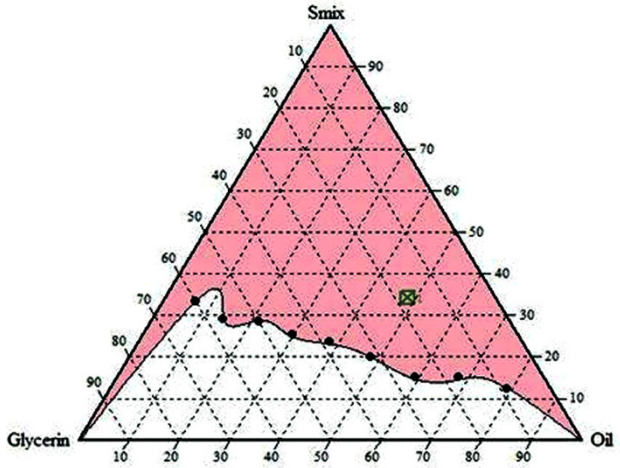

Fig. 3 Pseudo-ternary phase diagram of the griseofulvin non-aqueous nanoemulsion (NANE) region with combination of co-surfactant (ethanol) and surfactant (GMS), i.e., Smix; (c) Smix ratio 3:1

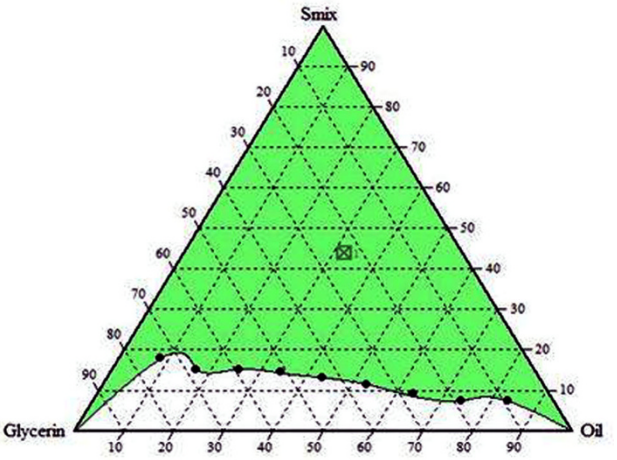

Fig. 5 Pseudo-ternary phase diagram of optimized griseofulvin non-aqueous nanoemulsion (NANE) region with co-surfactant (ethanol) and surfactant (GMS)

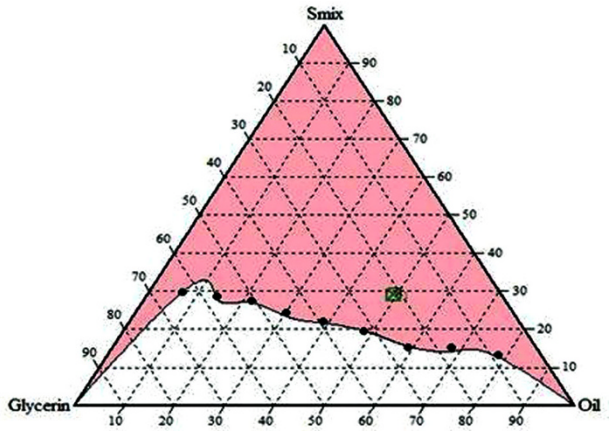

Fig. 4 Pseudo-ternary phase diagram of the griseofulvin non-aqueous nanoemulsion (NANE) region with combination of co-surfactant (ethanol) and surfactant (GMS), i.e., Smix; (d) Smix ratio 4:1

when surfactant concentration of Smix was increased from 1:1, 2:1, 3:1, 4:1, depletion in nanoemulsion region was observed. It might be because of insufficient co-surfactant concentration, resembling for reduction in an interfacial tension and provides the flexibility to the interface and nanoemulsion region. The literature [5] also supports that the Smix 1:1 possesses the maximum NANE area as compared to the other ratio indicating that surfactant and co-surfactant mass ratio has effect on phase ratio (Fig. 5).

Globule size analysis

A key distinctive property of nanoemulsion is its nanoscale particle size. The size distribution analysis of selected NANE was performed using Malvern nanosizer. A graphical representation of particle size distribution of freshly prepared griseofulvin loaded NANE (Fig. 6) shows a broader globule size distribution and globule size intensity ranges from 5.59 to 33.63 nm. The droplet size resembles for rate and extent of drug release, absorption and stability of NANE.

As the distance from the surface of nanoemulsion increases, the potential gradually decreases. The zeta potential can be related to the stability of colloidal dispersions for molecules and particles that are small enough; a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds the repulsion and dispersion will break out to flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. So the nanoemulsion shows the zeta potential −0.787 having low potential but having good stability.

Stability studies

Non-aqueous nanoemulsion droplets exhibit Brownian movement and no coalescence of droplets takes place unless droplets impinge upon each other owing to their Brownian movement. Agitation can contribute to the energy with which two droplets impinge upon each other.

After agitation on a reciprocating shaker for 24 h, there was no phase separation in NANE indicating that it has good stability and can withstand the mechanical forces during the transportation and handling.

Centrifugation was carried out to examine the effect of gravity on the NANE. NANE showed no phase separation after centrifugation for 30 min at 3500 rpm indicating that cream has a good stability over the gravitational forces.

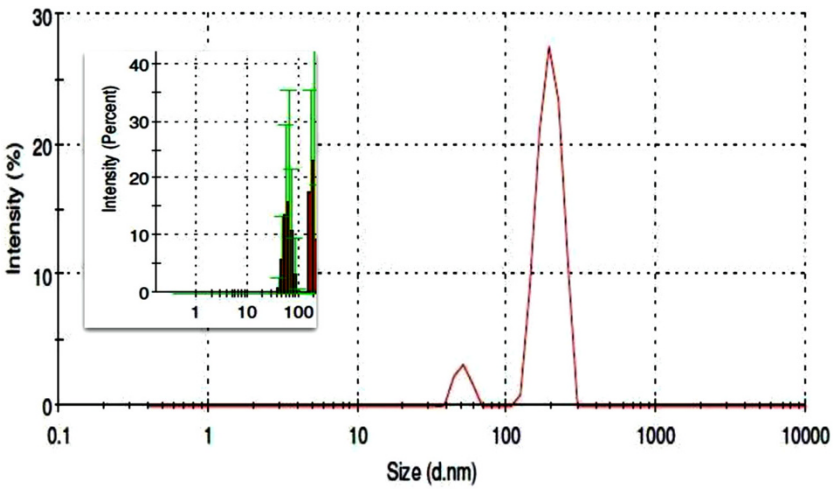

Fig. 6 Effect of % cumulative intensity (D10, D20, D30 and D40) on globule size distribution of optimized non-aqueous nanoemulsion (NANE) of griseofulvin

Freeze–thaw cycle is a stability test in which emulsions are subjected to two extreme temperature conditions. For the freeze–thaw cycling, samples were placed alternately at −10 and 25 °C for 48 h at each temperature. There was no phase separation after three freeze–thaw cycles indicating a good thermal stability of the formulation. Also there were no significant changes in viscosity measured before and after performing the test.

Non-aqueous nanoemulsion was monitored for changes in color, viscosity and drug content for the period of 3 months. During studies, formulation was kept at low-temperature (5 °C), moderate-temperature (25 °C) and high-temperature (40 °C) conditions.

It was observed that formulation was not sensitive to the low temperature. Drug content of the NANE was found to be decreased from 98.13 ± 0.56 to 97.50 ± 0.35 % within 3 months at 5 °C with no significant change in the chemical composition of the formulation. But there were some extent of changes in the viscosity of the formulation. Initial viscosity of the formulation was found to be 11043cPs up to 1 month; thereafter decrease in the viscosity was 10351 cPs. Initial increase in viscosity might be due to the gelation, because GMS at higher concentration causes the gelation. Color of the formulation did not change at the low temperature. So it was observed that NANE was stable at low temperature.

Stability study at 25 °C observed for color, drug content and viscosity. There was decrease in the drug content at 25 °C from 98.75 ± 0.13 to 97.50 ± 0.13 % within 3 months. As seen in the case of low temperature, there was an initial increase in viscosity of 11043 cPs followed by a decrease up to 9396 cPs (Fig. 7). Greater decrease in the viscosity was observed due to increase in the temperature. Also there was change in the color of the formulation; color was changed from fresh white to dull white.

At 40 °C, a distinct phase separation occurs within 48 h because of rise in temperature resembling for decrease in viscosity of formulation, leads to formation of larger globule sizes; therefore, stability study at higher temperature was terminated. From the stability studies, it was evident that NANE is stable at moderate and low temperatures. The globule size of NANE and aqueous formulation was found to be significant throughout the stability study.

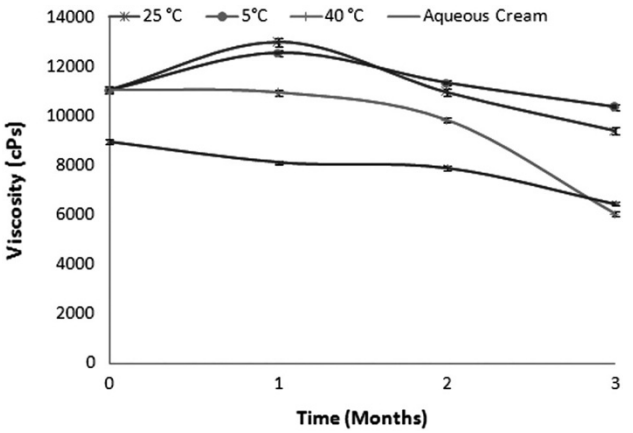

Fig. 7 Effect of changes in viscosity on conventional and non-aqueous nanoemulsion (NANE) of griseofulvin during stability study at 5, 25 and 45 °C at 3 months period

Conclusion

During formulation, it was found that emulsification was achieved when single surfactant was used, rather than surfactant combination, and hydrophobic surfactants was found to be more efficient than hydrophilic surfactants. Stable NANE can be obtained using glycerin as dispersed phase, olive oil as continuous phase and glycerol monostearate as surfactant. This emulsion has improved the stability of griseofulvin.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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