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Formulation and In Vivo Evaluation of Self-Nanoemulsifying Granules for Oral Delivery of a Combination of Ezetimibe and Simvastatin

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Self-nanoemulsifying granules were formulated with the objective of improving the bioavailability of the ezetimibe and simvastatin when administered together. Composition of self-nanoemulsifying system (SNS) was optimized using various modified oils, surfactant, and cosurfactant mixtures. SNSs were mixed with water and resultant emulsions were characterized for mean globule size and stability. SNSs were adsorbed on hydrophilic colloidal silicon dioxide to give free-flowing self-nanoemulsifying granules. Self-nanoemulsifying granules were characterized by X-ray diffraction pattern, scanning electron microscopy, dissolution profile, and for in vivo performance in hypercholesterolemic rats. X-ray diffraction studies and scanning electron microscopy indicated loss of crystallinity and/or solubilization of both drugs in the self-nanoemulsifying granules. Self-nanoemulsifying granules effected substantial increase in dissolution of the drugs as compared with pure powder of drugs. In vivo evaluation in rats showed significant decrease in the total cholesterol levels and triglyceride levels in rats as compared with positive control confirming potential of self-nanoemulsifying granules as a drug delivery system for the poorly water-soluble drugs.

Keywords self-nanoemulsifying granules; ezetimibe; simvastatin; colloidal silicon dioxide; hyperlipidemia

INTRODUCTION

A large number of new chemical entities are known to possess low aqueous solubility resulting in poor dissolution in gastrointestinal tract and very low bioavailability. Solubilization of drugs in the gastrointestinal tract is dependent on many complex factors including the effect of food, which results in erratic drug absorption. Lipid-based formulations are known to improve the bioavailability of poorly water-soluble drugs and decrease the variability in the absorption of drugs, which is beneficial to drugs with low therapeutic index (Hauss, 2007). These formulations can increase the bioavailability of drug by various mechanisms including lymphatic transport of drugs, which decreases the first pass effect (Humberstone & Charman, 1997), inhibition of presystemic degradation of drugs by enzymes (Wandel, Kim, & Stein, 2003), prevention of P-glycoprotein-related efflux (Cornaire et al., 2004), and by increasing the gut permeability (Kang et al., 2004). Self-nanoemulsifying systems (SNS) are mixtures of oil, surfactants, and cosurfactants that form fine oil in water nanoemulsions (NEs) when introduced into aqueous phases under gentle agitation (Nazzal, Smalyukh, Lavrentovich, & Khan, 2002). Nanoemulsions with smaller droplet size provide high surface area for fast and uniform absorption of drugs (Kang et al., 2004). SNS are filled into soft gelatin capsules or HPMC or gelatin based sealable capsules. However there are chances of physical instability with the hard gelatin capsule shells due to hygroscopicity of the excipients and may lead to integrity problems.

One of the approaches of presenting emulsions as more physically stable, elegant and patient compliant dosage form is to convert them into systems reported as solid state emulsions or dry emulsions (Myers & Shivley, 1993). Dry emulsions have been prepared by removing water from an ordinary emulsion using soluble or insoluble carriers by rotary evaporation, spray drying, lyophilization or by adsorbing the liquid emulsions or SNS on the surface of insoluble carriers (Chambin, Bellone, Champion, Rochat-Gonthier, & Pourcelot, 2000; Christensen, Pedersen, & Kristensen, 2001; Corveleyn & Ramon, 1998a, 1998b; Dollo et al., 2003; Dollo, Corre, Chevanne, & Verge, 2004; Pedersen, Faldt, Bergenstahl, & Kristensen, 1998). The type of liquid emulsion (o/w or w/o) and polarity of carrier will influence the release pattern of the drug from the system. Water-soluble carriers such as lactose, maltose, maltodextrin, sucrose, hydrophilic polymers like hydroxyl propyl methylcellulose, polyvinyl pyrrolidone were used for formulation of dry emulsions. Water insoluble carriers like hydrophilic and hydrophobic colloidal silicon dioxide as well as pH dependent polymers such as Eudragit E100 and hydroxyl propyl methylcellulose phthalate were also investigated for preparation of sustained release dry emulsion powders (Chambin, Berard, Rochat-Gonthier, & Pourcelot,

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2002; Cui, Wang, Wang, Feng, & Ning, 2007; Farah, Bouzan, Rollet, Taverdet, & Vergnaud, 1987; Toorisaka et al., 2005). These systems are attractive because they are physically and microbiologically stable solid formulations and can be utilized to protect photo unstable drugs against light and oxidation (Christensen, Pedersen, & Kristensen, 2002; Heinzelmann & Franke, 1999; Jang et al., 2006). Dry emulsions are easy to administer in the form of powders or pellets filled in capsules and tablets (Ahmed and Aboul-Einien, 2007; Christensen et al., 2001; Corveleyn & Ramon, 1998a, 1998b, 1999; Hansen, Holm, & Schultz, 2004; Hansen, Holm, Rohde, & Schultz, 2005; Newton, Petersson, Podczeck, Clarke, & Booth 2001).

Elevated low-density lipoprotein is a major risk factor for coronary heart disease. Many studies revealed that simvastatin (SIM), used to treat primary and secondary hypercholesterolemia could lead to substantial decrease in cardiovascular morbidity and mortality (Figure 1) (McKenney et al., 2007). Ezetimibe (EZE) is a first member of new class of cholesterol absorption inhibitors indicated for use as a monotherapy or in combination with statins for the treatment of primary hypercholesterolemia (Figure 1) (Kosoglou et al., 2005). Studies have been shown that EZE and SIM produced significantly greater reduction in LDL cholesterol relative to monotherapy of SIM and EZE and atorvastatin in patients with hypercholesterolemia. The superior cholesterol lowering ability of combination of SIM and EZE was observed with in all patients irrespective of age, gender and race (Davis, Pula, Alton, Burrier, & Watkins, 2001). Duration of 2 weeks was required for the 50% reduction of cholesterol levels in the patients suffering from hyperlipidemia when combination of SIM and EZE was used for treatment (Bays et al., 2004). Both the drugs are practically insoluble in water. Few papers have been reported to increase the solubility of SIM and thus decrease in the variability of bioavailability. (Ambike, Mahadik, & Paradkar, 2005; Kang et al., 2004; Patil, Patil, & Paradkar, 2007). Ezetimibe pharmacokinetics exhibited moderate intersubject variability with coefficient of variation ranging from 34 to 43% and 32 to 37% for C_{max} and AUC respectively. EZE slowly appears in the plasma with C_{max} occurring 4–8 h after ingestion. (Kosoglou et al., 2005).

The objective of the present study was to improve the dispersion of EZE and SIM and to reduce the variations in the bioavailability and to improve in vivo efficacy. EZE and SIM were incorporated into SNSs and these systems were converted into dry free flowing granules using insoluble hydrophilic colloidal silicon dioxide (Aerosil 200). After administration, granules in contact with gastrointestinal tract medium will release SNS and in turn get converted into nanoemulsions to facilitate improved absorption. The self-nanoemulsifying granules (SNGs) were optimized and evaluated for solid-state characteristics, surface and powder properties, and in vivo efficacy in experimental animal.

MATERIALS AND METHODS

Materials

EZE was a generous gift from Lupin Ltd. (Pune, India), SIM was obtained as a gift sample from Ipca Laboratories Ltd. (Mumbai, India), Capryol 90 (CAP), Transcutol P (TP), Lauroglycol 90 (LG), Labrasol (LAB), Labrafil 1944 CS (Colorcon Asia Pacific Pvt. Ltd., Mumbai, India), Migloyl 812 (S. Zhaveri & Co., Mumbai, India), Cremophor EL (CRE), Solutol HS 15 (SHS15) (BASF, Mumbai, India), Neobee M-5 (Stepan Company, Northfield, IL, USA). Hard gelatin capsules were gifted by Associated Capsules Ltd. (Mumbai, India). Colloidal silicon dioxide (Aerosil 200) was gifted by Aurobindo Pharmaceutical Pvt. Ltd. Propylene glycol (PG), PEG 400, Tween 80, and sodium lauryl sulfate (SLS) were purchased from S. D. Fine Chemical (Mumbai, India). All other reagents and solvents used were of analytical grade. Male S. D. Rats were procured from Glenmark Pharmaceuticals (Mumbai, India).

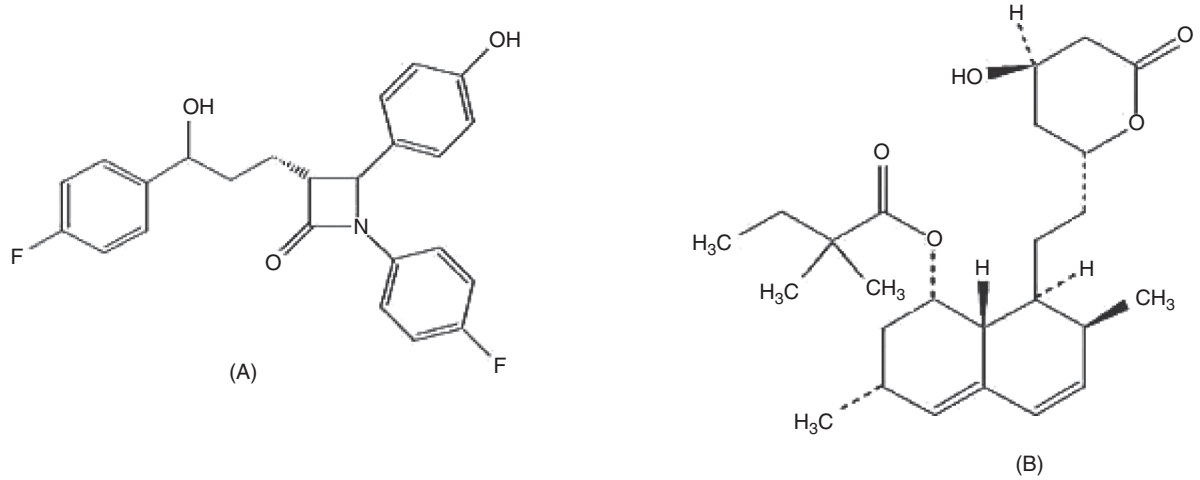

FIGURE 1. Chemical structure of drugs. (A) Ezetimibe, (B) Simvastatin.

Method of Analysis

EZE and SIM were analyzed by using high-performance liquid chromatography (HPLC). The chromatographic system consisted of the following components all from Jasco corporation (Tokyo, Japan): A UV/VIS detector (UV 2075 plus) covering the range of 200–400 nm and interfaced to a computer for data acquisition and a recorder model Star 800 interface module. A PU 2080 plus solvent delivery system. A rheodyne, 50 µL loop injector ODS Hypersil column (250 × 4.6 mm, 5 µm) was used (Thermo Electron Corporation). The mobile phase consists of acetonitrile and phosphate buffer pH 4.5 in the proportion of 65:35. The mobile phase was run through column at 1.0 mL/min. The column was maintained at ambient temperature. Detector was programmed at 232 nm for detection of EZE for 10 min and 238 nm for detection of SIM from 11 to 20 min SNG equivalent to 10:10 mg and 10:40 mg of EZE and SIM, respectively (hereafter referred to as 1,010 and 1,040 mg, respectively) were dispersed in suitable quantity of methanol. The samples were mixed thoroughly to extract the drugs in methanol. The samples were centrifuged using 12C micro centrifuge, (Remi motors, Mumbai, India) at 725 g for 15 min to separate the colloidal silicon dioxide particles. The supernatant was suitably diluted and analyzed.

Solubility Studies

Oils, surfactants, and cosurfactants were screened for solubility of EZE and SIM by shake flask method. An excess quantity of both was added to the 0.5 g of excipients and mixed in a vial for 5 min using CM101, cyclomixer (Remi, Mumbai, India). The mixture vial was shaken at 37 ± 1.0°C for 48 h using water bath shaker (Remi, Mumbai, India). The mixtures were centrifuged using 12C micro centrifuge, (Remi Motors, Mumbai, India) at 2016 g. The supernatant was separated, and EZE was extracted in methanol. The drug content was analyzed using HPLC method as described in the *Method of Analysis* section.

Construction of Ternary Phase Diagrams

Pseudo ternary phase diagrams were constructed in the absence and presence of drug. Phase diagrams were plotted for two strengths (1,010 and 1,040 mg) of EZE and SIM, that is, 10 mg of EZE and 10 mg of SIM, and 10 mg of EZE and 40 mg of SIM, respectively, in 500 mg of SNS. Nineteen experiments were carried out for preparation of phase diagram. The oil concentration was varied from 10 to 30% wt/wt surfactant and cosurfactant concentration was varied from 10 to 70% wt/wt. The above mixture was diluted 10 times with distilled water and observed for nanoemulsion formation. The nanoemulsions were kept under observation for 12 h. Nanoemulsions that showed precipitation of the drug or cracking were rejected. The area of nanoemulsion formation was identified for respective systems and phase diagrams were plotted. The mean globule size of most stable nanoemulsion was recorded by photon correlation spectroscopy (Beckmann N5 coulter, Miami, FL, USA). The nanoemulsion formed was checked for clarity, stability, and flow ability.

Preparation of Self-Nanoemulsifying Granules of EZE and SIM SNS

SNS were prepared by mixing the weighed quantity of drugs, oil, surfactant, and cosurfactant. The drugs were dissolved in the mixture of excipients by stirring using CM101, cyclomixer (Remi, Mumbai, India) at room temperature. The SNS (Table 1) was placed in a small bowl; colloidal silicon dioxide (Aerosil 200) was added slowly and mixed vigorously to get the granular mass. The ratio of 2:1 of SNS: colloidal silicon dioxide was used to prepare SNGs. The SNGs were passed through 500 µm mesh (B. S. S. 30 mesh) to get uniform free-flowing granules. The powder was stored over anhydrous calcium chloride in a dessicator until further evaluation.

Optimization of SNG

The SNGs were prepared with 2:1 proportion of SNS: colloidal silicon dioxide and evaluated for powder characteristics such as flow rate, bulk density, arithmetic mean particle diameter, and ability to produce nanoemulsions upon dilution. The flow rate was determined by measuring the time required for 1.0 g of SNG to flow through funnel with orifice of 1.5 cm diameter. The powder flow property was noted on the basis of time required to pass through orifice as less than 1 s (excellent), less than 5 s (good), less than 10 s (average), and more than 10 s (poor). Arithmetic mean particle size of powder was determined by sieve analysis. Mean globule size distribution of SNG mixed with aqueous media was determined by dispersing 100 mg in 4 mL of distilled water using cyclomixer. The mixtures were kept at rest for 10 min to allow colloidal silicon dioxide particles to sediment. The supernatant was filtered through a coarse filter (Whatman No. 1, 90 mm diameter), and the filtrate was used for globule size analysis using photon correlation spectroscopy (Beckmann N5 coulter). The optimized SNGs were considered for further evaluations.

TABLE 1
Optimized Formulae of SNS

	Formulations (Quantity in Milligram)					
Ingredients	F1	F2	F3	F4	F5	F6
Ezetimibe	10	10	10	10	10	10
Simvastatin	10	10	10	40	40	40
CAP	150	150	150	150	150	150
CRE	200	200	200	200	200	200
TP	150	–	–	150	–	–
LAB	–	150	–	–	150	–
SHS15	–	–	150	–	–	150

X-Ray Diffraction Studies

X-ray powder diffraction patterns were recorded on a Philips PW 17291 powder X-ray diffractometer using Ni-filtered, Cu K_ radiation, a voltage of 40 kV, and a 25-mA current. The scanning rate employed was 1° min^{-1} over the 10–40° 2θ range. The physical mixture of EZE, SIM, and colloidal silicon dioxide was made in the ratio of 1:1:25 and 1:4:25 for comparison. The XRD patterns of EZE and SIM mixture, colloidal silicon dioxide, physical mixture, and SNGs were recorded.

Scanning Electron Microscopy of SNGs

The morphological features of particles of EZE, SIM, colloidal silicon dioxide, and SNGs were investigated by Jeol JSM-840 scanning electron microscope. Gold sputter coating of all the samples was done to render the surface of particles electroconductive.

In Vitro Drug Release

SNGs containing 1,010 mg of EZE and SIM, respectively, were studied for drug release profiles in 500 mL, 0.05% wt/vol SLS solution. Dissolution testing of SNGs containing 1,040 mg of EZE and SIM was done in 0.05% and 0.5% wt/vol SLS solution. The dissolution studies were performed using type II apparatus (Electrolab, Mumbai, India) rotating at 75 rpm by powder dispersion technique at 37 ± 0.5°C. (Chambin et al., 2002). Samples were withdrawn at 30 min intervals ($\%DP_{30\ min}$) and analyzed by using HPLC for drug content.

Pharmacodynamic Activity in Rats

Animal study protocol was approved by the Institutional Animals Ethics Committee IAEC/CPCSEA, Mumbai. The antihyperlipidemic activity was evaluated in male Sprague-Dawley rats (200–250 g). Separate animal studies were performed for 1,010 and 1,040 mg strengths of EZE- and SIM-loaded SNGs. The animals were housed into groups of six and maintained on a standard diet with free access to water. Animals were fasted overnight before starting the experiment, anesthetized, and bled by retro-orbital puncture to obtain baseline values of total cholesterol (TC), high-density lipoproteins (HDL) in blood so that each animal served as its own control. For 1,010 mg of EZE and SIM strength, rats were divided into five groups namely control group receiving plain water, standard group receiving powder mixture of EZE and SIM in suspension form, and three test group receiving SNG formulation F1, F2, and F3, respectively. In the case of SNGs with strength of 1,040 mg of EZE and SIM, rats were divided in six groups namely control group receiving plain water, standard group receiving powder mixture of EZE and SIM in suspension form, placebo SNG group receiving placebo formulation of F5, and three test group receiving SNG formulation F4, F5, and F6, respectively. The hyperlipidemia was induced by feeding high fat diet (Ambike et al., 2005). The rats were fed daily with high fat diet consisting of 200 mg of cholesterol suspended in 2 mL of coconut oil. Two hours following high fat diet administration, animals were dosed with drugs. The blood samples were withdrawn after 4, 7, and 14 days of treatment. The serum was separated and analyzed for TC, (HDL), and triglycerides (TG) by in vitro diagnostic kit (Accurex diagnostics Pvt. Ltd., Mumbai, India). The statistical analysis for the determination of the difference in the lipid levels of control and treatment groups was performed by unpaired *t*-test and results with $P < 0.05$ were considered significant. The percentage of protection offered by the drug against control was calculated as follows:

$$\%\ \text{protection} = \frac{(\text{Mean \% increase in TC value of control group} - \text{Mean \% increase in TC value of drug treated group}) \times 100}{\text{Mean \% increase in TC value of control group}} \quad (1)$$

RESULTS AND DISCUSSION

Solubility Studies

The solubility of EZE and SIM in various oils and surfactants is given in Figure 2A and 2B. When SNS are in contact with aqueous media, they form clear to opalescent nanoemulsions. It is expected that components of SNS will contribute to solubilize drug and maintain in its solubilized form even when diluted with water. EZE had maximum solubility in CAP as compared with other lipid vehicles. Amongst the solubilizers and surfactants, TP showed highest capacity to dissolve the EZE. Excipients were selected on the basis of their ability to solubilize the drug and ability to form stable nanoemulsion. SIM had maximum solubility in CAP, LG 90 as compared with other oil phases. SIM showed around 60.0 mg/g solubility in all the surfactants. Amongst the cosurfactants, SIM demonstrated maximum solubility in TP as compared with other cosurfactants. It was observed that EZE showed good solubility in surfactants and cosurfactants as compared with modified oils, but SIM showed better solubility in oily phases as compared with EZE.

Pseudo Ternary Phase Diagrams

The shaded outer region is the area that was explored for the nanoemulsion formation in the presence of drugs. All 19 experiments to prepare SNS without drug gave stable nanoemulsions when diluted with mean globule size less than 300 nm. SNS prepared with incorporation of EZE and SIM demonstrate the effect of drug on the physical stability of nanoemulsions formed after mixing SNS with water. The objective was to identify composition which when diluted with water produces nanoemulsion devoid of precipitation of EZE and SIM and do not crack when stored at 12 h. Figures 3 and 4 show phase diagrams that identify area (dark shaded) of

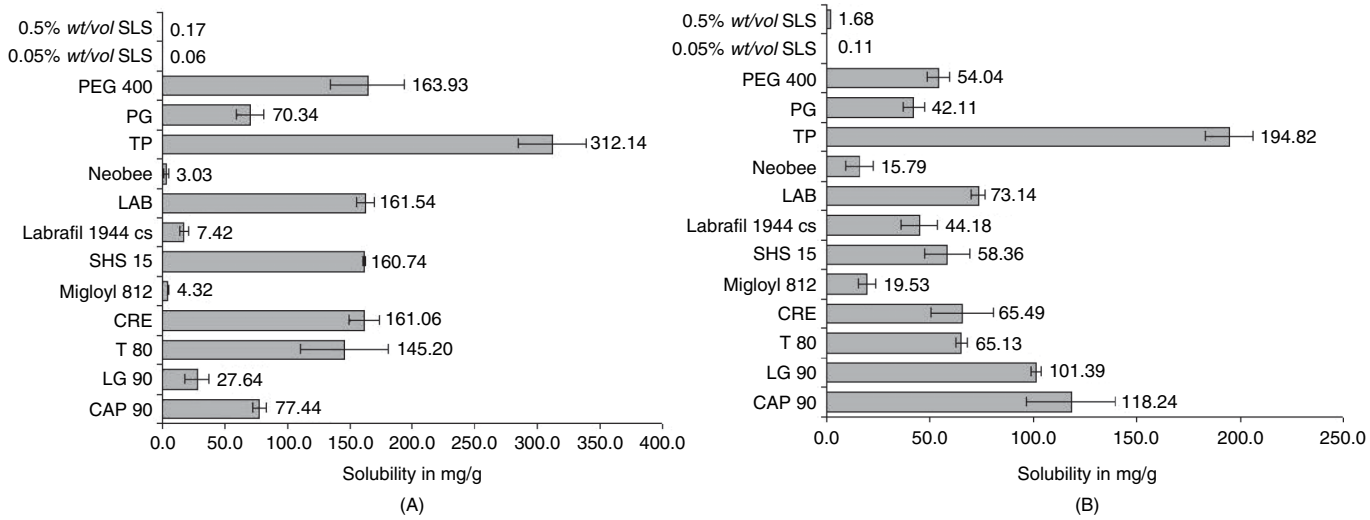

FIGURE 2. Solubility studies of ezetimibe (EZE) and simvastatin (SIM) in various excipients. (A) Solubility of EZE in mg/g of excipient, (B) Solubility of SIM in mg/g of excipient data expressed as mean ± *SD* (*n* = 3).

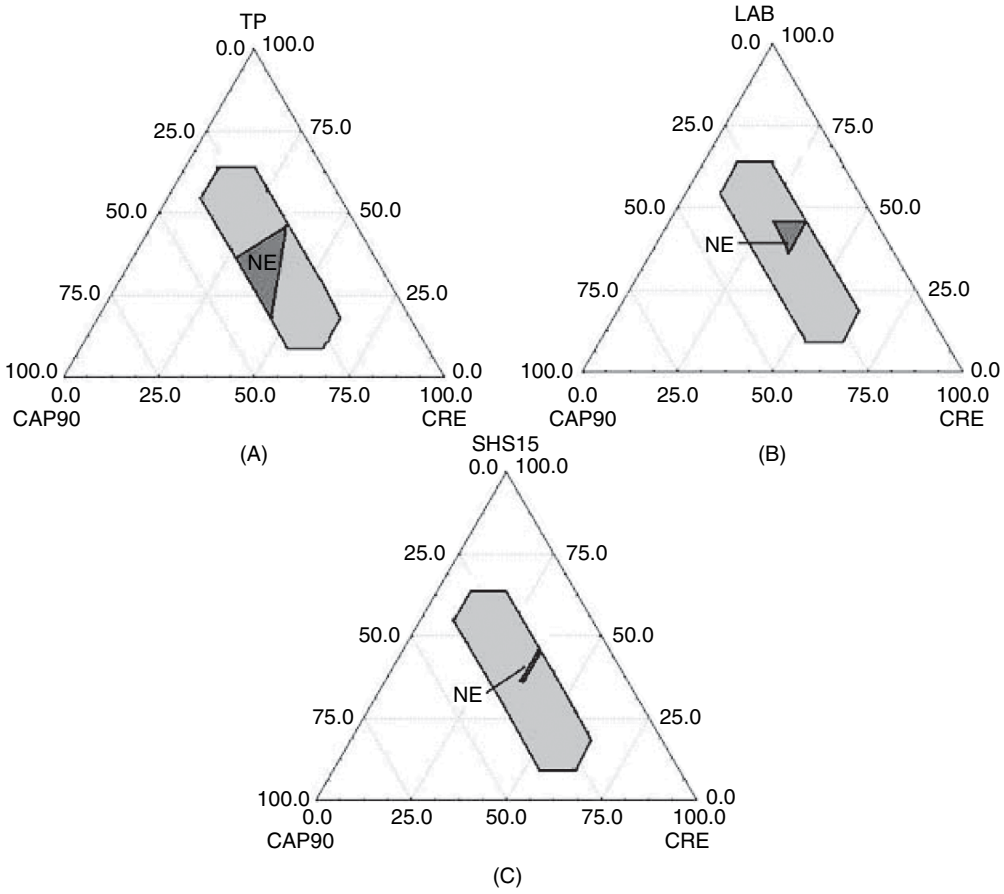

FIGURE 3. Phase diagram of self-nanoemulsifying system (SNS) containing 10 and 10 mg of SIM and EZE. (A) CAP, CRE, TP, (B) CAP, CRE, LAB, (C) CAP, CRE, SHS 15.

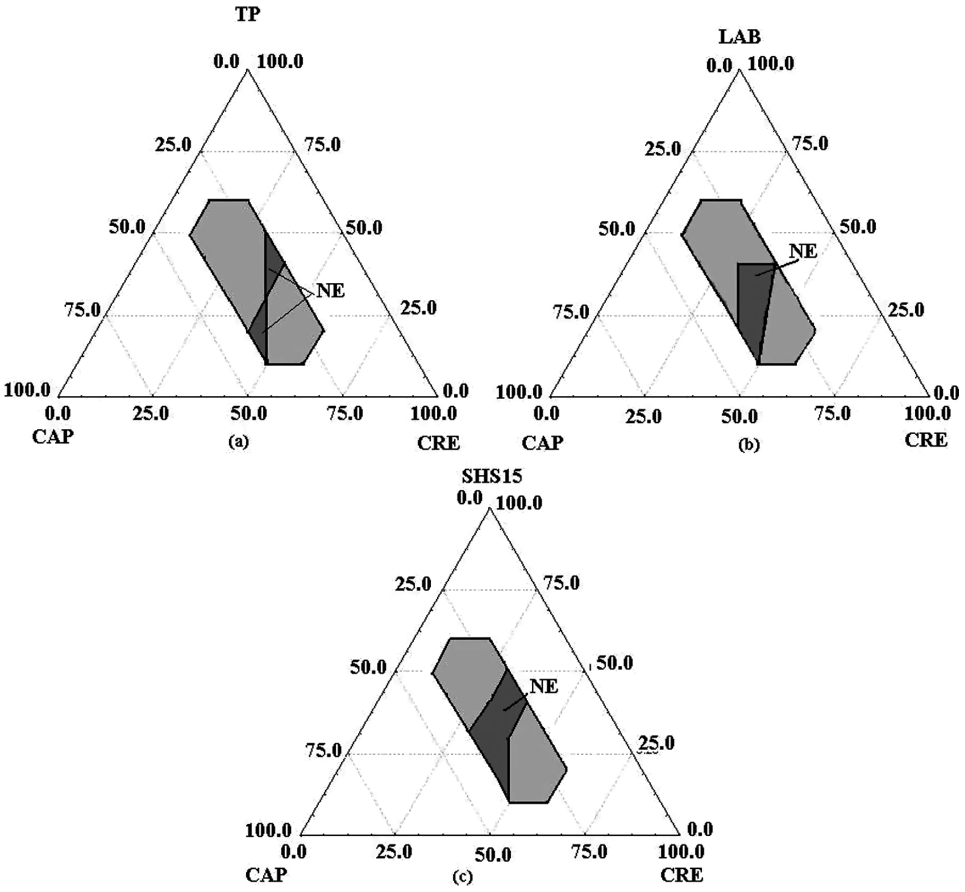

FIGURE 4. Phase diagram of self-nanoemulsifying system (SNS) containing 40 and 10 mg of SIM and EZE. (A) CAP, CRE, TP, (B) CAP, CRE, LAB, (C) CAP, CRE, SHS 15.

stable nanoemulsion in the presence of EZE and SIM. All the optimized formulations showed mean globule size in the range of 30–185 nm.

The area of stable nanoemulsion formation was increased when concentration of SIM was increased in the formulation from 10 to 40 mg. These results suggested SIM may have mild cosurfactant activity at the interface of oil and water because of its amphiphilic nature. The pH of the nanoemulsions after dilution with water was in acidic region from 4.0 to 4.5. The acidic nature was imparted to SNS by CAP, TP, LAB, and SHS15. In the presence of acidic condition, some part of SIM prodrug may get converted into SIM acid form, which is an active form (Alvarez- Lueje et al., 2005). In silico studies were performed to evaluate the ampiphilicity of SIM using Cerius 2, version 4.6, 1998 software (Accelrys Inc., San Diego, CA, USA). SIM is a prodrug that has five proton acceptor groups, and one proton-donating group and rest of the groups impart lipophilicity to the molecule. Whereas SIM acid is more hydrophilic in nature with six hydrogen bond-accepting groups and three hydrogen bond-donating groups. The lipophilicity of SIM acid is lower as compared with SIM prodrug. This amphiphilic nature of both SIM prodrug and SIM acid makes them suitable for molecular association with surfactants. The hydroxide groups of both forms of SIM and carboxylic acid group of SIM acid may interact with surfactant molecules to form hydrogen bonds. The lipophilic groups may get associated with the oily phase. More number of rotatable bonds (eight for SIM prodrug and 14 for SIM acid) make the molecule flexible enough to interact with the surfactant and cosurfactant molecules and form a closed pack, stable interfacial film resulting in highly stable nanoemulsions devoid of precipitation of drugs. This type of behaviour of amphiphilic drugs has been reported for ibuprophen (Formiga et al., 2007). The compositions of optimized SNS emulsions with reference to stability of nanoemulsions and lowest mean globule size are summarized in Table 1.

Optimization of SNGs

From the previous studies of our laboratory, 2:1 ratio of SNS : colloidal silicon dioxide was used for the preparation of SNGs (data not shown). The SNGs were free flowing and granular in nature. The bulk and tapped density of SNGs was found to be in the range of 0.33–0.36 g/mL and 0.46–0.5 g/mL and mean particle size of SNGs was in the range of 216–228 μm.

The mean globule size of nanoemulsions produced by mixing SNGs with water was in range of 150–600 nm. Significant increase in the mean globule size was observed when SNGs were mixed with various diluting media. The increase in the mean globule size may be due to coalescence of oil globules while converting it into SNGs or slow release of surfactants and cosurfactants from granules. However, the mean globule size after mixing with aqueous medium is still in nano range (less than 600 nm) retaining its advantages.

X-Ray Diffraction Studies

Figure 5 shows the X-ray diffraction patterns of EZE and SIM mixture, colloidal silicon dioxide, physical mixture of EZE, SIM and colloidal silicon dioxide, F1 SNG, F2 SNG, F3 SNG, F4 SNG, F5 SNG, and F6 SNG. In the X-ray diffraction pattern of EZE and SIM mixture, the sharp peaks at a diffraction angle (2θ) of 15.661, 15.840, 16.617, 17.297, 18.849, 19.423, 20.678, 20.905, 23.448, and 24.565° are present, and it reveals that drugs are present in crystalline form. Crystallinity peaks of drugs were still detectable in physical mixture with colloidal silicon dioxide. X-ray diffraction patterns of F1 SNG, F2 SNG, F3 SNG, F4 SNG, F5 SNG, and F6 SNG were characterized by diffuse spectra and no characteristic peaks of EZE and SIM. These results suggested that there was no crystallization of EZE and SIM while converting the liquid SNS into SNGs.

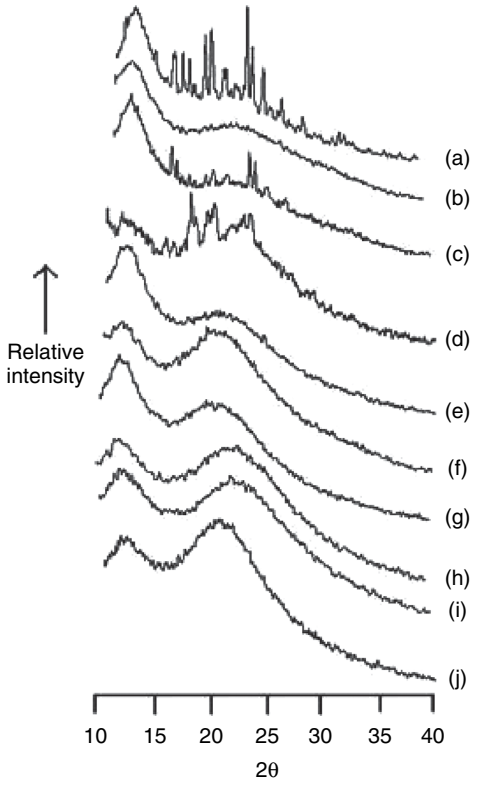

FIGURE 5. X-ray diffraction spectra of EZE, SIM, colloidal silicon dioxide, their physical mixture, and SNGs.(a) EZE and SIM in 1:1 proportion, (b) Colloidal silicon dioxide, (c) Physical mixture of EZE, SIM, and colloidal silicon dioxide in 1:1:25 proportion, (d) Physical mixture of EZE, SIM, and colloidal silicon dioxide in 1:4:25 proportion, (e) F1 SNG, (f) F2 SNG, (g) F3 SNG, (h) F4 SNG, (i) F5 SNG, (j) F6 SNG.

Scanning Electron Microscopy of SNGs

Scanning electron microscopy was undertaken to understand the surface characteristics of SNGs. Figure 6 revealed EZE and SIM as crystalline powder with rectangular plate-shaped crystals. Colloidal silicon dioxide was detected as aggregates of amorphous particles. The SNGs showed irregular shaped granular particles. No distinct crystals were evident on the surface of the granules after adsorbing the SNS on the surface of colloidal silicon dioxide confirms the presence of drug in its micronized/solubilized state.

In Vitro Drug Release

The drug content of all SNG formulations is summarized in Table 2. Because of insolubility of EZE and SIM in water, SLS solution was used as a dissolution medium. For 1,010 mg strength of EZE and SIM, 0.05% SLS wt/vol was used as discriminating dissolution medium. EZE being very insoluble in water, 0.05% wt/vol SLS was insufficient to dissolve it and only 3.0% of pure EZE powder was dissolved in 30 min (Table 3). SIM is relatively more soluble and 37% of SIM powder dissolved in 30 min. Because of insufficient solubility of EZE in 0.05% wt/vol SLS solution, 0.5% wt/vol SLS solution was used as a discriminating dissolution medium for higher strength of the SNGs. As SIM has high solubility in the 0.5% wt/vol SLS solution, it dissolved up to 80% in the medium and EZE dissolution was increased to 28% in 30 min. In 0.05% wt/vol SLS solution, formulation F1 SNG and F2 SNG provided fast release of drugs with around 23-fold enhancement in the dissolution of EZE and two times increase in the dissolution of SIM. Formulation F3 SNG showed lower values of $\%DP_{30\ min}$ as compared with F1 SNG and F2 SNG. This is in line with screening studies in which the ability to dissolve the drug was in order of TP > LAB > SHS 15. Formulation F4 SNG–F6 SNG provided approximately 13 times improvement in the dissolution of EZE and approximately seven times enhancement in the dissolution of SIM as compared with plain drug powders. In 0.5% wt/vol SLS solution, formulations provided threefold increased in the dissolution of EZE. As this medium is not a discriminating dissolution medium for SIM, no difference in the dissolution of SIM was observed from SNGs and plain drug powder. Overall all SNGs, F1 SNG–F6 SNG, facilitated higher dissolution of EZE in both the dissolution media as compared with plain drug powder. The enhancement in the dissolution of drugs from SNGs could be due to wetting and solubilization capacities of surfactant and cosurfactant mixture, which has helped to dissolve around 80% of drug in 30 min.

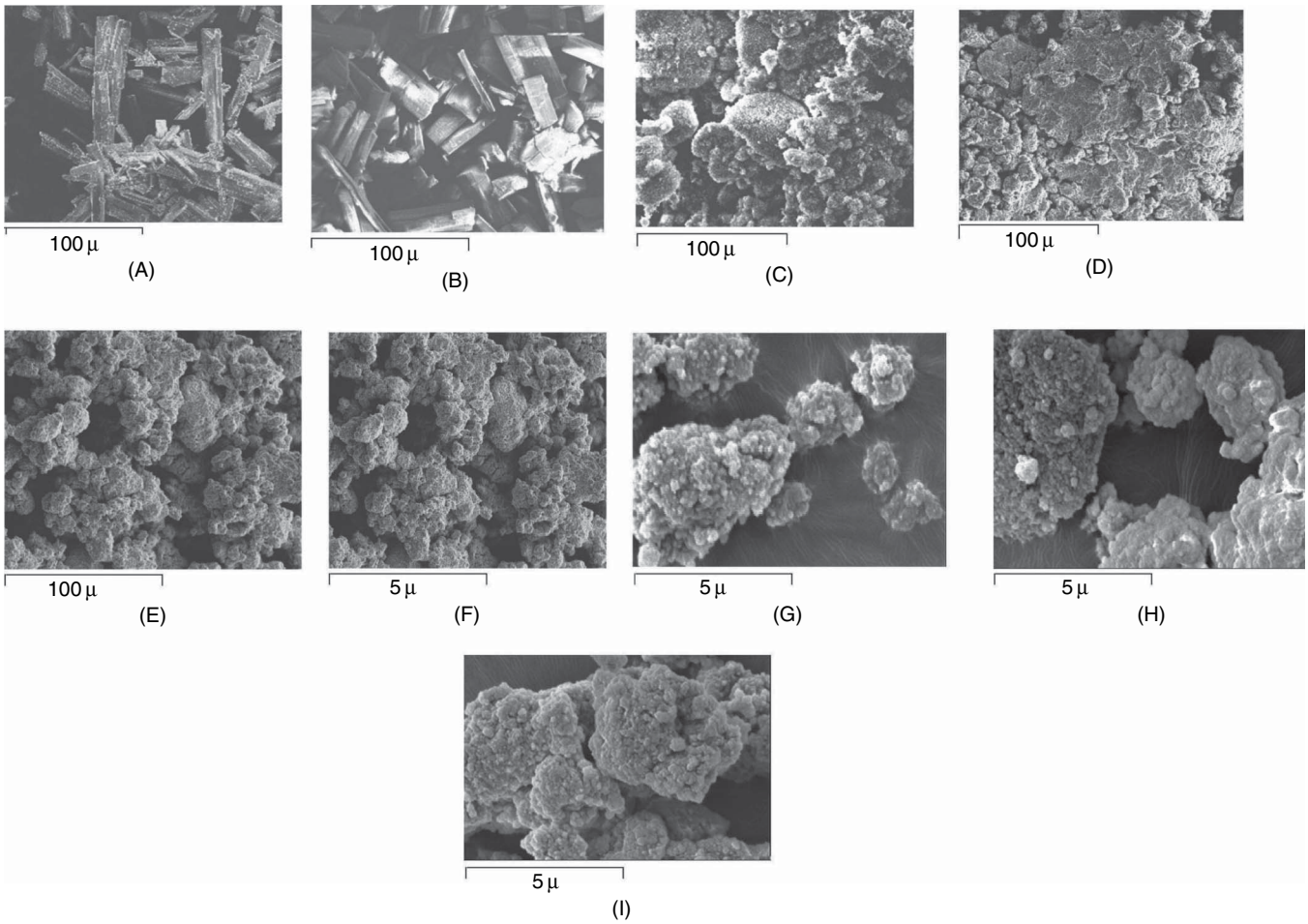

FIGURE 6. Scanning electron micrographs of SIM, EZE, colloidal silicon dioxide, and their SNGs. (A) SIM, (B) EZE, (C) Colloidal silicon dioxide, (D) F1 SNG, (E) F2 SNG, (F) F3 SNG, (G) F4 SNG, (H) F5 SNG, (I) F6 SNG.

TABLE 2
Percentage of Drug Content of EZE and SIM in SNGs

	Percentage of Drug Content					
	Formulation (Drug)					
	F1 (SNG)	F2 (SNG)	F3 (SNG)	F4 (SNG)	F5 (SNG)	F6 (SNG)
Ezetimibe	97.97 ± 0.168	101.64 ± 6.56	98.82 ± 0.185	99.58 ± 0.54	96.32 ± 4.28	102.38 ± 0.33
Simvastatin	102.43 ± 2.10	110.01 ± 6.15	99.26 ± 1.69	104.42 ± 1.65	102.80 ± 0.02	104.06 ± 1.28

Each Point Represents Mean ± *S D* (*n* = 3).

Pharmacodynamic Study in Rats

Pharmacodynamic evaluation in rats was done separately for both the strengths. Coadministration of EZE with SIM has proven to be more effective in reducing plasma cholesterol levels as compared with plain EZE and SIM. After 4 days of concomitant treatment with high fat diet, both F1 and F3 SNGs had significantly restricted the increase in the levels of TC as compared with rats treated with suspension of plain drugs ($p < 0.05$) (1,010 mg strength). F2 SNG was not superior to plain drug suspension, which is attributed to high standard deviation values ($p < 0.05$) (Figure 7). The percent protection offered by F1 SNG, F2 SNG, and F3 SNG was 216.23, 367.78, and 226.22%, respectively, as compared with no effect of plain drug suspension, which confirmed that formulations were better

TABLE 3
Comparison of Percentage of $DP_{30\ min}$ of EZE and SIM from SNGs in Various Dissolution Media

Formulations	Ezetimibe	Simvastatin
0.05% (wt/vol) SLS		
Plain EZE and SIM powder (1,010 mg)	3.27 ± 0.17	37.63 ± 2.26
F1 SNG (1,010 mg)	70.46 ± 3.50	79.05 ± 4.46
F2 SNG (1,010 mg)	72.81 ± 7.53	75.71 ± 5.53
F3 SNG (1,010 mg)	51.42 ± 1.02	52.54 ± 0.42
Plain EZE and SIM powder (1,040 mg)	3.17 ± 0.078	7.59 ± 7.59
F4 SNG (1,040 mg)	38.53 ± 2.01	44.32 ± 1.35
F5 SNG (1,040 mg)	39.20 ± 2.62	47.05 ± 0.93
F6 SNG (1,040 mg)	51.52 ± 0.8	54.95 ± 0.29
0.5% (wt/vol) SLS		
Plain EZE and SIM powder (1,040 mg)	28.76 ± 1.54	81.69 ± 0.47
F4 SNG (1,040 mg)	83.03 ± 1.08	75.28 ± 1.87
F5 SNG (1,040 mg)	81.70 ± 1.81	72.58 ± 1.47
F6 SNG (1,040 mg)	77.56 ± 4.19	70.50 ± 4.00

Each Point Represents Mean ± *SD* (n = 3). Using USP Apparatus II, 75 rpm, 37 ± 0.5°C.

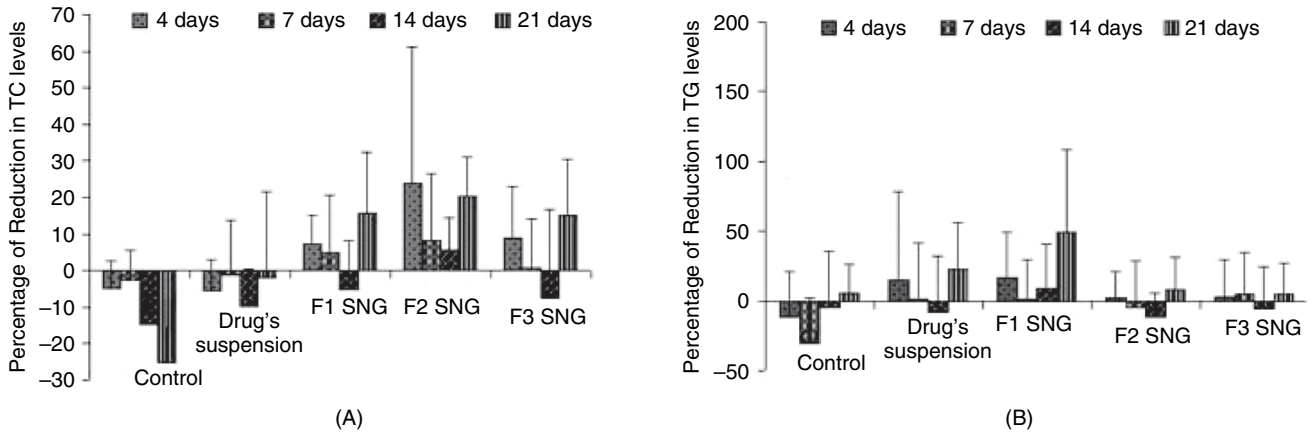

FIGURE 7. Percent changes in the levels of total cholesterol and triglycerides of experimental groups at different time intervals. (A) Percent changes in the levels of total cholesterol, (B) Percent changes in the levels of triglycerides. EZE and SIM were used in the concentration of 10 mg in the formulation.

for controlling the hyperlipidemia in rats (Table 4). Because of high standard deviation values, F1 SNG, F2 SNG, and F3 SNG did not show significant difference in the cholesterol levels as compared with rats treated with plain drugs suspension after 7 days of treatment. The percent protection offered by F1 SNG, F2 SNG, and F3 SNG was 183, 218, and 80% as compared with 22% protection offered by plain drug suspension. After 14 days of treatment, F2 SNG significantly limited the increase in the TC levels against plain drug suspension giving 127% protection as compared with 39% of plain drug suspension. All the drug-treated groups were better in controlling TC levels as compared with control group rats after 21 days of treatment ($p < 0.05$). The percent protection offered by formulations was two times more as compared with plain drug suspensions even after 21 days of treatment. The higher reduction in the TC offered by formulation could be due to increase in the dispersion of drugs leading to faster and complete absorption of drugs. The enhanced absorption of EZE and SIM led to reduced variability in the bioavailability resulting in faster onset of action and faster reduction in the cholesterol levels.

TABLE 4
Percent Protection Offered by Combination of EZE and SIM (10 and 10 mg, Respectively) in the Rats in Comparison with the Control Group Rats

Time in days	Plain EZE and SIM suspension (%)	F1 SNG (%)	F2 SNG (%)	F3 SNG (%)
4	−16.33	216.23	367.78	226.22
7	22.60	183.71	280.91	80.84
14	39.28	63.25	127.27	38.97
21	78.83	135.33	148.97	134.11

When HDL and TG levels were compared, there was no significant difference in the HDL and TG levels observed in the rats when compared with plain drugs treated rats even after 21 days of treatment.

The study was repeated for SNG with 1,040 mg strength for EZE and SIM. The study was performed at high dose level to investigate possibility of reduction in the variability in the results because of lower strength of drugs. After 4 days of treatment, all the rats treated with drug-containing formulations showed significant reduction in the TC levels as compared with control group rats ($p < 0.05$). The formulations F4 SNG and F6 SNG were better than plain drug suspension and provided 211 and 239% protection as compared with 129% provided by suspension of plain drugs (Table 5). Rats treated with placebo did not show significant effect on the cholesterol levels confirming that the excipients used in the study did not affect the cholesterol levels in vivo. Both F4 SNG and F6 SNG significantly prevented increase in the cholesterol levels after 7 and 14 days of treatment as compared with control group rats and offered better protection against the increase in TC levels than the rats treated with plain drug suspension (Figure 8). After 21 days of treatment, drug-containing formulations were

TABLE 5
Percent Protection Offered by Combination of EZE and SIM (10 and 40 mg, respectively) in the Rats in Comparison with the Control Group Rats

Time in Days	Plain EZE SIM Suspension (%)	Placebo SNG (%)	F4 SNG (%)	F5 SNG (%)	F6 SNG (%)
4	129.09	84.54	211.35	135.13	239.34
7	370.14	271.64	428.71	175.05	658.34
14	186.00	134.46	228.66	137.80	340.94
21	3308.22	1287.45	3451.83	2413.54	2533.17

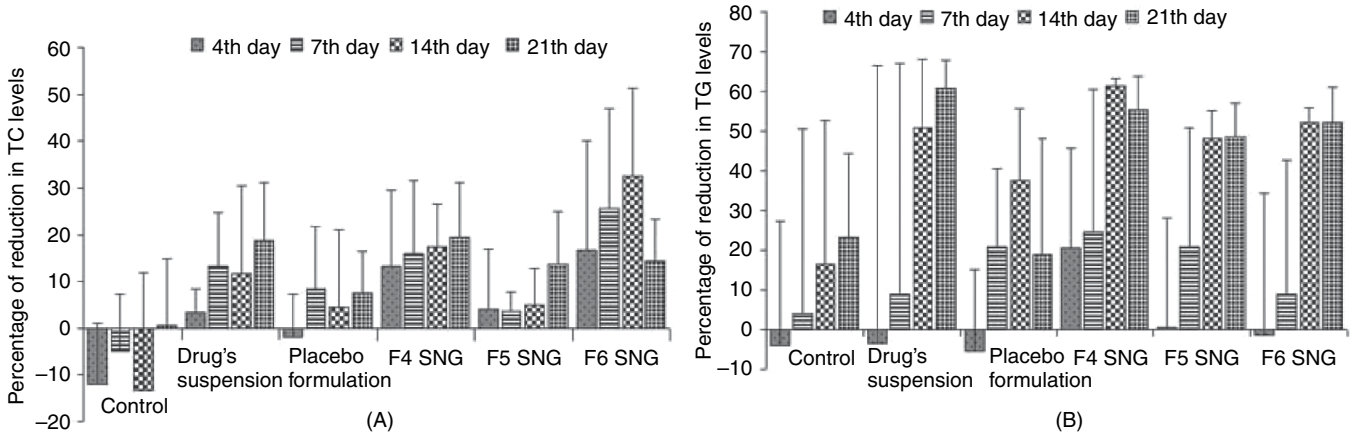

FIGURE 8. Percent changes in the levels of total cholesterol and triglycerides of experimental groups at different time intervals. (A) Percent changes in the levels of total cholesterol, (B) Percent changes in the levels of triglycerides. EZE and SIM were used in the concentration of 10 and 40 mg in the formulation.

significantly superior in controlling the TC levels as compared with control group rats and rats treated with placebo formulation. Formulation F5 SNG did not show improvement as compared with plain drug suspension. When TG levels were compared after 4 days of treatment, around 37 times percentage of protection was offered by F4 SNG as compared with plain drug suspension. F5 SNG and F6 SNG were also better in lowering TG levels as compared with plain drug suspension. Placebo formulation did not show protection confirming that the excipients did not lower the TG levels. After 7 days of treatment, the plain drug suspension also started showing action although SNG formulations were better in controlling the lipid levels. Rats treated with plain drugs or drug formulations showed no difference in the levels of TG after 14 and 21 days of treatment. There was no significant difference in the levels of high-density lipoproteins when the rats were treated with SIM and EZE for 21 days.

Overall F1SNG, F2 SNG, F4 SNG, and F6 SNG provided higher percentage of protection against increase in cholesterol levels as compared with plain drug suspension. This correlated well with enhanced in vitro drug release by the respective SNGs.

CONCLUSIONS

This study illustrated that the poorly water-soluble drugs with low and variable bioavailability can be formulated in the SNGs, which showed smooth conversion to nanoemulsion when mixed with aqueous media. The solubilizing ability of surfactants and cosurfactants was the key parameter to be considered for the superior in vivo performance of the drug. The superior dissolution profile of the drug from SNGs and enhanced in vivo activity establishes use of SNGs as a potential delivery system for water insoluble drugs.

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