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Nanocarrier for the enhanced bioavailability of a cardiovascular agent: *In vitro*, pharmacodynamic, pharmacokinetic and stability assessment

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ABSTRACT

The goals of the current study were to develop and characterize a nanoemulsion of ezetimibe, evaluate its stability, lipid lowering and pharmacokinetic profile. Solubility of the drug was estimated in various oils and surfactants. Existence of nanoemulsion region was confirmed by plotting phase diagrams. Various thermodynamic stability and dispersibility tests were performed on the formulations chosen from phase diagram. Percentage transmittance, refractive index, viscosity, droplet size and zeta potential of the optimized formulations were determined. Dialysis bag method was employed to study the release rate. The formulation selected for bioavailability estimation contained Capryol 90 (10%, v/v), Crempophor EL (11.25%, v/v), Transcutol® P (33.75%, v/v), and double distilled water (45%, v/v). The release rate from the nanoemulsion was highly significant ($p < 0.001$) in contrast to the drug suspension. The level of total cholesterol in the group receiving nanoemulsion CF1 was found to be highly significant ($p < 0.001$) in comparison to the group receiving drug suspension. Bioavailability studies in rats revealed superior absorption of ezetimibe from nanoemulsion as compared to the marketed formulation and drug suspension. The shelf life of the nanoemulsion was estimated to be 18.53 months. The present study corroborated nanoemulsion to be a promising choice to improve the bioavailability of ezetimibe.

1. Introduction

Whilst absolute definitions are difficult, up to 40% of the New Chemical Entities (NCEs) in development have been reported to be 'poorly water-soluble' (Porter et al., 2008). In case of orally administered formulations, lipophilic drugs show poor gastrointestinal absorption because of the low solubility or dissolution rate in aqueous media. Hence, the oral bioavailability is low and variation is large. Therefore, even if the NCE has powerful pharmacological activity, the clinical efficacy which is expected is sometimes not realized. This has led to increased efforts in the development of pharmaceutical formulations with enhanced oral bioavailability of these lipophilic compounds.

In fact, the most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles (Shafiq et al., 2007; Dixit and Nagarsenker, 2008a). Among these approaches, nanoemulsions are thermodynamically stable, transparent (or translucent) dispersions of oil in water stabilized by an interfacial film of surfactant and cosurfactant molecules having the droplet size less than 100 nm (Eccleston, 1994). Physicochemical properties and thermodynamic stability of the system permits nanoemulsion to be formed spontaneously by low energy emulsification method and also allows them to stay stable as long as the ingredients are intact (Bali et al., 2008a,b). Nanoemulsion is a good candidate for the oral delivery of poorly water-soluble drugs due to improvement in solubilization of these drugs and protection from enzymatic degradation. This system also aids in the absorption of drugs due to membrane fluidity and subsequent permeability changes induced by the surfactant (Kim et al., 2001). Nanoemulsion based systems lead to more reproducible plasma drug concentration profiles, bioavailability and have been reported to overcome inter and intra subject variations (Jadhav et al., 2006; Ghosh and Murthy, 2006). It has been reported in literature that non ionic surfactants like Cremohor EL and Tween 80 may be useful pharmaceutical excipients for inhibiting the function of the efflux transporter P-glycoprotein (P-gp) and thereby increasing the intestinal absorption of drugs, which are secreted by a P-gp-mediated efflux system in the intestine (Shono et al., 2004). Furthermore, it has been stated in literature reports that in contrast to Tween 80, Cremohor EL is a potent inhibitor of P-gp (Katneni et al., 2007).

Ezetimibe belongs to a new class of hypolipidemics that specifically inhibits the absorption of cholesterol as well as related phytosterols from the intestine. Logarithm of partition coefficient [$\log P_{(\text{octanol/water})}$] value of ezetimibe is 4.5. Ezetimibe comes under class II of Biopharmaceutics Classification System (BCS). By virtue of its extremely hydrophobic nature, the drug shows highly inconsistent dissolution profile in the gastrointestinal fluids (Bali et al., 2010a). Its bioavailability is highly unpredictable (Patel et al., 2008).

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As the drug is almost insoluble in aqueous media required for an i.v. preparation, it is not possible to establish absolute bioavailability of ezetimibe. Moreover, the drug shows intersubject variation in its pharmacokinetics. The coefficient of variation for C_{max} ranges from 34% to 43% and its value for AUC lies between 32 and 37% (Bali et al., 2010b). It has also been reported in literature that Ezetimibe and its glucuronide metabolite are substrates of P-gp (Oswald et al., 2006a). An assortment of methods such as cyclodextrin complexation and self nanoemulsifying systems has been explored to surmount these limitations (Dixit and Nagarsenker, 2008a,b; Patel et al., 2008). 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. Compared to a ready-to-use nanoemulsion, a nanoemulsion preconcentrate has improved physical stability profile upon long term storage and can be filled directly into soft or hard gelatin capsules for conventional oral delivery (Li et al., 2005). As a drug delivery system, however, the preconcentrate, has its limitations as a viable pharmaceutical dosage form. The most significant one is that the dosage form uses a large amount of surfactants for the purpose of forming nanoemulsions. The use of high concentration of surfactants is a legitimate concern from toxicological stand point, particularly when the indication for the therapy is chronic (Pouton, 1997). It was assumed that developing a nanoemulsion would facilitate in decreasing the intersubject variability seen in the pharmacokinetics of ezetimibe since nanoemulsion based systems lead to more reproducible plasma drug concentration profiles as well as bioavailability (Jadhav et al., 2006). Furthermore, it was hypothesized that substitution of Tween 80 by Cremophor EL as the surfactant in the nanoemulsion would help to provide enhanced intestinal uptake of the drug due to more potent inhibition of the efflux transporter P-gp by Cremophor EL over Tween 80. This would eventually lead to improvement in the absorption of the drug in comparison to earlier published report by our group (Bali et al., 2010b). Literature review shows that the studies performed until now on the nanoemulsion based drug delivery systems of ezetimibe are limited to the pharmacodynamic assessment of the developed system. The present study, in addition, describes bioavailability study in rats to specifically explain the plasma concentration–time profile of ezetimibe after oral administration of developed nanoemulsion. The current work also details stability studies on the optimized nanoemulsion and an effort has also been made to calculate shelf life of the developed nanoemulsion formulation.

Thus, the current study aimed at developing and characterizing a nanoemulsion of ezetimibe so as to augment its bioavailability by enhancing its solubility and inhibition of P-gp efflux. Other objectives were to evaluate the lipid lowering potential and shelf life of the developed formulation.

2. Materials and methods

2.1. Materials

Ezetimibe was gifted by Lupin Ltd. (Pune, India). Sefsol 218 (Propylene glycol mono caprylic ester) was a gift sample from Nikko Chemicals (Tokyo, Japan). Labrasol (Caprylo caproyl macrogol-8-glyceride), Capryol 90 (Propylene glycol monocaprylate), Labrafac (propylene glycol dicaprylocaprylate), Lauroglycol FCC (Propylene glycol laurate), Maisine (Glyceryl monolinoleate), Lauroglycol 90 (Propylene Glycol Monolaurate), Labrafil 1944 CS (Oleoyl macrogo-glyceride), and Transcutol® P (Diethylene glycol monoethyl ether) were gifted by Gattefosse (Saint Priest, Cedex, France). Tween 80 (Polyoxyethylene sorbitan monooleate), Triacetin (Glycerol triacetate), PEG (Polyethylene glycol) 400, and Tween 20 (Polyoxyethylene sorbitan monolaurate) were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Cremophor EL (Polyethoxylated castor oil) was obtained from BASF (Mumbai, India). Water was obtained from Milli-Q-water purification system (Millipore, MA, USA). All other chemicals and reagents were of analytical reagent grade.

2.2. Solubility studies

Solubility of ezetimibe was ascertained in oils, surfactants and cosurfactants. An excess amount of drug was added in 2 mL of selected vehicle in stoppered vials and mixed with the help of a vortex mixer (Nirmal International, Delhi, India). These vials were then kept at $25 \pm 1\,^{\circ}C$ in an isothermal shaker (Nirmal International, Delhi, India) for 72 h. The resulting samples were centrifuged at 3000 rpm for 15 min (REMI International, Mumbai, India). The supernatant was filtered through a 0.22 μm filter. The content of drug was determined using HPLC at 232 nm (Sistla et al., 2005). The system used was Shimadzu LC-10AT VP having a UV detector (Shimadzu, Kyoto, Japan) and the software used was Class VP, version 5.032. A RP C_{18} column (25 cm × 4.6 mm, 5 μm particle size) was used as the stationary phase along with a mixture of water (pH 6.8, adjusted with 0.5% w/v aqueous solution of 1-hexane sulphonic acid) and acetonitrile (30:70, v/v) as the mobile phase. A flow rate of 0.5 mL/min was employed along with a run time of 10 min. The retention time of ezetimibe was found to be 7.5 ± 0.1 min.

2.3. Construction of pseudo ternary phase diagrams

Based on the results of solubility studies, Capryol 90 having HLB value of 6.0 was used as the oil phase for the development of nanoemulsion. Cremophor EL was used as surfactant along with Transcutol® P as the cosurfactant. Double distilled water was used as the aqueous phase. Surfactant and cosurfactant were mixed (S_{mix}) in different volume ratios (1:1, 1:2, 1:3, 2:1, 3:1 and 4:1). The ratios were chosen so as to have increasing concentration of surfactant with respect to cosurfactant and vice versa. For every phase diagram, oil and specific S_{mix} ratio was mixed in volume ratios ranging from 1:9 to 9:1 to obtain 16 different combinations like 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. Aqueous titration method was employed to construct the phase diagrams. The amount of aqueous phase was incremented by 5% to provide concentration of aqueous phase in the range of 5–95% of total volume. After each addition of aqueous phase, physical state of the mixture was marked on a pseudo three-component phase diagram where one axis symbolized the aqueous phase, one axis corresponded to the oil and the third indicated the S_{mix} (Shafiq et al., 2007). The software employed to make the phase diagrams was PCP Disso V2.08 (Anant Ketkar, Vinay Patil and A.R. Paradkar, Pune, Maharashtra, India). Phase diagrams were also constructed in the presence of drug, using drug-enriched oil as the oil phase, to observe the effect of drug addition on the nanoemulsion boundary.

2.4. Selection of formulation

Different formulations were chosen, from each of the constructed phase diagram, based on the following criteria:

1. 10 mg was chosen as the dose for the development of nanoemulsion as the recommended dose of ezetimibe is 10 mg/day (Kosoglou et al., 2005).
2. The concentration of oil should be capable of dissolving 10 mg of the drug easily.

3. Different concentrations of oil which could solubilize 10 mg of ezetimibe were selected from every phase diagram to prepare 2 mL of nanoemulsion.
4. For a particular percentage of oil selected, that formula was taken from the phase diagram, which used minimum concentration of S_{mix} for the formation of nanoemulsion.

2.5. Thermodynamic stability studies

2.5.1. Centrifugation study

Centrifugation study was performed at 5000 rpm for 30 min and the formulations were checked for phase separation, creaming or cracking. The formulations that did not show any signs of instability were chosen for heating–cooling cycle.

2.5.2. Heating–cooling cycle

Heating cooling cycle involved six cycles between 4 °C and 40 °C with storage at each temperature for not less than 48 h. The formulations that were stable at these temperatures were chosen for freeze–thaw stress test.

2.5.3. Freeze–thaw cycle (accelerated ageing)

Freeze–thaw cycle involved three freeze–thaw cycles at temperatures between −21 °C and +25 °C with storage at each temperature for not less than 48 h. The formulations that passed this test were selected for the dispersibility study.

2.6. Dispersibility studies

Dispersibility studies were performed to evaluate the efficiency of dispersibility of oral nanoemulsion. 2 mL of each formulation was added to 500 mL of distilled water and 0.1 N HCl in a standard USP XXII dissolution apparatus 2 (Veego, Mumbai, India). Speed of the paddle was adjusted to 50 rpm and the temperature was maintained at 37 ± 0.5 °C (Pouton, 1997). The formulations were visually evaluated using the grading system reported by Bali et al. (2010a,b).

One formulation was chosen from each S_{mix} ratio used, having the least S_{mix} concentration and passing the dispersibility test in distilled water as well as in 0.1 N HCl with Grade A (Table 1). To prepare the selected formulations, 10 mg of the drug was dissolved in oil, the respective S_{mix} ratio was added and mixed on a vortex mixer. Finally the aqueous phase was added and the mixture was mixed on the vortex mixer again to yield the nanoemulsion.

2.7. Characterization of nanoemulsion

2.7.1. Percentage transmittance

Percentage transmittance was determined using Shimadzu UV-Vis spectrophotometer (Shimadzu, Japan). One milliliter of the formulation was diluted 100 times using double distilled water and analyzed at 500 nm using double distilled water as blank.

2.7.2. Refractive index

Abbe type refractrometer was used to determine the refractive index.

2.7.3. Viscosity

Viscosity of the prepared nanoemulsion was determined using R/S CPS Plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Spindle # C 50-1 having diameter of 50 mm was employed for the estimation of viscosity. 1 mL of the formulation was used and the speed of the spindle was adjusted to 70 rpm. A single run was performed at a temperature of 25 ± 0.5 °C and wait time for the operation was 50 min. A shear rate of 413 min^{-1} was applied.

2.7.4. Droplet size analysis

Dynamic light scattering (DLS) was used to assess droplet size of the prepared nanoemulsion. DLS analyzes fluctuations in the intensity of light scattering due to Brownian movement of the particles (Attwood et al., 1992). 0.1 mL of the nanoemulsion was dispersed in 50 mL of double distilled water and mixed gently. DLS was carried out at an angle of 90° on a digital correlator (Photocor Instruments Inc., MD, USA) using a He–Ne laser having a wavelength of 632.8 nm at 25 °C.

2.7.5. Determination of zeta potential

0.1 mL of the formulation was diluted to 10 mL using double distilled water and zeta potential was determined using zeta potential measuring instrument, ZC-2000 (Zeecom-2000, Microtec Co. Ltd., Chiba, Japan).

2.7.6. Transmission electron microscopic (TEM) analysis

Shape of the dispersed oil globules was studied by transmission electron microscopic (TEM) analysis. The nanoemulsion was diluted 100 times and a drop was applied to 300-mesh copper grid. The grid was left for 1 min. The grid was 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 Morgagni 268D (FEI Company, OR, USA) operated at 60–80 kV at 1550× magnification.

2.8. In vitro drug release study

In vitro drug release study was carried out using dissolution apparatus 2 (Veego, Mumbai, India). 500 mL of double distilled water at 37 ± 0.5 °C was used as the dissolution medium. 2 mL of the nanoemulsion was placed in treated dialysis bag (MWCO 12,000 g/mole; Sigma, St. Louis, USA) and the speed of the paddle was adjusted to 50 rpm. 2 mL samples were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h) and equivalent amount of double distilled water was replaced (Kang et al., 2004). Drug content in the withdrawn samples was estimated using HPLC at 232 nm. The drug release from optimized formulations was compared to that from the drug suspension. Mathematical models were applied to study the release kinetics from the nanoemulsion formulation.

2.9. Animal handling and care

Studies pertaining to effects in rat model of hypercholesterolemia and bioavailability of ezetimibe formulations were performed after obtaining consent from the Jamia Hamdard Institutional Animal Ethics Committee (IAEC), New Delhi. The guidelines were adhered to throughout the study. Albino Wistar rats were used as the animal model and were kept under standard laboratory conditions (temperature = 25 ± 2 °C and 55 ± 5% RH). Six animals were kept in each polypropylene cage with open access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. Dose for the rats was determined after consideration of surface area ratio of a rat to that of a human being (Freireich et al., 1966; Ghosh, 2005). The blood samples were withdrawn from the tail vein of rat and collected in microcentrifuge tubes containing EDTA as an anticoagulant. The collected blood was centrifuged at 5000 rpm for 20 min after mixing with the anticoagulant properly. The plasma was separated and stored at −21 °C until analysis was carried out.

2.10. Effects in rat model of hypercholesterolemia

Animals were placed in four groups as delineated in previous reports by our group (Bali et al., 2010a,b). Negative control, model

Table 1
Dispersibility study of different formulations selected from phase diagrams.

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	0.1 N HCl	
1:1	10	40	50	Grade B	Grade B	Failed
	10	45	45	Grade A	Grade B	Failed
	10	50	40	Grade A	Grade A	Passed
	15	35	50	Grade C	Grade D	Failed
	20	40	40	Grade C	Grade D	Failed
	20	45	35	Grade B	Grade C	Failed
1:2	10	50	40	Grade A	Grade A	Passed
	20	45	35	Grade A	Grade A	Passed
1:3	10	45	45	Grade A	Grade A	Passed
	10	50	40	Grade A	Grade A	Passed
	25	50	25	Grade B	Grade C	Failed
2:1	10	40	50	Grade B	Grade B	Failed
	10	50	40	Grade A	Grade A	Passed
	25	50	25	Grade C	Grade D	Failed
3:1	10	35	55	Grade B	Grade C	Failed
	10	50	40	Grade A	Grade B	Failed
	25	50	25	Grade C	Grade D	Failed
4:1	25	50	25	Grade C	Grade D	Failed

[a] S_{mix} indicates mixture of surfactant and cosurfactant in specific volume ratio; S, surfactant; CoS, cosurfactant. Failed indicates the results of dispersibility studies of the formulation in either of the media were in grade other than A.

(toxic control), standard and test received distilled water, cholesterol, drug suspension along with cholesterol and nanoemulsion CF1 along with cholesterol respectively. Six animals were kept in each group. Hyperlipidemia was induced by the use of high fat diet (Ambike et al., 2005; Bolkent et al., 2005). The animals were given high fat diet containing 200 mg of cholesterol suspended in 2 mL of coconut oil for 14 days. Animals were dosed with the drug 2 h after the administration of high fat diet. The formulations were provided orally using 18-gauge oral feeding needle. Following fourteen days of treatment, the rats were anaesthetized using ether anaesthesia and blood samples were withdrawn. The plasma was separated and *in vitro* assessment of total cholesterol and HDL (high density lipoprotein) cholesterol was performed using Cogent diagnostic kit (Span Diagnostics Ltd., Surat, India).

2.11. Bioavailability study of ezetimibe formulations in rats

All the animals used in the study were fasted over night. Animals were separated into three groups with six animals in every group. The formulations were provided orally using 18-gauge oral feeding needle. Average weight of marketed tablets of ezetimibe providing 10 mg dose was found to be 125.63 mg. So, for a 200 mg rat requiring 0.18 mg of drug, the weight of tablet to be used was 2.26 mg. Hence, fraction of tablet corresponding to the weight of the tablet required to give the desired dose was administered to rats. The rats were anaesthetized using ether anaesthesia. 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. The plasma was separated and drug content was estimated using LC/MS/MS (LC/MS/MS API 3000, International Equipment Trading Ltd., Vernon Hills, IL, USA). The method was based on reported methods by Li et al. (2006) and Oswald et al. (2006b). The integrated system used was Shimadzu Controller using software, Analyst 1.4. In this method, 10 mM ammonium phosphate buffer and methanol (20:80, v/v) was used as the mobile phase and Ascentis C_{18} (4.6 mm × 50 mm), 5 μm packing (Supelco, St. Louis, MO, USA) was used as the stationary phase. Ezetimibe d_4 was used as the internal standard. All frozen plasma samples were thawed at ambient temperature. 200 μL of plasma sample was transferred to a 2 mL polypropylene test tube. Ezetimibe-glucuronide was converted to ezetimibe by the addition of 50 μL of β-glucuronidase (100,000 IU/mL) into the tube. After vortexing for 30 s, the tube was incubated at 50 °C for 60 min. After the addition of 10 μL of internal standard solution, the tube was briefly vortexed and then extraction was carried out with 1 mL of methyl *tert*-butyl ether. The supernatant was transferred to a clean polypropylene test tube and dried with a stream of nitrogen gas at 40 °C. The residue was reconstituted with 100 μL of methanol and 5 μL volume was injected into the LC/MS/MS system. The retention time and detection of ezetimibe and internal standard (ezetimibe d_4) were as follows:

Retention time: Ezetimibe: 1.95 ± 0.05 min, Ezetimibe d_4: 1.94 ± 0.05 min
Detection: Ezetimibe: *m/z* 408.4 (parent) and 270.9 (product)
Ezetimibe d_4: *m/z* 402.4 (parent) and 271.0 (product)

The mean calibration curve was given by the equation $y = 0.0065x - 0.0007$, with a correlation coefficient, $r^2 = 0.9995$, where y represents peak area ratio and x the concentration of ezetimibe in ng/mL.

2.12. Pharmacokinetic analysis

PK functions for Microsoft Excel software (developed by Joel I. Usanky, Atul Desai and Diane Tang-Liu, Allergan, CA, U.S.A.) was used to determine pharmacokinetic parameters. C_{max}, t_{max}, $AUC_{0\rightarrow 128\,h}$ and $AUMC_{0\rightarrow 128\,h}$ were calculated in Microsoft Excel worksheets. Mean residence time (MRT) was computed as $MRT_{0\rightarrow 128\,h} = AUMC_{0\rightarrow 128\,h}/AUC_{0\rightarrow 128\,h}$.

2.13. Stability studies on optimized nanoemulsion and determination of shelf life

For stability studies, nanoemulsion CF1 was kept at a temperature of 40 ± 2 °C and 75 ± 5% RH for three months. Samples were withdrawn at the end of 0, 30, 60, and 90 days. Withdrawn samples were checked for any change in the refractive index, viscosity, droplet size and remaining drug content using HPLC at 232 nm. Percentage label claim (% drug remaining) was plotted against the time in months to determine the shelf life. Shelf life was determined as the time at which the 95% one-sided confidence limit for the mean curve intersected the acceptance criterion of 90% percentage label claim. The data was evaluated using Sigmaplot™ 10 software (Cranes Software International, Bangalore, India).

2.14. Statistical application for the analysis of data

Statistical significance of the data from the characterization of nanoemulsion formulations, *in vitro* release study, effects in rat model of hypercholesterolemia and bioavailability study was evaluated 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

Solubility of the poorly water soluble drug in oil was the decisive factor for the screening of components. The superior solubility of drug in the oil phase was important for the nanoemulsion to keep the drug in the solubilized form. For the design of nanoemulsions, both long and medium chain triglyceride oil with different degrees of saturation have been employed (Ahmed et al., 2008). In the current study, oil from different classes like long chain triglyceride, medium chain triglyceride and synthetic monoglyceride was chosen in order to attain the maximum solubility of ezetimibe. Higher is the solubility of the drug in the oil phase, lower is the volume of oil required to dissolve a single dose of drug. This in turn would lead to lower consumption of surfactants and cosurfactants for the preparation of nanoemulsion. Considering the fact that most of the surfactants are toxic, minimal use of surfactants is desirable during the preparation of nanoemulsion. In the previously published reports by our group, highest solubility (68.22 ± 0.81 mg/mL) of the drug was found in Capryol 90 and hence Capryol 90 was selected as the oil phase for the development of nanoemulsion in the present study as well (Bali et al., 2010a,b).

3.2. Construction of pseudo ternary phase diagrams

Phase diagrams are generally utilized to learn the phase behaviour of nanoemulsion systems. Pseudo ternary diagrams involving more than one component in the vertices of a ternary phase diagram are one of the approaches to describe these multicomponent systems. Selection of oil, surfactant, and the mixing ratio of oil to surfactant/cosurfactant are vital in the formation of nanoemulsions (Patel and Vavia, 2007). Due to low toxicity as well as resistance to pH and ionic strength changes of non-ionic or zwitterionic surfactants, they are frequently used for nanoemulsion formulation (Constantinides, 1995). In the current study, Cremophor EL having hydrophilic–lipophilic balance (HLB) value of 12 and Transcutol® P having HLB of 4.2 were used as the surfactant and cosurfactant respectively. Transcutol® P is a non-ionic surfactant and is also known to increase the permeability of drugs.

A more comprehensive study was carried out in the current research for improved elucidation of the association between the phase behaviour of the mixture and its composition. This would also assist in the improved selection of formulation. Thus, in this study, the concentration of both oil and S_{mix} were varied from 10% to 90% (v/v) (Fig. 1) in contrast to the previously reported studies involving just 10–30% (w/w) of oil (Dixit and Nagarsenker, 2008a,b). Very small nanoemulsion region was observed when surfactant alone was used [Fig. 1(a)]. When cosurfactant was used along with the surfactant in equal proportion [Fig. 1(b)], a tremendous increase in the nanoemulsion region was observed along with the maximum amount of oil that could be emulsified and 45% (v/v) of oil could be emulsified using 45% (v/v) of S_{mix}. When the concentration of cosurfactant was increased to two parts [Fig. 1(c)], the maximum amount of oil that could be emulsified was 54.55% (v/v) using 36.36% (v/v) of S_{mix}. On further increasing the proportion of Transcutol® P in the S_{mix} to 3:1 [Fig. 1(d)], the nanoemulsion region decreased with the maximum amount of oil that could be emulsified still remaining 54.55% (v/v) using 36.36% (v/v) of S_{mix}. When the concentration of Cremophor EL was increased in the S_{mix} from 1:1 to 2:1 [Fig. 1(e)], slight change in the nanoemulsion region was observed. On further increasing the concentration of surfactant in the S_{mix} from 2:1 to 3:1 [Fig. 1(f)] and then to 4:1 [Fig. 1(g)], it was observed that the nanoemulsion region remained almost same. It was found that 36% (v/v) of oil could be emulsified using 54% (v/v) of S_{mix}.

The degree of reduction in the surface tension at the oil–water interface brought about by the surfactant and alteration in dispersion entropy were found to affect the free energy of nanoemulsion formation (Lawrence and Rees, 2000). Thus, the system in which the surfactant or the S_{mix} concentration employed is able to enhance the dispersion entropy, reduce the interfacial tension, augment the interfacial area, lower the free energy of system to a very small value would lead to the prospective nanoemulsion for oral drug delivery.

3.3. Selection of formulation

Only non-ionic surfactants were employed in the current study since an important concern with regard to the selection of surfactants was their toxicity and non-ionic surfactants have been reported to be the least toxic. It was crucial to use the least feasible concentration of surfactant as large amount of surfactants have been reported to cause GI irritation (Lawrence and Rees, 2000). 54.55% (v/v) of oil was found be emulsified on the basis of phase diagrams. Different concentrations of oil capable of solubilizing single dose of ezetimibe were selected from the phase diagrams at a difference of 5%. Formula for the formation of nanoemulsion was optimized from the phase diagram after taking care to use minimum concentration of S_{mix} (Table 1). Since development and stability of nanoemulsions utilizing non-ionic surfactants have been reported to remain unaltered by pH and ionic strength modifications (Shafiq et al., 2007), no variation was observed when drug was included in the oil employed for the construction of phase diagram. As a result, the drug is less liable to precipitate because of phase separation occurring due to variation in pH and ionic strength during its movement through the biological milieu.

3.4. Thermodynamic stability studies

Nanoemulsions are thermodynamically stable in contrast to emulsions that possess kinetic stability and will ultimately phase separate (Vyas and Khar, 2002). Various thermodynamic stability tests such as centrifugation study, heating–cooling cycle and freeze–thaw cycle were carried out so as to eliminate metastable systems. Dispersibility study was conducted on the formulations that passed the thermodynamic stability tests.

3.5. Dispersibility studies

Formation of nanoemulsions occurs at a specific concentration of oil, water and surfactant. Thus, when the formulation undergoes infinite dilution in the GI fluids, it is very probable that it might phase separate resulting in the precipitation of the drug owing to its poor aqueous solubility. To avoid such a situation, dispersibility studies in double distilled water and in 0.1 N HCl were vital. Formulations passing the dispersibility test in both the media in grade A were considered to pass the dispersibility test (Table 1). Since these formulations were certain to remain as nanoemulsion upon dilution in the aqueous environment of the gastrointestinal tract (GIT), these were selected for further study. Eventually, three

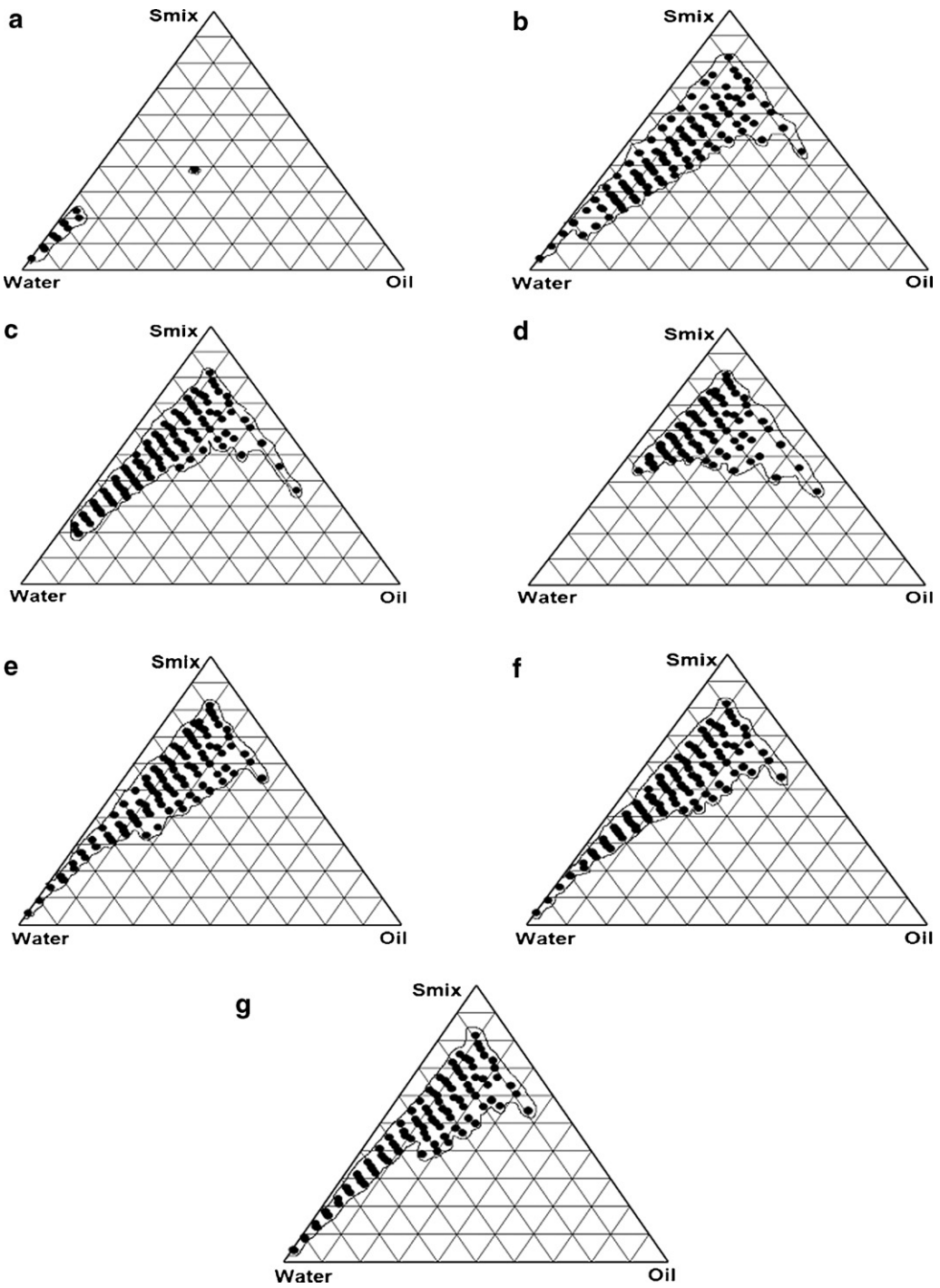

Fig. 1. Pseudo-ternary phase diagrams of system containing the following components: Capryol 90 as oil, Cremophor EL as surfactant, Transcutol® P as cosurfactant. Ratio (v/v) of surfactant to cosurfactant in (a) is 1:0, (b) is 1:1, (c) is 1:2, (d) is 1:3, (e) is 2:1, (f) is 3:1, (g) is 4:1. Dotted area shows oil in water nanoemulsion region.

formulations were selected (Table 2) for globule size determination, refractive index assessment, viscosity assessment and *in vitro* release studies.

3.6. Characterization of nanoemulsion

Since nanoemulsions possess a complex and varied structure as well as composition, their characterization is a difficult exercise. However, a variety of techniques have been exploited for the successful characterization of nanoemulsions as such knowledge is essential for their successful commercial exploitation (Vyas and Khar, 2002).

3.6.1. Percentage transmittance

No significant ($p > 0.05$) difference was observed among the percentage transmittance of formulations CF1, CF2 and CF3 (Table 2) and formulation CF1 was found to have the highest percentage transmittance. A value of percentage transmittance closer to 100% signified that all of the optimized formulations were clear and transparent. Nanoemulsion being a liquid formulation, clarity and transparency of the formulation are significant attributes in imparting elegance to the formulation and enhancing its acceptability by the patient. Besides signifying clarity of the formulation, a percentage transmittance closer to 100% also implies that the size of the globules in the nanoemulsion formulation approximates the

Table 2
Composition, mean ± S.D. ($n = 3$) percentage transmittance, refractive index, viscosity, droplet size, polydispersity index and zeta potential of nanoemulsion formulations from a batch.

Batch code	S_{mix} ratio	Percentage v/v of different components in formulation				Oil:S_{mix} ratio	Percentage transmittance ± S.D.	Mean refractive index ± S.D.	Mean viscosity (cP) ± S.D.	Mean droplet size ± S.D. (nm)	Mean PDI[a] ± S.D.	Mean zeta potential ± S.D. (mV)
		Oil	S	CoS	Water							
CF1	1:3	10	11.25	33.75	45	1:4.5	99.41 ± 0.21	1.407 ± 0.003	42.82 ± 2.38	37.22 ± 7.75	0.208 ± 0.011	−26.65 ± 1.13
CF2	1:2	20	15	30	35	1:2.25	98.97 ± 0.42	1.423 ± 0.002	46.09 ± 3.14	132.49 ± 27.94	0.210 ± 0.011	−22.48 ± 1.26
CF3	1:1	10	25	25	40	1:5	99.03 ± 0.08	1.412 ± 0.002	51.43 ± 1.27	102.8 ± 23.58	0.229 ± 0.030	−19.70 ± 0.54

[a] PDI indicates polydispersity index.

nanometer range. This in turn indicates that the drug in the formulation has a large surface area for release and absorption in biological milieu.

3.6.2. Refractive index

Formulation CF1 was found to have significant ($p < 0.001$) difference in the refractive index as compared to formulations CF2 but not with respect to CF3 when refractive index of the selected formulations was determined (Table 2). Refractive index of all the nanoemulsion formulations was found to be closer to the refractive index of neat Capryol 90 (1.422 ± 0.002). This led to the conclusion that the optimized nanoemulsion formulations were not only thermodynamically stable but also isotropic in nature. Similarity of the values of refractive index is a sign of the uniform nanoemulsion structure. A uniformity of the structure of nanoemulsion is significant in ensuring dose uniformity of the formulation. This in turn can help in minimising variations in bioavailability during the course of the therapy.

3.6.3. Viscosity

Results from the viscosity studies revealed that there was no significant ($p > 0.05$) difference among the viscosity of the nanoemulsion CF1, CF2 and CF3. Nanoemulsion CF1 was found to have the lowest viscosity (Table 2). In general, the selected nanoemulsion formulations had a very low viscosity. Low viscosity of the formulations is significant in its production and transfer. Moreover, geriatrics being the most vulnerable population to hyperlipidemia and associated disorders, low viscosity of the nanoemulsions is also favorable considering its ease of intake by the elderly.

3.6.4. Droplet size analysis (particle size distribution)

Formulation CF1 was found to have the smallest globule size and least polydispersity index (PDI) of 37.22 ± 7.75 nm and 0.208 respectively when droplet size distribution was studied using DLS (Table 2). The difference in globule size of formulation CF1 was also found to be significant ($p < 0.001$) as compared to formulations CF2 and CF3. Significant increase in the mean globule size of the formed nanoemulsion upon dilution has been reported in earlier studies (Dixit and Nagarsenker, 2008a,b) which could negatively affect the release of the drug from the developed formulation when the formulation undergoes infinite dilution in the GI fluids. Since PDI of the developed nanoemulsion formulations in the present study was small, this indicates uniformity in the size distribution of the dispersed oil globules. The difference in the globule size of formulation CF1 was also found to be significant ($p < 0.001$) in comparison to the globule size of the nanoemulsion reported in earlier study by our group (Bali et al., 2010b). Small sizes of the dispersed oil globules in the present study imply a large surface area for the faster release of drug from the formulation and subsequent absorption in the biological system. Due to their subcellular and submicrometer size, nanoemulsions are expected to penetrate deep into tissue through fine capillaries. This would allow efficient delivery of therapeutic agent to target site in the body (Tamilvanan, 2009).

3.6.5. Determination of zeta potential

Table 2 shows the results from the determination of zeta potential. Since zeta potential signifies degree of repulsion between neighbouring, like charged particles in dispersion, it can be related to the stability of colloidal dispersions. For molecules that are small enough a high zeta potential will confer stability, i.e. the solution or dispersion will oppose aggregation. Charge interactions are governed by zeta potential. Typically, −30 mV or +30 mV would be regarded as high zeta potential. Negative values of zeta potential of the optimized formulations showed that the formulations were negatively charged and high values of zeta potential of all the formulations denoted stability of the system. The high negative charge of the prepared nanoemulsions is probably due to the anionic groups of the fatty acids and glycols present in the oil, surfactant and cosurfactant. Thus, there are minimal chances of coagulation or flocculation of the system in the biological environment and during its shelf life.

3.6.6. Transmission electron microscopic (TEM) analysis

Since TEM directly generates images at high resolution and can also capture any concomitant structures as well as microstructure transitions, it is the most vital tool for the study of microstructures (Ghosh and Murthy, 2006). Transmission electron microscopy was employed to establish morphology and structure of the optimized nanoemulsion formulations. The nanoemulsion droplets emerged as dark and the surroundings were found to be bright. Fig. 2 shows droplet sizes of some of the dispersed oil globules of nanoemulsion CF1 measured using TEM. The droplet sizes were in proximity with the results obtained using DLS.

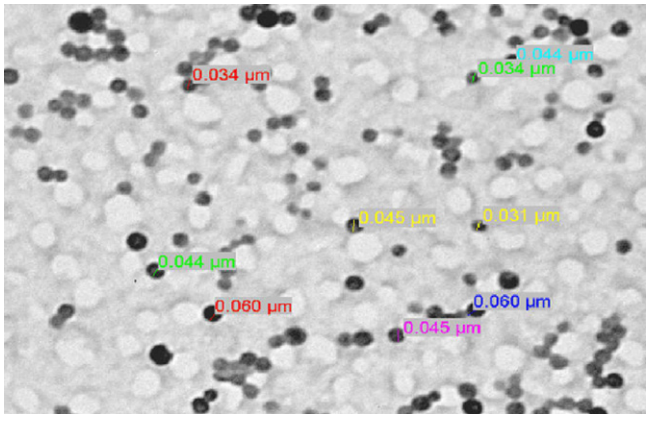

Fig. 2. Transmission electron microscopic positive image of ezetimibe nanoemulsion CF1, showing size of some oil droplets.

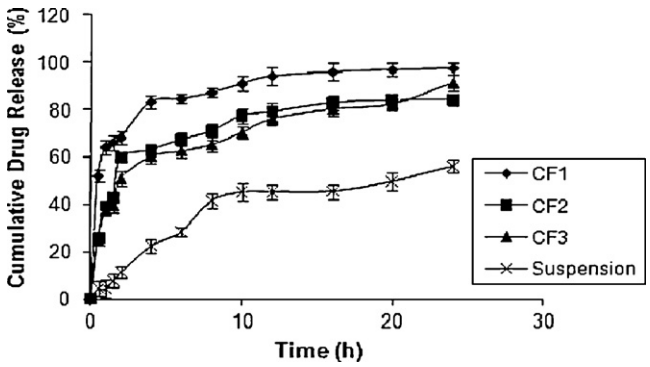

Fig. 3. Dissolution profile of ezetimibe (mean percent release ± S.D., $n = 3$) from nanoemulsion formulations CF1 to CF3 and drug suspension.

3.7. In vitro drug release study

The release of drug from the selected nanoemulsion formulations is shown in Fig. 3. The release of drug from the nanoemulsion formulations was found to be highly significant ($p < 0.001$) in contrast to the drug suspension. The dissolution profile of ezetimibe from the nanoemulsions CF1, CF2 and CF3 was found to follow first order release kinetics (data not shown). The maximum release, i.e., 97.6% was obtained in case of formulation CF1. As compared to the drug suspension that showed a release of merely 4.5%, the nanoemulsion CF1 was found to release more than 60% of the drug in the first hour of the study. Both the maximum drug release and percent cumulative drug release in the first hour were found to be higher from nanoemulsion CF1 in comparison to the earlier published report by our group (Bali et al., 2010b). Smaller globule size in case of nanoemulsion CF1 in the current study offers a large surface area for the release of drug thereby facilitating quicker rate of drug release. The *in vitro* drug release of the optimized formulation in earlier studies has been performed in 0.5% (w/v) solution of sodium lauryl sulphate (Dixit and Nagarsenker, 2008a,b). In the presence of a wetting agent, the solubilization potential of a formulation prepared with the objective of augmenting solubility of a poorly water-soluble drug may not be displayed accurately. This may cause confounding interpretation of the release behaviour. Moreover, the reported reduction in the rate of dissolution of the self nanoemulsifying formulation on filling into capsules in the same study may also lower the potential of the developed system. In contrast to this, a nanoemulsion formulation helps to administer a poorly water-soluble compound in a dissolved state and by virtue of its liquid nature, the energy related with the solid–liquid transition is also reduced. In addition, the preconcentrate has its drawbacks as a drug delivery system. The most noteworthy one is that the dosage form utilizes a large amount of surfactants so as to form nanoemulsion. High amount of surfactants impose clinical liabilities due to the toxicity issues associated with surfactants when used at high levels. This becomes significant considering the fact that treatment of hyperlipidemia involves chronic therapy. Eventually, formulation CF1 was chosen for the evaluation of antihyperlipidemic potential and bioavailability studies owing to its maximum drug release (97.6%), smallest droplet size (37.22 ± 7.75 nm), least polydispersity (0.208) and lowest viscosity (42.82 ± 2.38 cP).

3.8. Effects in rat model of hypercholesterolemia

In vitro estimation of total cholesterol and HDL cholesterol in plasma revealed that the value of total cholesterol in the group provided with the formulation CF1 was highly significant ($p < 0.001$) in comparison to the values achieved in the group receiving cholesterol only and the group administered with the pure suspension of the drug (Fig. 4). This could be attributed to the fact that ezetimibe decreases total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B and enhances HDL cholesterol in patients with hypercholesterolemia (Basha et al., 2007; Basha et al., 2007). Furthermore, the reduction in the value of total cholesterol and HDL cholesterol by nanoemulsion CF1 was found to be better than that observed in earlier study by our group (Bali et al., 2010b). This implies a superior protection against hyperlipidemia from nanoemulsion CF1 than that by nanoemulsion TF1. The values of HDL-cholesterol in the group provided with the formulation CF1 was not significant in comparison to the values achieved in the group receiving cholesterol only and the group administered with the pure suspension of the drug.

3.9. Bioavailability study of ezetimibe formulations in rats

Bioavailability study was performed with the objective of estimating ezetimibe after oral administration of ezetimibe formulations. Ezetimibe is rapidly absorbed after oral administration and undergoes conjugation by the action of UDP-glucuronosyltransferases (UGT) in the small intestine and liver to ezetimibe-glucuronide. Ezetimibe and ezetimibe-glucuronide account for 10–20 and 80–90% of the total drug in plasma respectively. The glucuronide metabolite of ezetimibe has been reported

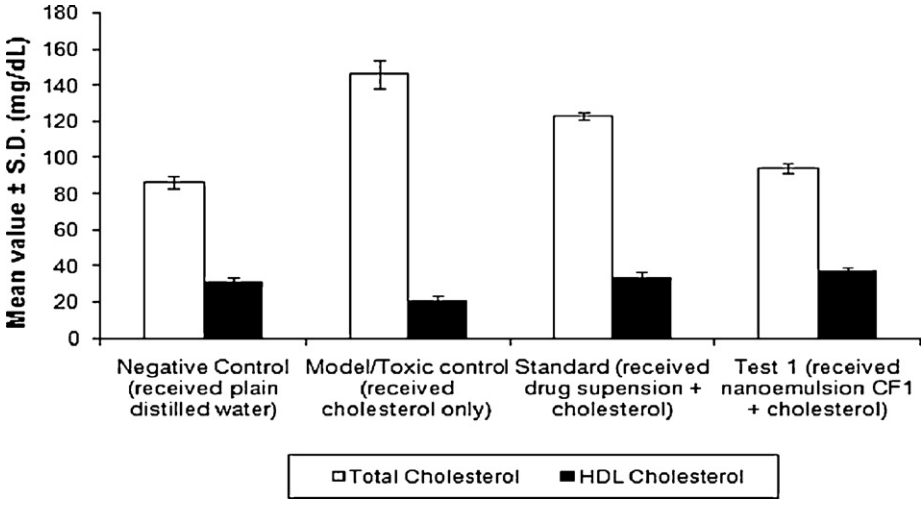

Fig. 4. Mean ± S.D. ($n = 6$) value of total cholesterol and HDL-cholesterol (mg/dL).

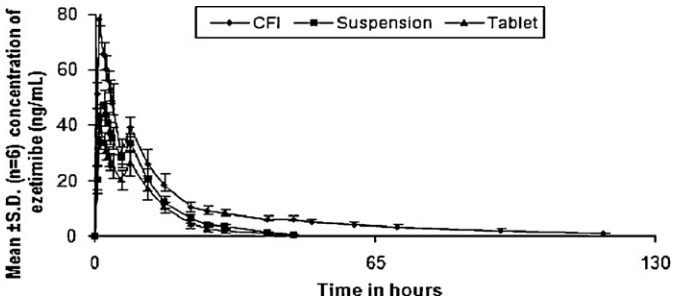

Fig. 5. Plasma concentration (mean ± S.D., $n = 6$) profile of ezetimibe after oral administration of different formulations to adult wistar rats.

to be more effective in inhibiting the cholesterol transport than ezetimibe itself (Ezzet et al., 2001; Patrick et al., 2002; Jeu and Cheng, 2003; Lipka, 2003). Total ezetimibe, which includes both ezetimibe and ezetimibe-glucuronide, was estimated. For this purpose, ezetimibe-glucuronide was converted back to ezetimibe by using the enzyme β-glucuronidase. It was observed that the plasma concentration–time profile of ezetimibe for the nanoemulsion corresponded to better improvement of absorption of drug as compared to the marketed formulation and drug suspension (Fig. 5).

The C_{max} of CF1 was found to be 78.91 ± 6.65 ng/mL whereas its value for the drug suspension and tablet formulation was found to be 47.42 ± 5.28 ng/mL and 43.74 ± 2.59 ng/mL, respectively. Statistically, the C_{max} of CF1 was found to be extremely significant ($p < 0.001$) in comparison to the drug suspension and tablet formulation (Table 3). $AUC_{0\rightarrow 128\,h}$ values for the formulation CF1, drug suspension and tablet were found to be 1073.44 ± 83.72 ng h/mL, 293.64 ± 65.79 ng h/mL, 222.01 ± 42.48 ng h/mL respectively. $AUC_{0\rightarrow 128\,h}$ value for CF1 was found to be extremely significant ($p < 0.001$) as against that of drug suspension and marketed tablet. $AUMC_{0\rightarrow 128\,h}$ values for the nanoemulsion CF1, drug suspension and tablet formulation were observed to be 25700.75 ± 552.23 ng h^2/mL, 6031.50 ± 99.92 ng h^2/mL and 4458.03 ± 95.43 ng h^2/mL respectively. Statistically this value for CF1 was extremely significant ($p < 0.001$) in comparison to the drug suspension and tablet formulation. T_{max} of nanoemulsion CF1 was found to be 1 h whereas its value for drug suspension and marketed tablet was found to be 2 h and 2.5 h respectively. The reduction in value of T_{max} observed in case of nanoemulsion could be due to the fact that nanoemulsion presents the drug in a solubilized form to the gastrointestinal tract and the major rate-limiting step in the absorption, drug dissolution is absent. In case of suspension, the drug is suspended in the form of fine particles and is yet to undergo dissolution in GI fluids to get absorbed. In case of tablets, the drug is not available for absorption until the dosage form undergoes disintegration and further deaggregation into fine particles which can undergo subsequent dissolution. Since in suspension dosage form, the drug is present in the form of fine particles having large surface area for dissolution, the process of dissolution and subsequent absorption is faster in suspension in comparison to tablets. In nanoemulsion, the nano size range of the globules ensures an enormously large surface area for the dissolution of the drug leading to fastest drug release and subsequent absorption. Thus, the number of steps involved in the dissolution of the drug from the dosage form and the surface area available for the release of drug from the dosage form could be the contributing factors towards the observed order of T_{max}. $MRT_{0\rightarrow 128\,h}$ values for the nanoemulsion CF1, drug suspension and tablet formulation were observed to be 23.39 ± 2.37 h, 20.54 ± 1.99 h and 20.08 ± 1.01 h 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 CF1 was compared to that of suspension and tablet formulations. MRT is an intrinsic property of the drug and there is no change in the intrinsic property when a drug is formulated into different formulations. The percent relative bioavailability of CF1 with respect to drug suspension was found to be 313.34% whereas with respect to marketed tablet was found to be 462.78%.

Both the value of C_{max} and AUC from nanoemulsion CF1 in the current study were found to be highly significant ($p < 0.001$) in comparison to the values reported in the earlier study by our group (Bali et al., 2010b). The percent relative bioavailability of CF1 with respect to drug suspension and marketed tablet was also found to be higher than that of naneomulsion TF1 reported previously (Bali et al., 2010b). These figures corroborate a better absorption of ezetimibe from nanoemulsion CF1 in comparison to nanoemulsion TF1. The superior bioavailability of nanoemulsion CF1 in the present study is most likely due to the enhanced solubility and instant dispersion of the drug in the GIT (Constantinides, 1995). In addition, the presence of a surfactant Cremophor EL in the present study which is also reported to be a potent inhibitor of P-gp over Tween 80, and a permeability enhancer Transcutol® P, in the nanoemulsion CF1 might have resulted in better alteration in the membrane permeability due to the inhibition of an apocally polarised efflux system (Neurkar et al., 1996). Since the drug is already available as a once a day formulation in the market, decrease in value of T_{max} observed in case of nanoemulsion over suspension and tablet might not change the dosing frequency. However, enhancement in oral bioavailability of the drug from the nanoemulsion in comparison to suspension and tablet formulation might considerably reduce the dose of the drug when administered in the form of a nanoemulsion, thus affecting the dosing regimen.

The concentration of ezetimibe from the study of plasma concentration–time profile of ezetimibe from CF1, drug suspension and tablet formulations, was found to reach its peak followed by a swift decline and then increased resulting in multiple peaks. Ezetimibe is excreted into bile after undergoing extensive glucuronidation in the intestine to a phenolic glucuronide. It is probable that after going through absorption in the ileum, ezetimibe is repeatedly delivered back to its site of action, the lumen of the intestinal tract via enterohepatic recirculation (EHC). This may consecutively enhance the residence time of the drug in the lumen of

Table 3
Mean pharmacokinetic parameters of ezetimibe from CF1, 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)
CF1	1.00	78.91 ± 6.65[f]	1073.44 ± 83.72[f]	25700.75 ± 552.23[f]	23.39 ± 2.37
Drug suspension	2.00	47.42 ± 5.28	293.64 ± 65.79	6031.50 ± 99.92	20.54 ± 1.99
Tablet	2.50	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.
[f] $p < 0.001$ when compared with drug suspension and tablet using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test.

Table 4
Mean ± S.D. ($n = 3$) refractive index, viscosity, droplet size and concentration of drug remained in nanoemulsion CF1 stored at 40 ± 2 °C and 75 ± 5% RH.

Time (in days)	Mean refractive index ± S.D.	Mean viscosity ± S.D. (cP)	Mean droplet size ± S.D. (nm)	Mean concentration of drug remained ± S.D (μg/ml)	% drug remained	Log % drug remained
0	1.404 ± 0.003	42.84 ± 2.13	92.09 ± 8.54	50.07 ± 0.08	100	2.0000
30	1.407 ± 0.004	42.86 ± 2.22	92.31 ± 8.72	49.79 ± 0.04	99.44	1.9976
60	1.410 ± 0.004	42.89 ± 2.31	92.72 ± 8.93	49.59 ± 0.07	99.04	1.9958
90	1.413 ± 0.005	42.93 ± 2.42	92.93 ± 9.12	49.46 ± 0.06	98.78	1.9947

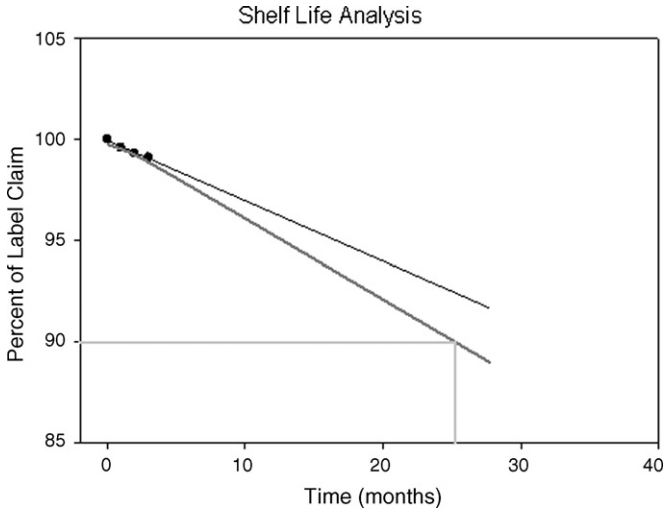

Fig. 6. Shelf life determination of nanoemulsion CF1. The thin line shows the curve obtained by plotting the percent drug remaining versus time whereas the bold line shows the plot of 95% one sided confidence limit for the percent drug remaining values versus time.

the intestinal tract and thus may potentiate its antihyperlipidemic action [11,28–30].

3.10. Stability studies on optimized nanoemulsion

No significant ($p > 0.05$) change was observed in the values of droplet size, viscosity and refractive index estimated at the end of 0, 30, 60, and 90 days (Table 4). A degradation of 1.22% was observed in the content of ezetimibe in the formulation CF1 at the end of 90 days. Shelf life of the formulation CF1 and was found to be 18.53 months (Fig. 6).

4. Conclusions

A nanoemulsion having the formula as Capryol 90 (10%, v/v), Cremophor EL (11.25%, v/v), Transcutol® P (33.75%, v/v), and double distilled water (45%, v/v) was successfully optimized to achieve higher release as well as bioavailability of ezetimibe in comparison to previously prepared nanoemulsion TF1. Stability of the formulation was confirmed by zeta potential determination. The optimized formulation also demonstrated superior lipid lowering properties in contrast to nanoemulsion TF1. The bioavailability studies in rats revealed improved bioavailability of ezetimibe form the optimized formulation as compared to nanoemulsion TF1 reported previously. Absence of significant changes in the observed physical parameters during stability studies indicated stability of the formulation. The shelf life of the optimized nanoemulsion was calculated to be 18.53 months. The present study corroborated use of inhibitors of P-glycoprotein (P-gp) efflux transporter in oral nanoemulsion formulations to be a promising alternative for increasing the intestinal absorption of drugs secreted by a P-gp-mediated efflux so as to achieve enhanced absorption and oral bioavailability.

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