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Study of surfactant combinations and development of a novel nanoemulsion for minimising variations in bioavailability of ezetimibe

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ABSTRACT

The present study aimed at developing an optimal nanoemulsion of ezetimibe and evaluating its stability, pharmacodynamic and pharmacokinetic potential. Solubility of ezetimibe was determined in various vehicles. Surfactants and cosurfactants were grouped in two different combinations to construct pseudoternary phase diagrams. Formulations were selected from the o/w nanoemulsion region and were subjected to various thermodynamic stability and dispersibility tests. Optimized formulations were characterized for their percentage transmittance, refractive index, viscosity, droplet size and zeta potential. Release rate of optimized formulations was determined using an *in vitro* dissolution test. The formulation used for assessment of lipid lowering potential and bioavailability contained Capryol 90 (10%, v/v), Tween 20 (33.33%, v/v), PEG 400 (16.67%, v/v), double distilled water (40%, v/v). The release of drug from the nanoemulsion formulations was extremely significant ($p < 0.001$) in comparison to the drug suspension. More than 60% of the drug was released in the initial 1 h of the dissolution study in comparison to the drug suspension. The value of total cholesterol in the group administered with the formulation PF1 was highly significant ($p < 0.001$) with respect to the group administered with the suspension of the drug. The plasma concentration time profile of ezetimibe from nanoemulsion represented greater improvement of drug absorption than the marketed formulation and simple drug suspension. The shelf life of the nanoemulsion was found to be 5.94 years at room temperature. The present study established nanoemulsion formulation to be one of the possible alternatives to traditional oral formulations of ezetimibe to improve its bioavailability.

1. Introduction

Ezetimibe is the first member of a novel class of lipid lowering agents that selectively inhibits the absorption of biliary and dietary cholesterol as well as related phytosterols from the intestine without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids. Ezetimibe is a class II molecule as per Biopharmaceutics Classification System (BCS). It is a highly lipophilic molecule having log *p* (octanol/water) of 4.5. Due to its highly hydrophobic character, ezetimibe exhibits highly erratic and very low-dissolution profile in the gastrointestinal fluids [1]. Its bioavailability is highly variable [2]. Moreover, its absolute bioavailability cannot be determined since the drug is virtually insoluble in aqueous media suitable for injection. Ezetimibe exhibits moderate intersubject variability in its pharmacokinetics with coefficient of variation ranging from 34% to 43% and 32% to 37% for C_{max} and AUC respectively [3]. Researchers have tried various methods to overcome these limitations [1,4,5]. It was hypothesized that formulating a nanoemulsion would help to reduce the intersubject variability observed in the pharmacokinetics of ezetimibe since nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible [6,7].

Ezetimibe being a class II molecule as per BCS, solubility of the drug will be the limiting step in the absorption process. In earlier studies, the *in vitro* drug release studies on the optimized formulation have been reported in 0.5% (w/v) solution of sodium lauryl sulphate at 75 rpm [4,5]. This may lead to confounding interpretation of the release profile since in the presence of a wetting agent, the exact solubilisation potential of the system developed with the aim of increasing solubility of a poorly water-soluble drug may not be fully exhibited. In order to have better interpretation of the relationship between the phase behaviour of the mixture and its composition and to aid in the better selection of the formulation, a more exhaustive study was carried out where the concentration of both oil and S_{mix} (mixture of surfactant and cosurfactant in specific volume ratios) were varied from 10% to 90% (v/v) in comparison to previously reported studies involving only 10–30% (w/w) of oil [4,5]. Significant increase in the mean globule size of formed nanoemulsion upon dilution reported in earlier studies could adversely affect the release of the drug from the developed

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formulation when the formulation undergoes infinite dilution in the GI fluids. The reported decrease in the rate of dissolution of the self nanoemulsifying formulation filled in capsules may lower the potential of the developed system as compared to a liquid nanoemulsion since by administering a poorly water-soluble compound in a dissolved state and in a liquid formulation, one is able to reduce the energy associated with the solid–liquid transition. Moreover, as a drug delivery system, the preconcentrate has its own limitations as a viable dosage form. The most significant one is that the dosage form uses a large amount of surfactants for the purpose of forming nanoemulsions. This has posed clinical liabilities as surfactants often have potential toxic effects when used at high levels. It has been revealed by literature review that the studies conducted so far on the nanoemulsion based drug delivery systems of ezetimibe are restricted to the pharmacodynamic evaluation of the developed system. In addition to the pharmacodynamic evaluation of the nanoemulsion formulation, the present study also reports pharmacokinetic study in rats to precisely delineate the plasma concentration time profile of ezetimibe after oral administration of developed nanoemulsion formulation. Furthermore, the present work also reports stability studies on the optimized nanoemulsion formulation to evaluate the integrity of the formulation. Such studies have not been reported till date in the literature pertaining to the nanoemulsion based drug delivery systems of ezetimibe. An attempt has also been made to predict shelf life of the developed nanoemulsion formulation by performing accelerated stability studies.

Thus, the objectives of the present study were to develop and characterize an optimal nanoemulsion formulation of ezetimibe using minimum surfactant concentration with an aim to increase the solubility of ezetimibe and reduce the variations in bioavailability. Other objectives were to assess the lipid lowering potential, pharmacokinetic parameters and shelf life of the developed nanoemulsion formulation.

2. Materials and methods

2.1. Materials

Ezetimibe was obtained as a gift sample from Lupin Ltd. (Pune, India). Labrafac (propylene glycol dicaprylocaprylate), Labrasol (caprylo caproyl macrogol-8-glyceride), Lauroglycol 90 (propylene glycol monolaurate), Lauroglycol FCC (propylene glycol laurate), Capryol 90 (propylene glycol monocaprylate), Maisine (glyceryl monolinoleate), Labrafil 1944 CS (oleoyl macrogoglyceride) and Transcutol® P (diethylene glycol monoethyl ether) were donated by Gattefosse (Saint Priest, Cedex, France) and were used as received. Sefsol 218 (propylene glycol mono caprylic ester) was a gift sample from Nikko Chemicals (Tokyo, Japan). Cremophor EL (polyethoxylated castor oil) was obtained from BASF (Mumbai, India). Triacetin (Glycerol triacetate), Tween 80 (polyoxyethylene sorbitan monooleate), Tween 20 (polyoxyethylene sorbitan monolaurate), and PEG (polyethylene glycol) 400 were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Water was obtained from Milli-Q-water purification system (Millipore, MA, USA). All other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1. Solubility studies

Solubility of ezetimibe in various components (oils, surfactants and cosurfactants) was determined by adding an excess amount of drug in 2 ml of selected vehicle in 5 ml capacity stopperred vials, and mixed using a vortex mixer (Nirmal International, Delhi, India). These vials were then kept at 25 ± 1 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min (REMI International, Mumbai, India). The supernatant was taken and filtered through a 0.45 μm filter. The concentration of drug was determined using HPLC at 232 nm.

2.2.2. Construction of pseudoternary phase diagrams

On the basis of solubility studies, Capryol 90 was used as the oil phase for the development of nanoemulsion. Tween 20 and Labrasol were used as surfactant along with PEG 400 and Transcutol® P as the cosurfactant. Double distilled water was used as the aqueous phase. Oil, surfactant and cosurfactant were grouped in two different combinations for phase studies. One combination (group I) comprising of Capryol 90, Labrasol and Transcutol® P as the oil, surfactant and cosurfactant respectively was selected to have a high HLB surfactant with a low HLB cosurfactant. Other combination (group II) comprising of Capryol 90, Tween 20 and PEG 400 as the oil, surfactant and cosurfactant respectively was selected to have a high HLB surfactant with a high HLB cosurfactant. Surfactant and cosurfactant were mixed (S_{mix}) in different volume ratios (1:1, 1:2, 1:3, 2:1, 3:1 and 4:1). For each phase diagram, oil and specific S_{mix} ratio was mixed well in different volume ratios ranging from 1:9 to 9:1. 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. Phase diagrams were developed using aqueous titration method. Physical state of the nanoemulsion was marked on a pseudo three component phase diagram with one axis representing the aqueous phase, one representing oil and the third representing a mixture of surfactant and cosurfactant at fixed volume ratio (S_{mix}) [8] using PCP Disso V2.08 software (Anant Ketkar, Vinay Patil and A.R. Paradkar, Pune, Maharashtra, India). To determine the effect of drug addition on the nanoemulsion boundary, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the oil phase.

2.2.3. Selection of formulation

From each of the phase diagram constructed, different formulations were selected from the nanoemulsion region based on the following criteria:

(1) The most frequently used dose of ezetimbe is 10 mg. Therefore, 10 mg was selected as the dose for the development of nanoemulsion formulation.
(2) The concentration of oil should be such that it is able to dissolve 10 mg of the drug easily.
(3) From each phase diagram, different concentration of oil which could solubilise 10 mg of ezetimibe was selected at a difference of 5% (10%, 15%, 20%, 25% and 30%) to prepare 2 ml of nanoemulsion.
(4) The emphasis for the selection of formulations from the phase diagram was given to the minimum concentration of S_{mix}.

2.2.4. Thermodynamic stability studies

2.2.4.1. Centrifugation study. The prepared formulations were centrifuged (REMI International, Mumbai, India) at 5000 rpm for 30 min and observed for phase separation, creaming or cracking. The formulations which did not show any instability (creaming, cracking, phase separation) were selected and subjected to heating–cooling cycle.

2.2.4.2. Heating–cooling cycle. It was used to see the effect of variations in temperature on the stability of nanoemulsions. Six cycles between 4 and 40 °C with storage at each temperature for not less than 48 h were performed. The formulations that were found to be stable at these temperatures were subjected to freeze–thaw stress test.

Table 1
Observation table of dispersibility study.

S. no.	Observation	Grade
1	Rapidly forming (within 1 min) nanoemulsion, having a clear or slight bluish appearance	A
2	Rapidly forming, slightly less clear nanoemulsion, having a bluish appearance	B
3	Fine milky emulsion that formed within 2 min	C
4	Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min)	D
5	Formulation exhibiting either poor or minimal emulsification with large oil globules present on the surface	E

2.2.4.3. Freeze–thaw cycle (accelerated ageing). In this study formulations were subjected to three freeze–thaw cycles at temperatures between −21 and +25 °C with storage at each temperature for not less than 48 h. The formulations that passed these tests were selected for the dispersibility study in order to estimate the efficiency of dispersibility.

2.2.5. Dispersibility studies

The efficiency of dispersibility of oral nanoemulsion was assessed using a standard USP XXII dissolution apparatus 2 (Veego, Mumbai, India). One ml of each formulation was added to 500 ml of distilled water and 0.1N HCl respectively at 37 ± 0.5 °C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation [8]. The *in vitro* performance of the formulations was visually assessed using the grading system shown in Table 1 and the results of dispersibility studies are shown in Tables 2 and 3.

One formulation was selected from each S_{mix} ratio used, having the least S_{mix} concentration and passing the dispersibility test in Grade A in distilled water as well as in 0.1N HCl. The selected formulations were prepared by dissolving the drug (10 mg) in the oil, adding the respective S_{mix} ratio and mixing on a vortex mixer. Finally the aqueous phase was added and the mixture was mixed on the vortex mixer again to yield the nanoemulsion. The composition of the optimized formulations is given in Table 4.

2.2.6. Characterization of nanoemulsion

2.2.6.1. Percentage transmittance. Percentage transmittance of the prepared nanoemulsion formulations was determined spectrophotometrically using Shimadzu UV–vis spectrophotometer (Shimadzu, Japan). One ml of the formulation was diluted 100 times using methanol and analyzed at 232.6 nm using methanol as blank (Table 4).

2.2.6.2. Refractive Index. Refractive index of selected formulations was determined using an Abbe type refractrometer (Table 4).

2.2.6.3. Viscosity. The viscosity of the prepared nanoemulsion formulations was determined as such without dilution by R/S CPS Plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using spindle # C 50-1 at 25 ± 0.5 °C. The software used was RHEO3000. One ml of the formulation was used for viscosity determination. The speed of the spindle was adjusted to 70 rpm and a single run was performed at a temperature of 25 ± 0.5 °C. Wait time for the operation was 50 min. Shear rate applied was 413 per min and diameter of the spindle used was 50 mm.

2.2.6.4. Droplet size analysis (particle size distribution). Droplet size of the prepared nanoemulsion was determined using dynamic light scattering, which analyzed fluctuations in the intensity of light scattering due to Brownian movement of the particles [9]. The formulation (0.1 ml) was dispersed in 50 ml (500 times dilution) of double distilled water in a volumetric flask and gently mixed by inverting the flask. Dynamic light scattering was performed at 25 °C, using He–Ne laser having wavelength 632.8 nm at an angle

Table 2
Dispersibility study of different formulations selected from group I (Fig. 1a–g).

S_{mix}[a] ratio (S:CoS)	Percentage v/v of different components in formulation			Observations based on the dispersibility studies		Inference
	Oil	S_{mix}	Water	Distilled water	0.1N HCl	
1:0 Fig. 1(a)	10	40	50	Grade A	Grade B	Failed
	10	50	40	Grade A	Grade B	Failed
2:1 Fig. 1(e)	10	40	50	Grade A	Grade A	Passed
3:1 Fig. 1(f)	10	40	50	Grade A	Grade A	Passed
	10	50	40	Grade A	Grade A	Passed
	15	35	50	Grade A	Grade B	Failed
4:1 Fig. 1(g)	10	40	50	Grade A	Grade A	Passed
	10	45	45	Grade A	Grade A	Passed
	10	50	40	Grade A	Grade A	Passed

[a] S_{mix} indicates mixture of surfactant and cosurfactant in specific volume ratio, S indicates surfactant, CoS indicates cosurfactant.

Table 3
Dispersibility study of different formulations selected from group II (Fig. 2a–g).

S_{mix}[a] ratio (S:CoS)	Percentage v/v of different components in formulation			Observations based on the dispersibility studies		Inference
	Oil	S_{mix}	Water	Distilled water	0.1N HCl	
1:1 Fig. 2(b)	10	50	40	Grade A	Grade B	Failed
	25	50	25	Grade C	Grade C	Failed
1:2 Fig. 2(c)	10	50	40	Grade A	Grade B	Failed
	15	45	40	Grade C	Grade E	Failed
1:3 Fig. 2(d)	10	50	40	Grade A	Grade B	Failed
	25	50	25	Grade D	Grade E	Failed

[a] S_{mix} indicates mixture of surfactant and cosurfactant in specific volume ratio; S indicates surfactant; CoS indicates cosurfactant.

Table 4
Composition, mean (±S.D., $n = 3$) percentage transmittance, refractive index, viscosity, droplet size, polydispersity index and zeta potential of optimized formulations.

Code	S_{mix} ratio	Percentage v/v of different components in formulation				Oil:S_{mix} ratio	Mean percentage transmittance ± S.D.	Mean refractive index ± S.D.	Mean viscosity (cP) ± S.D.	Mean droplet size ± S.D. (nm)	PDI[a] ± S.D.	Mean zeta potential ± S.D. (mV)
		Oil	S	CoS	Water							
PF1	2:1	10	33.33	16.67	40	1:5	99.85 ± 0.005	1.412 ± 0.003	25.85 ± 0.80	46.53 ± 8.24	0.177 ± 0.011	−27.46 ± 3.97
PF2	3:1	10	30	10	50	1:4	99.63 ± 0.03	1.415 ± 0.001	41.41 ± 1.96	128.46 ± 29.27	0.227 ± 0.030	−22.47 ± 1.21
PF3	4:1	10	32	8	50	1:4	99.38 ± 0.03	1.418 ± 0.002	49.92 ± 3.74	161.83 ± 29.53	0.182 ± 0.011	−19.83 ± 0.27

Oil used: Capryol 90, surfactant used: Tween 20, cosurfactant used: PEG 400, aqueous phase: double distilled water.
[a] PDI indicates polydispersity index.

of 90° on a digital correlator (Photocor Instruments Inc., MD, USA). The results are shown in Table 4.

2.2.6.5. Determination of zeta potential. The formulation (0.1 ml) was diluted 100 times using double distilled water and analyzed using zeta potential measuring instrument, ZC-2000 (Zeecom-2000, Microtec Co. Ltd., Chiba, Japan) (Table 4).

2.2.6.6. Transmission electron microscopic (TEM) analysis. Transmission electron microscopic (TEM) analysis was done to determine the shape of the dispersed phase. A drop of diluted nanoemulsion was applied to a 300 mesh copper grid and was left for 1 min. After this the grid was kept inverted and a drop of phosphotungstic acid (PTA) was applied to the grid for 10 s. Excess of PTA was removed by absorbing on a filter paper and the grid was analyzed using the Morgagni 268D (FEI Company, OR, USA) operated at 60–80 kV at 1550× magnification.

2.2.7. *In vitro* drug release study

In vitro drug release test was performed in dissolution apparatus 2 (Veego, Mumbai, India) containing 500 ml of double distilled water at 37 ± 0.5 °C. The speed of the paddle was adjusted to 50 rpm. Two ml of the nanoemulsion formulation (containing 10 mg of ezetimibe) was placed in treated dialysis bag (MWCO 12,000 g/mole; Sigma, St. Louis, USA) and 2 ml sample was withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h) and same amount of double distilled water was replaced [10]. The withdrawn samples were analyzed for the drug content using HPLC at 232 nm. The release of drug from different optimized nanoemulsion formulations was compared with that of the pure drug suspension.

2.2.8. *Pharmacodynamic studies*

Approval to carry out pharmacodynamic studies was obtained from the Jamia Hamdard Institutional Animal Ethics Committee (IAEC), New Delhi and their guidelines were followed throughout the study. Albino Wistar rats were used for the pharmacodynamic study and were divided into four groups with six animals in each group. Control group received distilled water, model (toxic control) group received cholesterol, standard group received drug suspension along with cholesterol and test group received formulation PF1 along with cholesterol. The animals were kept under standard laboratory conditions, temperature at 25 ± 2 °C and relative humidity (RH) of 55 ± 5%. The animals were housed in polypropylene cages, six per cage with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. To perform the study, hyperlipidemia was induced by feeding high fat diet [11,12]. The animals were fed daily for 14 days with high fat diet consisting of 200 mg of cholesterol suspended in 2 ml of coconut oil. Animals were dosed with the drug after 2 h of administration of high fat diet. The formulations were given orally using 18-gauge oral feeding needle. Dose for the rats was calculated after taking into consideration surface area ratio of a rat to that of a human being [13,14]. After 14 days of the treatment, the rats were anaesthetized using diethyl ether and blood samples were withdrawn from the tail vein in microcentrifuge tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. The blood collected was mixed with the anticoagulant properly and centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at −21 °C until estimation of total cholesterol and high-density lipoprotein (HDL) cholesterol was carried out *in vitro* by Cogent diagnostic kit (Span diagnostics Ltd., Surat, India).

2.2.9. *Bioavailability study of ezetimibe formulations in rats*

Approval to carry out *in vivo* studies was obtained from the Jamia Hamdard Institutional Animal Ethics Committee, New Delhi and their guidelines were followed throughout the study. The animals

Table 5
Mean pharmacokinetic parameters (±S.D., $n = 6$) of ezetimibe from formulation PF1, drug suspension and tablet.

Formulation	t_{max}[a] (h)	C_{max}[b] (ng/ml)	$AUC_{0\rightarrow 128\,h}$[c] (ng h/ml)	$AUMC_{0\rightarrow 128\,h}$[d] (ng h^2/ml)	$MRT_{0\rightarrow 128\,h}$[e] (h)
PF1	2.00	69.53*** ± 5.71	948.53*** ± 38.95	19797.31*** ± 758.61	23.38 ± 2.55
Drug suspension	2.00	47.42 ± 5.28	293.64 ± 65.79	6031.50 ± 99.92	20.54 ± 1.99
Tablet	1.00	43.74 ± 2.59	222.01 ± 42.48	4458.03 ± 95.43	20.08 ± 1.01

[a] Time of peak plasma concentration.
[b] Peak plasma concentration.
[c] Area under the plasma concentration versus time curve until the last observation.
[d] Area under the moment curve computed upto last observation.
[e] Mean residence time.
*** $p < 0.001$ when compared with drug suspension and tablet using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test.

used for the *in vivo* study were Albino Wistar rats. The animals were kept under standard laboratory conditions, temperature at 25 ± 2 °C and RH of 55 ± 5%. The animals were housed in polypropylene cages, six per cage with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. The formulations were given orally using 18-gauge oral feeding needle. Dose for the rats was calculated after taking into consideration surface area ratio of a rat to that of a human being [13,14]. The rats were anaesthetized using diethyl ether and blood samples were withdrawn from the tail vein of rat at 0 (pre-dose), 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 16, 22, 26, 30, 40, 46, 50, 60, 70, 94, 118 and 128 h in microcentrifuge tubes containing EDTA as an anticoagulant. The blood collected was mixed with the anticoagulant properly and centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at −21 °C until drug analysis was carried out using LC/MS/MS (LC/MS/MS API 3000, International Equipment Trading Ltd., Vernon Hills, IL, USA). The results are shown in Table 5.

2.2.10. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using PK Functions for Microsoft Excel software (Joel I. Usanky, Atul Desai and Diane Tang-Liu, Allergan, CA, U.S.A.). Following pharmacokinetic functions were calculated in Microsoft Excel worksheets: C_{max}, t_{max}, $AUC_{0\rightarrow 128\,h}$ and $AUMC_{0\rightarrow 128\,h}$.

Mean residence time is the average amount of time a particle remains in a compartment or system and was computed as $MRT_{0\rightarrow 128\,h} = AUMC_{0\rightarrow 128\,h}/AUC_{0\rightarrow 128\,h}$.

2.2.11. Stability studies on optimized nanoemulsion

Three batches of the nanoemulsion formulation PF1 were prepared. These batches were kept at a temperature of 40 ± 2 °C and 75 ± 5% RH for three months. Samples were withdrawn after specified time intervals (0, 30, 60, and 90 days) and examined visually for any physical change in the formulation. Refractive index, viscosity, droplet size and the remaining drug content was determined using HPLC at 232 nm at the end of 0, 30, 60, and 90 days. Logarithm of percent drug remaining versus time (in days) was plotted.

2.2.12. Determination of shelf life of optimized nanoemulsion

Accelerated stability studies were also performed for the determination of shelf life. Nanoemulsion formulations were kept at three different temperatures and ambient humidity conditions (30 ± 0.5, 40 ± 0.5 and 50 ± 0.5 °C) for three months. Samples were withdrawn after specified time intervals (0, 30, 60, and 90 days) and the remaining drug content was measured (Table 7) using a stability indicating HPLC method at 232 nm [15]. Zero time samples were used as controls. Order of the reaction was determined. After determination of order of the reaction, the reaction rate constant (K) for the degradation was measured from the slope of the lines at each elevated temperature using the equation

$$\text{slope} = \frac{-K}{2.303}$$

Plot of the logarithm of K values at various elevated temperatures against the reciprocal of absolute temperature was drawn (Arrhenius plot). From the plot, K value at 25 °C was determined and was used to calculate shelf life by substituting in the equation

$$t_{0.9} = \frac{0.1052}{K_{25}}$$

where $t_{0.9}$ is the time required for 10% degradation of the drug and is referred to as shelf life.

2.2.13. Statistical application for analysis of data

The data from the characterization of nanoemulsion formulations, *in vitro* release study, pharmacodynamic and pharmacokinetic study were compared for statistical significance by one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test using GraphPad Instat software (GraphPad Software Inc., CA, USA).

3. Results and discussion

3.1. Solubility studies

The most important criterion for the screening of components is the solubility of poorly soluble drug in oil. The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in the solubilised form. Both long and medium chain triglyceride oil with different degree of saturation have been used for the design of nanoemulsions [16]. In the present study, at least one oil from different categories such as long chain triglyceride, medium chain triglyceride as well as synthetic monoglyceride oil was selected so that highest solubility of ezetimibe could be achieved. Since the drug exhibited highest solubility in Capryol 90, it was selected as the oil phase for the development of nanoemulsion.

3.2. Construction of pseudoternary phase diagrams

Phase behaviour studies are essential for the study of surfactant systems and are performed by constructing phase diagrams that provide information on the boundaries of the different phases as a function of composition variables and temperatures, and, more important structural organization can also be inferred. One approach to characterize these multicomponent systems is by means of pseudoternary diagrams that combine more than one component in the vertices of the ternary diagram. Surfactant and cosurfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in free energy required for the nanoemulsion formation consequently improves the thermodynamic stability of the nanoemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to surfactant/cosurfactant play an important role in the formation of nanoemulsions [17]. Non-ionic or zwitterionic surfactants are often

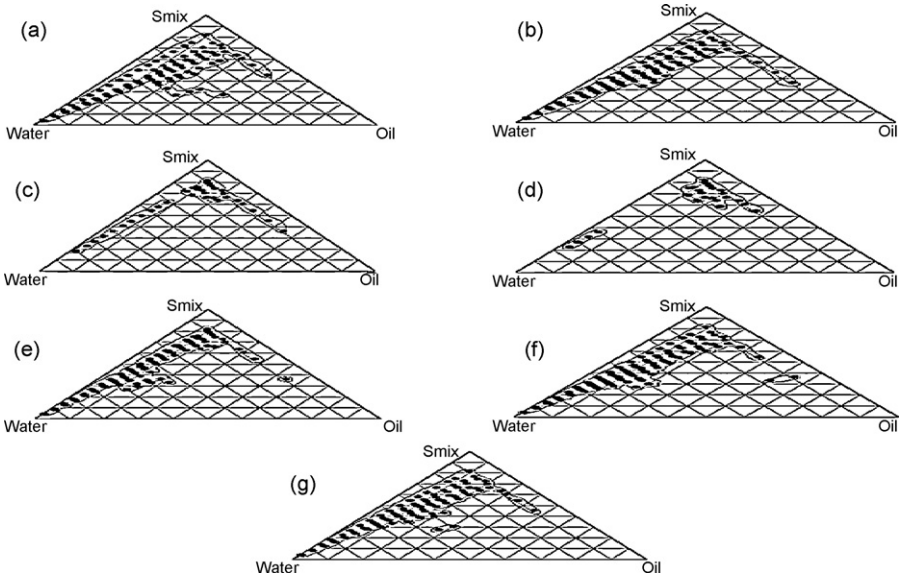

Fig. 1. Pseudoternary phase diagrams involving Capryol 90, Tween 20 and PEG 400 as the oil, surfactant and cosurfactant respectively (group I). Ratio of surfactant to cosurfactant in (a) is 1:0, (b) is 1:1, (c) is 1:2, (d) is 1:3, (e) is 2:1, (f) is 3:1 and (g) is 4:1. Dotted area shows oil in water nanoemulsion region.

considered for pharmaceutical applications and nanoemulsion formulation since these are less toxic and less affected by pH and ionic strength changes [6]. Thus, for the present study, Tween 20 and Labrasol were used as surfactants having HLB values of 16.7 and 14 respectively. It is generally not possible to achieve the required interfacial area with the use of single surfactant. If, however, a second amphiphile is added to the system, the effects of the two surfactants can be additive provided that the absorption of one does not adversely affect the absorption of the other and that mixed micelle formation does not reduce the available concentration of surfactant molecule. The second amphiphile is referred to as the cosurfactant [18]. In the present study, PEG 400 and Transcutol® P were used as cosurfactants. These are non-ionic surfactants having HLB of 11.3 and 4.2 respectively. To study the relationship between the components of the nanoemulsion and their phase behaviour, phase diagrams were constructed (Figs. 1 and 2).

In group I, When Tween 20 alone was used in the S_{mix} [Fig. 1(a)], an appreciable nanoemulsion region was obtained. The maximum amount of oil that could be emulsified was found to be 45% (v/v) using 45% (v/v) of S_{mix}. When cosurfactant was incorporated along with the surfactant in equal proportion, i.e., S_{mix} ratio 1:1 [Fig. 1(b)], nanoemulsion region was somewhat reduced and the maximum amount of oil that could be emulsified was found to be 54% (v/v) using 36% (v/v) of S_{mix}. When the proportion of cosurfactant was doubled in the S_{mix} [Fig. 1(c)], the nanoemulsion region was drastically reduced. The maximum amount of oil that could be emulsified was observed to remain the same, i.e., 54% (v/v) of oil could be emulsified using 36% (v/v) of S_{mix}. On further increasing the proportion of cosurfactant in the S_{mix} from 1:2 to 1:3 [Fig. 1(d)], it was observed that the nanoemulsion region was further reduced. The maximum amount of oil that could be emulsified was found to be 36% (v/v) using 54% (v/v) of S_{mix}. When the concentration of surfactant was increased in the S_{mix} to 2:1 [Fig. 1(e)], it was observed

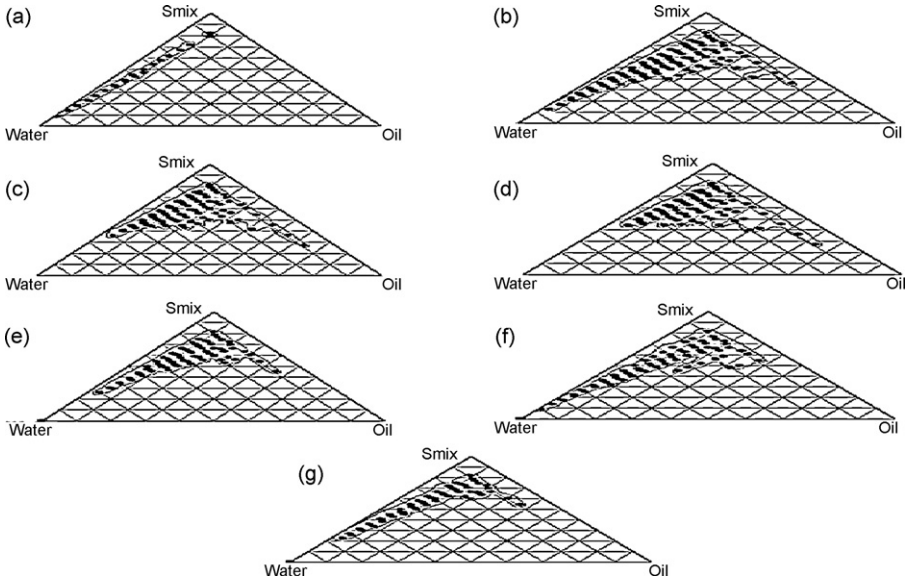

Fig. 2. Pseudoternary phase diagrams involving Capryol 90, Labrasol and Transcutol® P as the oil, surfactant and cosurfactant respectively (group II). Ratio of surfactant to cosurfactant in (a) is 1:0, (b) is 1:1, (c) is 1:2, (d) is 1:3, (e) is 2:1, (f) is 3:1 and (g) is 4:1. Dotted area shows oil in water nanoemulsion region.

that the nanoemulsion region was somewhat reduced as compared to that observed in the S_{mix} ratio of 1:1. There was no change in the maximum amount of oil that could be emulsified using this S_{mix} ratio. On further increasing the concentration of surfactant in the S_{mix} to 3:1 [Fig. 1(f)], it was observed that there was little increase in the nanoemulsion region but the maximum amount of oil that could be emulsified still remained the same as that of 1:1. No appreciable increase in the nanoemulsion region was observed on further increasing the proportion of surfactant in the S_{mix} to 4:1 [Fig. 1(g)] and the maximum amount of oil that could be emulsified also remained the same.

In case of group II consisting of Capryol 90 as oil, Labrasol as surfactant, and Transcutol® P as cosurfactant, when Labrasol was used alone without cosurfactant [Fig. 2(a)], very small amount (8%, v/v) of oil could be emulsified using very high concentration (72%, v/v) of surfactant. When surfactant and cosurfactant were used in equal proportion, i.e., 1:1 [Fig. 2(b)], the nanoemulsion region increased tremendously along with an increase in the maximum amount of oil that could be emulsified and 54.55% (v/v) of oil could be emulsified using considerably lower, i.e., 36.36% (v/v) of the S_{mix}. When the concentration of cosurfactant was further increased, S_{mix} ratio 1:2 [Fig. 2(c)], the amount of oil that could be emulsified increased further and 63% (v/v) of oil could be emulsified using still lower amount (27%, v/v) of the S_{mix}. When the proportion of cosurfactant was further increased, i.e., S_{mix} ratio 1:3 [Fig. 2(d)], not much change was observed in the maximum amount of oil that could be emulsified. When the proportion of surfactant was increased in the S_{mix}, the nanoemulsion region was found to extend towards the aqueous rich apex. When the proportion of surfactant was doubled, i.e., S_{mix} ratio 2:1 [Fig. 2(e)], 45% (v/v) of oil could be emulsified using 45% (v/v) of the S_{mix}. When the concentration of surfactant was further increased in the S_{mix}, i.e., S_{mix} ratio 3:1 [Fig. 2(f)], maximum amount of oil that could be emulsified decreased and only 36% (v/v) of oil could be emulsified using 54% (v/v) of the S_{mix}. On further increasing the concentration of surfactant in the S_{mix}, i.e., S_{mix} ratio 4:1 [Fig. 2(g)], 36.36% (v/v) of oil could be emulsified using relatively higher amount (54.55%, v/v) of the S_{mix}.

While studying phase diagrams (Figs. 1 and 2), it can be seen that the free energy of nanoemulsion formation depends on the extent to which the surfactant lowers the surface tension of the oil–water interface and the change in dispersion entropy. Thus, a negative free energy of formation is achieved when large reduction in surface tension is accompanied by significant favorable entropic change. In such cases, nanoemulsification is spontaneous and the resulting dispersion is thermodynamically stable [7]. Therefore, the potential system being explored for oral drug delivery would be the one in which the surfactant or the S_{mix} concentration used should be able to increase the dispersion entropy, lower the interfacial tension, increase the interfacial area, lower the free energy of system to a very low value with minimum concentration resulting in a thermodynamically stable spontaneous dispersion.

3.3. Selection of formulation

Toxicity is an independent issue, and is important with regard to the choice of surfactants. All surfactants are potentially irritant or poorly tolerated. In general terms cationic surfactants are more toxic than anionic surfactants which in turn are more toxic than non-ionic surfactants [19]. Thus, in the present study only non-ionic surfactants were used. It has been established that large amount of surfactants cause GI irritation [7]. Hence, it is imperative to calculate the concentration of surfactant properly and use the minimum possible concentration. From each phase diagram, different concentration of oil which could solubilise 10 mg (single dose) of ezetimibe was selected at a difference of 5% (10, 15, 20, 25 and 30%). For each percentage of oil selected, that formula was taken from the phase diagram, which used minimum concentration of S_{mix} for the formation of nanoemulsion. No difference was observed when drug was incorporated into the oil used for the preparation of phase diagram which was in accordance with the observation that the formation and stability of nanoemulsions comprising non-ionic surfactant is not affected by pH and ionic strength changes [8]. Therefore, the chances of precipitation of the drug due to phase separation with variation in pH and ionic strength would be less during transport through the biological environment.

3.4. Thermodynamic stability studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nanoemulsion from emulsions that have kinetic stability and will eventually phase separate [20]. In order to avoid inclusion of metastable systems, various thermodynamic stability studies like centrifugation study, heating cooling cycle and freeze–thaw cycle were performed. The formulations that passed these tests were selected for the dispersibility study in order to estimate the efficiency of dispersibility.

3.5. Dispersibility studies

Since the objective of the present research work was to develop an oral nanoemulsion formulation of ezetimibe, dispersibility studies in double distilled water and in 0.1N HCl were of paramount importance. The formulations that passed the dispersibility test in double distilled water as well as in 0.1N HCl in grade A (as specified in Table 1) were considered to pass the dispersibility test and were selected for further study as these formulations (Table 2) were certain to remain as nanoemulsion upon dispersion in the aqueous environment of the GIT. It was surprising to find that all the formulations from group II failed the dispersibility test (Table 3). As nanoemulsions are formed at a particular concentration of oil, water and surfactant, it is very likely that when the formulation undergoes infinite dilution in the GI fluids, there might be phase separation of the formulation leading to precipitation of the drug due to its poor aqueous solubility. For oral nanoemulsion the process of dilution by the GI fluids will result in the gradual desorption of the surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration [7]. Group II was dropped from further study and finally three formulations were selected from group I (Table 4). Selected formulations were subjected to globule size analysis, refractive index determination, viscosity determination and *in vitro* release studies.

3.6. Characterization of nanoemulsion

Nanoemulsions have been characterized using a wide variety of techniques. The characterization of nanoemulsions is a difficult task due to their complexity, variety of structures and components involved in these systems, as well as the limitations associated with each technique but such knowledge is essential for their successful commercial exploitation [20].

3.6.1. Percentage transmittance

The percentage transmittance of the optimized formulations was determined. The results are shown in Table 4. Amongst the selected formulations, formulation PF1 had the highest percentage transmittance which was highly significant ($p < 0.001$) in comparison to other formulations. A value of percentage transmittance

closer to 100% indicated that all of the optimized formulations were clear and transparent.

3.6.2. Refractive index

Refractive index of the selected formulations was determined using an Abbe type refractrometer. The results are shown in Table 4. No significant difference ($p > 0.05$) was observed in the refractive indices of formulation. In all the nanoemulsion formulations, the refractive index was closer to 1.422 ± 0.002 (refractive index of neat Capryol 90). Similarity of the refractive index value is a sign of the uniform nanoemulsion structure. This led to the conclusion that the optimized nanoemulsion formulations were not only thermodynamically stable but also isotropic in nature.

3.6.3. Viscosity

Viscosity studies are necessary for nanoemulsion to characterize the system physically and to control its physical stability. The viscosity of the optimized formulations was determined and the values are shown in Table 4. It can be seen from the table that the selected nanoemulsion formulations had a very low viscosity. Formulation PF1 had significant ($p < 0.001$) difference in viscosity as compared to other formulations and its viscosity was also the lowest amongst the selected formulations.

3.6.4. Droplet size analysis (particle size distribution)

The information on droplet size is particularly important for understanding the behaviour of nanoemulsion. Moreover, in addition to composition, the bioacceptability of the delivery system is also influenced by the particle size. Droplet size of the prepared nanoemulsion was determined and the results are shown in Table 4 along with the polydispersity indices. From the table, it can be seen that formulation PF1 has the smallest particle size (46.53 ± 8.24 nm) which is highly significant ($p < 0.001$) in comparison to other formulations of the group. The polydispersity index of formulation PF1 is lowest having a value of 0.177. Since the diameter of the dispersed oil droplets of the nanoemulsion PF1 was much smaller than the smallest blood capillary (400 nm), there are minimal chances of capillary blockage during transport of the droplets. A small size distribution of the droplets is also favorable for higher circulation time after *in vivo* application.

3.6.5. Determination of zeta potential

Zeta potential of the optimized formulations was determined using zeta potential measuring instrument, ZC-2000 (Zeecom-2000, Microtec Co. Ltd., Chiba, Japan). The results are shown in Table 4. The significance of zeta potential is that it can be related to the stability of colloidal dispersions. Zeta potential indicates degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules that are small enough a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. Zeta potential controls charge interactions. Conventionally, a high zeta potential can be high in positive or negative sense, i.e., −30 mV or +30 mV would be considered as high zeta potential. Negative values of zeta potential of the optimized formulations indicated that the formulations were negatively charged and high values of zeta potential of all the formulations signified stability of the system.

3.6.6. Transmission electron microscopic (TEM) analysis

Transmission electron microscopy is the most important technique for the study of microstructures, because it directly produces images at high resolution and it can capture any coexistent structures and microstructure transitions [18]. Morphology and structure of the optimized nanoemulsion formulations were determined using transmission electron microscopy. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. The nanoemulsion droplets appeared as dark and the surroundings were bright. The droplet sizes were in agreement with the results obtained using photon correlation spectroscopy.

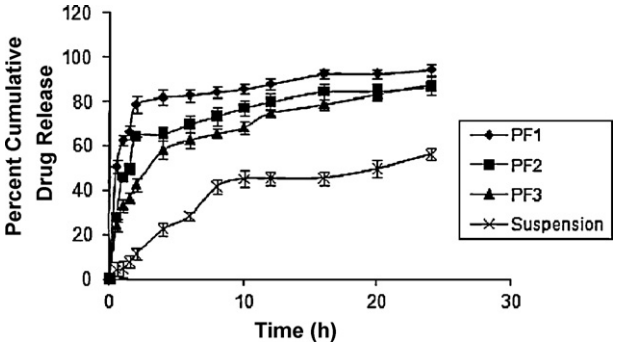

Fig. 3. Dissolution profile of ezetimibe (percent cumulative release ± S.D., $n = 3$) from nanoemulsion formulations PF1 to PF3 and drug suspension.

3.7. In vitro drug release study

The release of drug from the nanoemulsion formulations was extremely significant ($p < 0.001$) in comparison to the drug suspension. Nanoemulsion PF1 provided the highest release of 94.2% amongst the group I formulations (Fig. 3). More than 60% of the drug was released in the initial 1 h of the dissolution study in comparison to the drug suspension which showed a release of only 4.5%. This could be attributed to the small globule size in case of nanoemulsion formulations which provided large surface area for the release of drug and thus permitting faster rate of drug release. The rate of drug release from the nanoemulsion formulation PF3 was slow in comparison to PF2 although the difference was not statistically significant ($p > 0.05$). This could be attributed to the fact that the formulation PF3 had higher globule size than PF2. Thus, the drug had a larger surface area for release in PF2 than in PF3. Another reason for this observation could be the low viscosity of PF2 than PF3. Thus, on the basis of dissolution study, the formulation PF1 providing the highest drug release (94.2%), least droplet size (46.53 ± 8.24 nm), minimum polydispersity (0.117), and lowest viscosity (25.85 ± 0.80 cP) were selected for the *in vivo* studies.

3.8. Pharmacodynamic studies

Pharmacodynamic studies were performed to carry out *in vitro* determination of total cholesterol and high-density lipoprotein (HDL) cholesterol in plasma following oral administration of high fat diet and ezetimibe formulations for a time period of 14 days. The results are shown in Fig. 4. It can be seen that the value of total cholesterol in the group administered with the formulation

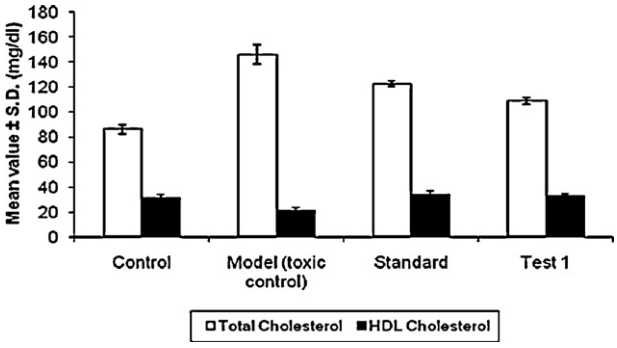

Fig. 4. Mean ± S.D. ($n = 6$) value of total cholesterol and HDL cholesterol (mg/dl).

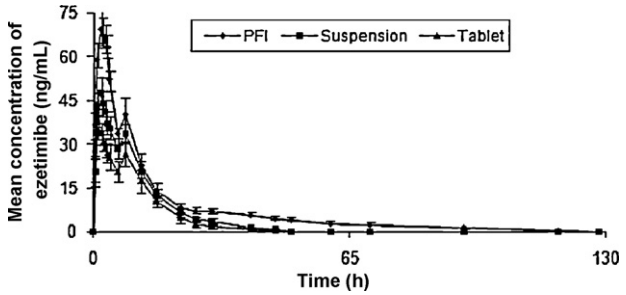

Fig. 5. . Plasma concentration (mean ± S.D.) profile of ezetimibe after oral administration of different formulations to adult Wistar rats ($n = 6$ and dose = 0.9 mg/kg).

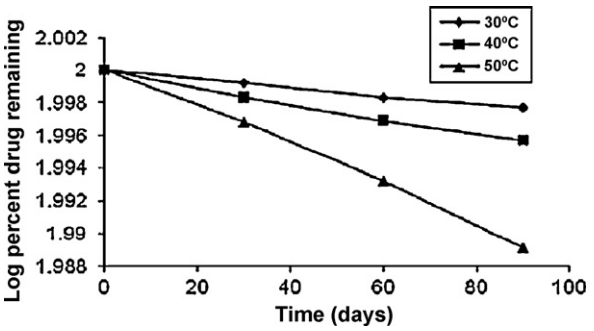

Fig. 6. Log percent concentration of drug remaining (±S.D., $n = 3$) versus time plot for nanoemulsion PF1.

PF1 was highly significant ($p < 0.001$) with respect to the group administered with the pure suspension of the drug. This could be due to the reason that ezetimibe reduces total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B and increases high-density lipoprotein cholesterol in patients with hypercholesterolemia [21]. However, the values of HDL cholesterol observed in the group administered with the formulation PF1 was not significant in comparison to the value obtained in the group administered with the pure suspension of the drug.

3.9. Bioavailability study of ezetimibe formulations in rats

The *in vivo* study was performed to quantify ezetimibe after oral administration of ezetimibe formulations. After oral administration, ezetimibe is rapidly absorbed and primarily conjugated by the action of UDP-glucuronosyltransferases (UGT) in the small intestine and liver to pharmacologically active ezetimibe–glucuronide. Ezetimibe and its glucuronide are the major drug-derived compounds in plasma, constituting approximately 10–20% and 80–90% of the total drug in plasma, respectively. The glucuronide is more potent in inhibiting the cholesterol transport than the parent compound [2,22–24]. For the estimation of ezetimibe–glucuronide, it was back converted to ezetimibe by the use of enzyme β-glucuronidase. The plasma profiles of ezetimibe in adult Albino Wistar rats following oral administration of nanoemulsion formulations PF1, marketed tablet (Ezedoc® 10, Lupin Ltd., Pune, India), and drug suspension of ezetimibe were compared. It can be seen that the plasma concentration time profile of ezetimibe for nanoemulsion represented greater improvement of drug absorption than the marketed formulation and simple drug suspension (Fig. 5).

The C_{max} of PF1 was 69.53 ± 5.71 ng/ml as compared to the C_{max} of drug suspension and tablet which were found to be 47.42 ± 5.28 and 43.74 ± 2.59 ng/ml respectively (Table 5). Statistically the C_{max} of nanoemulsion formulation PF1 was extremely significant ($p < 0.001$) with respect to drug suspension and tablet formulation. $AUC_{0\rightarrow 128\,h}$ of PF1 was found to be 948.53 ± 38.95 ng h/ml in comparison to the drug suspension and tablet which were 293.64 ± 65.79 and 222.01 ± 42.48 ng h/ml respectively. $AUC_{0\rightarrow 128\,h}$ of PF1 was found to be extremely significant ($p < 0.001$) in comparison to the drug suspension and tablet formulation. Values for $AUMC_{0\rightarrow 128\,h}$ for the nanoemulsion PF1, drug suspension and tablet formulation were found to be 19797.31 ± 758.61, 6031.50 ± 99.92 and 4458.03 ± 95.43 ng h^2/ml respectively. Value of $AUMC_{0\rightarrow 128\,h}$ for the nanoemulsion PF1 was found to be extremely significant ($p < 0.001$) in comparison to the drug suspension and tablet respectively. The difference in the values of $MRT_{0\rightarrow 128\,h}$ was not statistically significant ($p > 0.05$) when its value for the nanoemulsion formulation PF1 was compared with that of suspension and tablet formulation. The relative bioavailability of PF1 with respect to drug suspension was found to be 323.02% whereas with respect to marketed tablet was found to be 477.09%. The enhanced bioavailability is probably due to the increase in solubility and immediate dispersion of the drug in the GI tract [6]. Furthermore, the presene of a surfactant in the nanoemulsion system might have caused changes in membrane permeability by the inhibition of an apocally polarised efflux system, which could lead to enhancement of the oral absorption [25].

As can be seen from the concentration time profile of ezetimibe in PF1, drug suspension and tablet formulations, the concentration of ezetimibe reached its peak followed by a rapid decline and then increased leading to multiple peaks in the plasma concentration time profile. These multiple peaks could be attributed to the fact that ezetimibe is excreted into bile after undergoing extensive glucuronidation in the intestine to a phenolic glucuronide. It is possible that ezetimibe is repeatedly delivered back to the site of action, the lumen of the intestinal tract, via enterohepatic recirculation (EHC) after undergoing reabsorption in the ileum. This, in turn, has the potential to enhance the residence time of the compound in the lumen of the intestinal tract, thereby potentiating its cholesterol-lowering activity [2,22–24].

3.10. Stability studies on optimized nanoemulsion

During stability studies, droplet size, viscosity and refractive index were determined at 0, 30, 60, and 90 days. As can be seen from Table 6, these parameters were slightly increased with respect to time but the changes in the observed parameters were not found to be statistically significant ($p > 0.05$). Stability studies at 40 ± 2 °C and 75 ± 5% RH predicted a degradation of 0.90% of ezetimibe in the formulation PF1 at the end of 90 days.

3.11. Determination of shelf life of optimized nanoemulsion

Accelerated stability studies were performed for the determination of shelf life of the nanoemulsion formulation PF1. The amount of drug remaining undecomposed in the formulation PF1 at each time interval is shown in Table 7. It can be seen from the table that the concentration of drug remaining undecomposed in the formulation PF1 at the end of 90 days was 49.82 ± 0.04, 49.6 ± 0.09 and 48.76 ± 0.04 μg/ml at 30 ± 0.5, 40 ± 0.5 and 50 ± 0.5 °C respectively. The order of degradation of ezetimibe in the nanoemulsion formulations was found to follow first-order kinetics (Fig. 6). The reaction rate constant '*K*' for the degradation was measured from the slope of the lines at each elevated temperature. Plot of the logarithm of *K* values for the nanoemulsion PF1 (Table 8) at each elevated temperature against the reciprocal of absolute temperature was drawn (Arrhenius plot) as shown in Fig. 7. From the plot, *K* value at 25 °C was determined and was used to calculate shelf life. The shelf life of the nanoemulsion PF1 at room temperature was calculated to be 5.94 years.

Table 6
Mean (±S.D., $n = 3$) refractive index, viscosity, droplet size and concentration of drug remained in nanoemulsion PF1 stored at 40 ± 2 °C and 75 ± 5% RH.

Time (in days)	Mean refractive index ± S.D.	Mean viscosity ± S.D. (cP)	Mean droplet ± S.D. (nm)	Mean concentration of drug remained ± S.D. (μg / ml)	% Drug remained	Log % drug remained
0	1.412 ± 0.003	25.81 ± 0.77	47.01 ± 9.21	50.00 ± 0.05	100	2.0000
30	1.414 ± 0.003	25.85 ± 0.79	47.05 ± 9.32	49.79 ± 0.36	99.58	1.9981
60	1.417 ± 0.004	25.88 ± 0.83	47.09 ± 9.41	49.65 ± 0.06	99.30	1.9969
90	1.420 ± 0.004	25.93 ± 0.91	47.13 ± 9.52	49.55 ± 0.06	99.10	1.9961

Table 7
Percent drug remaining (±S.D., $n = 3$) in nanoemulsion PF1 stored at elevated temperatures (30 ± 0.5, 40 ± 0.5 and 50 ± 0.5 °C).

Time (in days)	Temperature (°C)	Mean concentration of drug remaining ± S.D. (μg/ml)	Percent drug remaining	Log percent drug remaining
0	30 ± 0.5	50.08 ± 0.08	100	2.0000
30	30 ± 0.5	49.98 ± 0.09	99.21	1.9990
60	30 ± 0.5	49.89 ± 0.08	99.62	1.9978
90	30 ± 0.5	49.82 ± 0.04	99.48	1.9963
0	40 ± 0.5	50.08 ± 0.08	100	2.0000
30	40 ± 0.5	49.55 ± 0.10	99.69	1.9983
60	40 ± 0.5	49.64 ± 0.08	99.26	1.9969
90	40 ± 0.5	49.60 ± 0.09	99.02	1.9957
0	50 ± 0.5	50.08 ± 0.08	100	2.0000
30	50 ± 0.5	49.64 ± 0.02	99.26	1.9968
60	50 ± 0.5	49.23 ± 0.03	98.45	1.9932
90	50 ± 0.5	48.76 ± 0.04	97.52	1.9891

Table 8
Degradation rate constant for ezetimibe in nanoemulsion PF1.

Temperature (°C)	Temperature (K)	$1/T \times 1000(K^{-1})$	Slope	$K \times 10^{-4}$	Log K
30	303	3.3003	−0.00003	0.6909	−4.1606
40	313	3.1948	−0.00005	1.15	−3.9387
50	323	3.0959	−0.0001	2.303	−3.6377
25	298	3.3557	–	0.4864	−4.3130

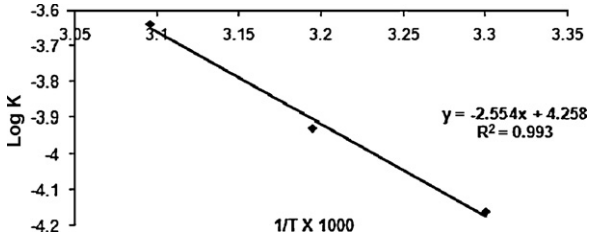

Fig. 7. Arrhenius plot for nanoemulsion PF1.

4. Conclusions

A nanoemulsion formulation of ezetimibe containing Capryol 90 (10%, v/v), Tween 20 (33.33%, v/v), PEG 400 (16.67%, v/v) and double distilled water (40%, v/v) was successfully optimized based on increased dissolution rate, optimum globule size, minimum polydispersity, lower viscosity, lower surfactant concentration, higher solubility as well as higher bioavailability. Results from the determination of zeta potential established stability of the system. The developed formulation showed higher lipid lowering potential as compared to the pure suspension of the drug. The *in vivo* studies revealed significantly greater extent of absorption than the conventional tablet (Ezedoc® 10, ezetimibe 10 mg) formulation. The absorption of ezetimibe from ezetimibe nanoemulsion resulted in 3.23-folds increase in bioavailability as compared to drug suspension and 4.77-folds increase in bioavailability as compared to the conventional tablet. Results from the stability studies at 40 ± 2 °C and 75 ± 5% RH indicated stability of the optimized formulation as there was no significant change in the observed physical parameters. Results from the accelerated stability studies indicated that the degradation of ezetimibe in the nanoemulsion formulation followed first order kinetics. The shelf life of the nanoemulsion at room temperature was calculated to be 5.94 years. The present study confirmed that the nanoemulsion formulation could be used as a possible alternative to traditional oral formulations of ezetimibe with the advantages of improved absorption and reduced variations in bioavailability.

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