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FORMULATION AND EVALUATION OF SELF MICROEMULSION DRUG DELIVERY SYSTEM OF LOW SOLUBILITY DRUG "SIMVASTATIN" FOR IMPROVED SOLUBILITY AND BIOAVAILABILITY

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ABSTRACT

The aim of the present work was to prepare a novel self microemulsion drug delivery system (ME) to enhance the solubility, dissolution rate and ultimately the oral bioavailability of a poorly water soluble drug, Simvastatin. The prepared self microemulsion of simvastatin was charactriesed by using techniques like FTIR analysis for investigating the drug-excipients interactions, zeta potential, viscosity determination, drug entrapment efficiency and thermodynamic stability studies. The invitro drug release profile of self microemulsion was carried in phosphate buffer Ph 7.4 for 1 hr by using USP dissolution apparatus type-II device. From the invitro dissolution data, F5 formulation was found that the drug release is best and the cumulative % of drug release was 98.62% respectively. The promising formulation F5 was found by evaluation studies were compared with Marketed product (Simvas 10mg), the F5 formulation gave 98.62% of the drug release and the Marketed product gave 45.19 % of drug release in 1 hr of dissolution study. The in-vitro intestinal permeability results exhibits the drug diffused at a faster rate from the self microemulsion system than from the tablet dosage form. After 1 hour of diffusion, 76.54% of drug was diffused from the self microemulsion system, as compared with 34.23% diffused from the tablets. Therefore the developed ME formulation improved the Solubility and *in-vitro* drug release of Simvastatin when compared with commercial tablet formulation.

Key Words:- Simvastatin, Self Microemulsion, Characterization, *In vitro* release studies, Stability studies.

INTRODUCTION

The oral route is the most acceptable route for drug delivery, but delivery of hydrophobic drugs through this route is nearly hampered to 50%. This is because of poor solubility, poor bioavailability, lack of dose proportionality, and unacceptable patient variability. As a result, one of the most important challenges facing the pharmaceutical research and development sector today is designing appropriate oral dosage forms for new chemical moieties that are poorly soluble in water. Among the various drug delivery systems that have been developed and explored, colloidal drug delivery systems offer great potential for solving problems encountered in the normal drug development process. The most widely used colloidal drug delivery systems are micelles, microemulsions, liposomes and nanoparticles. (Amidon G L *et al*., 1995; Dressman J B *et al*., 1998; Horter D *et al*., 2001; Bhatt PP *et al*., 2004; Aungust B *et al*., 1993; Burcham D L *et al*.,

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1997; Charman SA *et al*., 1992; Constantinides PP *et al*., 1995; Craig DQM *et al*., 1993; Craig DQM *et al*., 1995; Kararli TT *et al*., 1992; Kommuru TR *et al*., 2001; McClelland CA *et al*., 1991).

Self Microemulsions have been successfully designed to deliver hydrophobic drug and have been shown to increase their oral absorption and bioavailability. Self Microemulsions are mixtures of oils, (natural or synthetic), surfactants (solid or liquid at room temperature) and possibly co-surfactants that show some hydrophilicity and water. The mixtures are clear and isotropic microemulsions that are fine drops or globules with particle sizes below 100 nm. When these Self microemulsions used in the drug delivery system, drugs are incorporated into the oil or surfactant, and then water is added to form Self microemulsion spontaneously. As on today, there are four marketed formulations that are based on this technology- Sandimmune and Sandimmun Neoral (cyclosporine A), Norvir (ritonavir) and Fortovase (saquinavir). (Myers RA *et al*., 1992; Palin KJ *et al*., 1986; Schwendener, RA *et al*., 1996; Charman S *et al*., 1992; Constantinides P *et al*., 1994).

MATERIALS AND METHODS

Simvastatin and Cremophore RH 40 were obtained as a gift samples from (Bright Labs, Hyderabad, India). Transcutol p was purchased from Sd fine chemicals, ltd, Mumbai, India. Oleic acid was purchased from Merck specialities Pvt limited, Mumbai, India. HPLC Grade Acetonitrile and all other buffering agents of analytical grade were purchased from Sd fine chemicals, ltd, Mumbai, India. HPLC grade water prepared by using SG-LABOSTAR^{TM} 3 TWF-UV ultra pure water system.

Solubility studies

The solubility of simvastatin in various oils, surfactants, and cosurfactants was determined. An excess amount of simvastatin was added into each vial containing 10 mL of selected vehicle. Then, the mixture was heated at 40 °C in a water bath to facilitate the solubilization. Mixing of the systems was performed using a cyclo mixer (CM 101, Remi, India) for 10 min in order to facilitate proper mixing of drug with the vehicles. Then, the formed suspensions were shaken for 48 h in a mechanical shaker (Remi, India). After reaching equilibrium, the mixtures were centrifuged at 2500g for 20 min to remove undissolved simvastatin, followed by filtration through a 0.45-µm millipore membrane filter paper. The concentration of simvastatin was quantified by HPLC (Kovarik J *et al*., 1994). The solubility of simvastatin in various oils and surfactants were represented in graph.

Construction of Phase Diagrams

The pseudo-ternary phase diagrams of oil, surfactant: cosurfactant and water were developed using surfactant titration method: the mixtures of oil and water at certain weight ratios were titrated with surfactant/co-surfactant mix in a dropwise manner. three types of surfactant phases were prepared Cremophore RH40 + Transcutol p (1:1,2:1,3:1)] For each phase diagrams at a specific ratio of surfactant/cosurfactant transparent and homogenous mixture of oil and drug was formed under the mixing by magnetic stirring. Then, visually observed for phase clarity and flow ability. After the identification of self-microemulsion region in the phase diagrams, the SMEDDS formulations were selected at desired component ratios. In order to form the self-microemulsion. (Shah N *et al*., 1994; Matuszewska B *et al*., 1996).

Preparation of SMEDDS formulations

On the basis of the "Solubility studies" section, the oil (Oleic acid), surfactant (Cremophore RH-40), and cosurfactants (Transcutol-p) were selected due to their greater solubility enhancement effect on Simvastatin. Various formulations were tried as shown in Table-1. The formulations were prepared by dissolving Simvastatin in the mixture of oil, surfactant, and cosurfactant and were heated at 50°C in an isothermal water bath. This mixture was mixed well and subjected to vortexing using cyclomixer (Remi, India), until a transparent preparation was obtained. All the mixtures were stored at ambient temperature for further use.

CHARACTERIZATION AND EVALUATION OF SMEDDS

Self-emulsification and precipitation assessment

In brief, various compositions were categorized on the basis of clarity and apparent stability of the resultant emulsion. Visual assessment was performed by dropwise addition of the preconcentrate (SMEDDS) into 250 mL of distilled water taken in a glass beaker at room temperature. The contents were gently stirred either using glass rod or magnetically at ~100 rpm. They were observed immediately after dilution for assessment for self-microemulsification efficiency, appearance (transparency), phase separation, and precipitation of drug. Precipitation was evaluated by visual inspection of the resultant microemulsion after 24 h. The formulation were then categorized as clear (transparent or transparent with bluish tinge), non clear (turbid), stable (no precipitation at the end of 24 h), or unstable (showing precipitation within 24 h).

Emulsion droplet size analysis/particle size determination

The droplet size and surface charge of the emulsions was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using Nano Zeta sizer (Horiba Instruments, Japan) able to measure sizes between 10-3000 nm. Light scattering was monitored at 25^{o}C at a 90^{o} angle. The dispersed formulations were measured after dilution (1:100) to produce the required count rate (50-200) to enable the accurate measurement. (Matuszewska B *et al*., 1996)

Percent drug content estimation

Simvastatin from preweighed SMEDDS was extracted by dissolving in 20 mL of The mobile phase prepared by using 0.1% Triethylamine buffer (pH 7.5) combined with HPLC grade Acetonitrile in the ratio of 20:80 v/v. Simvastatin content in the mobile phase extract was analyzed using HPLC (Shimadzu) at 238 nm.

Zeta potential determination

The zeta potential of the diluted SMEDDS formulations was measured using a Nano Zeta sizer (Horiba Instruments, Japan)). The SMEDDS were diluted with a ratio of 1:2500 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. Zeta potential of each SMEDDS was determined in triplicate.

Viscosity

The rheological property of the self-microemulsion was evaluated by BROOKFIELD-DV-II+pro viscometer using spindle 00 UL adaptor at 25+_0.5 °C, at 5 rpm. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation. (Bok K.K *et al*., 2004)

FTIR studies

FTIR spectrums of simvastatin and drug–self-microemulsion formulation were obtained by means of a FTIR spectrophotometer (Bruker-Alpha T). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 cm−1 over the range of 400–4000cm−1. After running the spectra, significant peaks relating to major functional groups were identified; spectra of the subsequent sample of the same compound were compared with the original. (Mingtao Liang A *et al*., 2008)

Thermodynamic Stability Studies

The self-microemulsion formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 months for intermediate and accelerated conditions and 6 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating HPLC method. (Bennett K E *et al*., 1982)

***In vitro* drug release studies**

The release of Simvastatin from the optimized SMEDDS and marketed tablet was determined according to USP dissolution apparatus type-II. To permit the quantitative drug release from SMEDDS and marketed tablet, 900 ml of phosphate buffer PH-5.5 was placed in the dissolution vessel and then the SMEDDS formulation filled in hard gelatin capsule and tablet was placed in the dissolution medium and was agitated at 50 rpm at 37oC. At predetermined time intervals of 5min (up to 1 hour), 5 ml of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 238nm. The volume withdrawn was replaced each time with fresh dissolution medium. Cumulated released amounts were plotted as a function of time (Mingtao Liang A *et al*., 2008).

***In Vitro* Intestinal Permeation Studies**

The methods employed were modified from experimental procedures well described in the literature. Male Sprague- Dawley rats (250-300g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intra duodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The SMEDDS sample was diluted with 1 mL of distilled water (outside mixing for 1 minute by vortex mixer), and for the tablet sample a suspension of tablet was made in distilled water. The resultant sample (1 mg/mL) was injected into the lumen of the duodenum using a syringe, and the 2 sides of the intestine were tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37^{o}C. The receiver compartment was filled with 30mL of phosphate-buffered saline (pH 5.5). At predetermined time intervals of 5min (up to 1 hour), 2 ml of the samples were withdrawn and the drug concentration was determined by HPLC at maximum wavelength 238nm the percent diffusion of drug was calculated against time and plotted on a graph. (Ashok R Patel *et al*., 2007)

RESULTS AND DISCUSSION

Solubility studies

Solubility studies were performed to identify suitable oily phase, surfactants, and cosurfactants for the development of SMEDDS of Simvastatin. Because an important consideration when formulating a self-emulsifying formulation is avoiding precipitation of the drug on dilution in the gut lumen *in vivo*. The components used in the system should have high-solubilization capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion.

The results of solubility studies are reported in figure-1. It is evident from the results that, among surfactants Cremophore RH 40 and Transcutol P provided higher solubility than other vehicles and Oleic acid as oil was selected respectively, for the optimal self-microemulsion formulation resulting in improved drug loading capabilities. Hence, for the preparation of SMEDDS, Oleic acid, Cremophore RH-40, and Transcutol-p were chosen as an oil, surfactant, and cosurfactant.

Figure 1. Graph showing solubility of Simvastatin in various Oils and Surfactants, The solubility of Simvastatin was determined in various vehicles by HPLC. The solubility of Simvastatin in surfactant was found to be high in cremophore RH40 & Transcutol P, among oils oleic acid exhibited the highest solubility

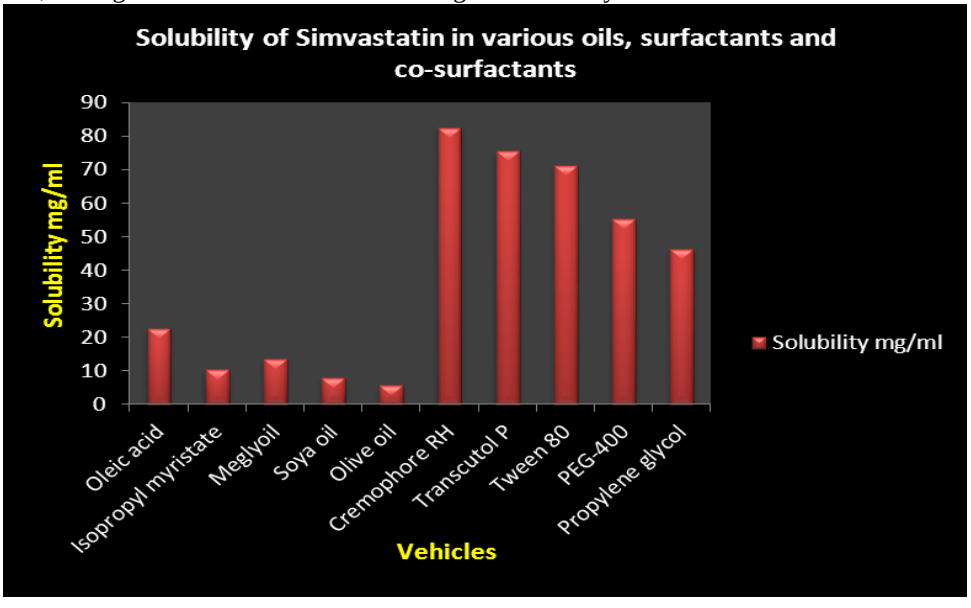
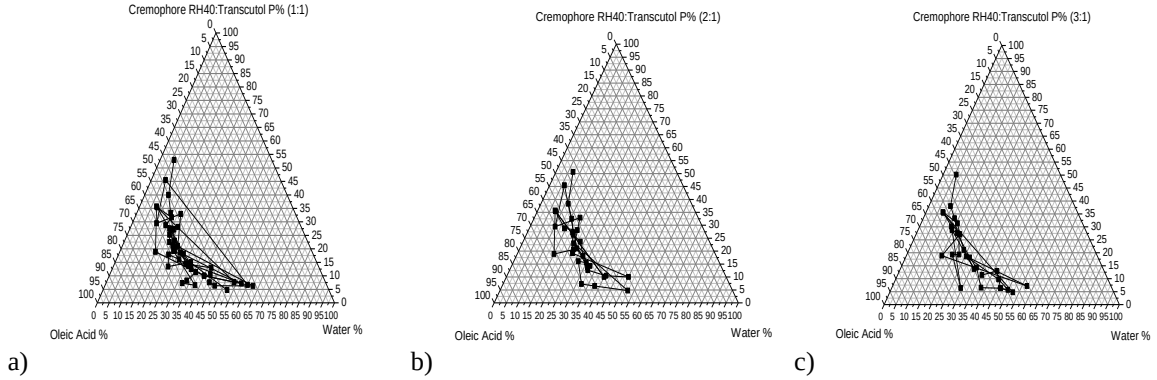


Figure 2. Pseudo-ternary phase diagrams indicating the efficient self-microemulsion region containing (Cremophore RH 40/Transcutol p) = (a) 1:1 (w/w), (b) 2:1 (w/w), (c) 3:1(w/w)



a) b) c)

Table 1. Composition of self-microemulsifying drug delivery systems formulations of simvastatin

Ingredients (%w/w)	F1	F2	F3	F4	F5
Simvastatin	9.04	9.04	9.04	9.04	9.04
Oleic acid	38.85	36.32	33.59	29.75	24.12
Cremophore RH-40	38.83	40.79	43.25	47.76	52.38
Transcutol-p	13.28	13.85	14.12	13.45	14.46

Table 2. Evaluation parameters of self-microemulsifying drug delivery systems formulation of simvastatin, F5 (n = 3)

Evaluation Parameter	Results
Mean droplet size (nm)	154.6± 3.45
Mean Zeta potential (mv)	27.7±4.26
% Drug found (mg/ml^{-1})	99.63±5.32
Viscosity	25.2632

Table 3. Evaluation data of formulation subjected to stability studies

Condition	Sampling point	Droplet size(nm)	% drug content
A= (25^{o}C/60% RH)	0 days	154.6	99.63
	45 days	154.1	97.54
	3 months	152.5	96.25
	6 months	151.4	95.34
B= (30^{o}C/65% RH)	0 days	154.6	99.63
	45 days	152.2	97.22
	3 months	150.9	95.64
C=(40^{o}C/75% RH)	0 days	154.6	99.63
	45 days	151.2	96.91
	3 months	149.4	94.48

Figure 3. Droplet size graph

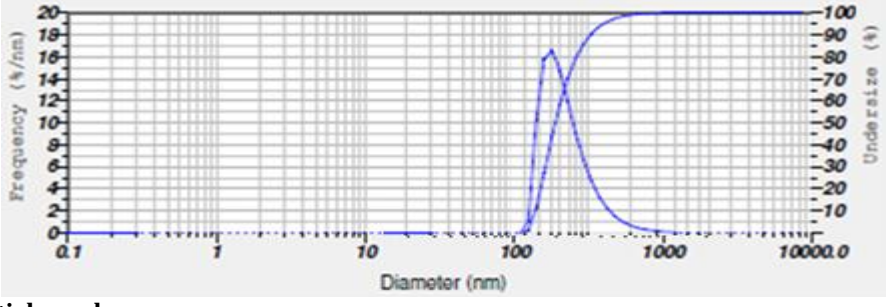


Figure 4. Zeta potential graph

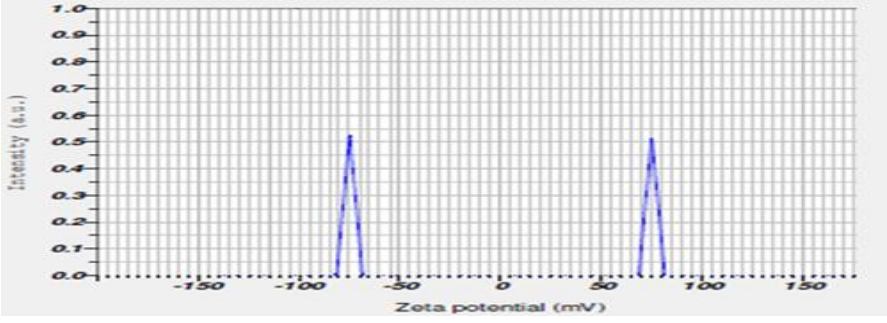


Figure 5. Comparitive FTIR Spectra of Simvastatin self-microemulsion with individual excipients

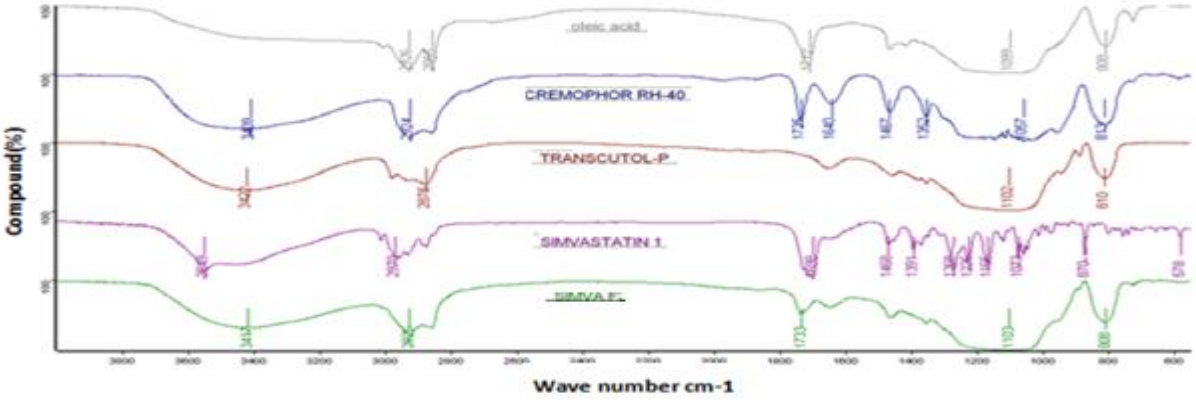


Figure 6. Comparative in vitro dissolution profile of simvastatin (–♦–) SMEDDS (F5) and (–■–) tablet

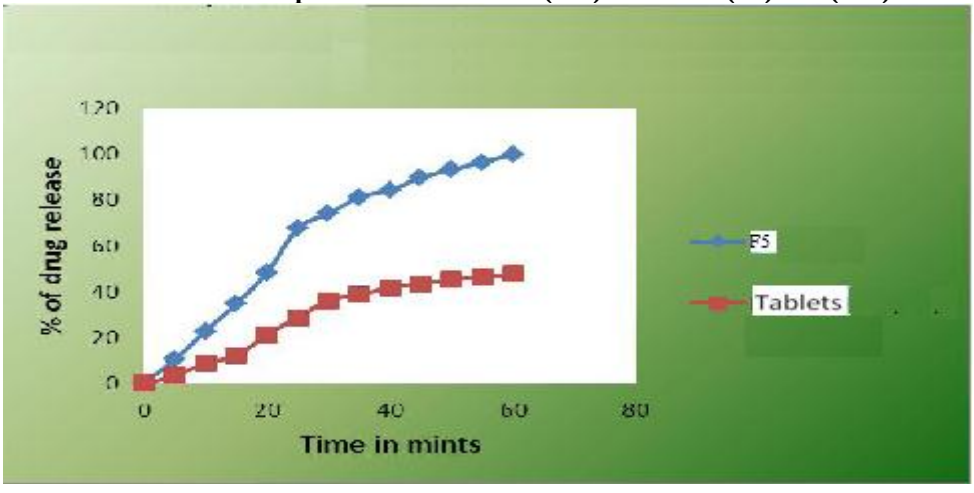
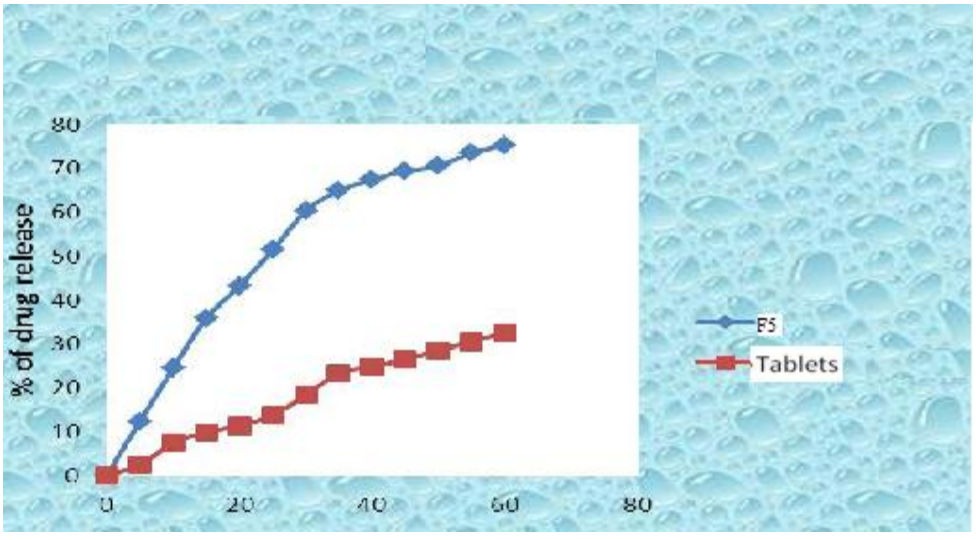


Figure 7. Comparative in vitro diffusion profile of simvastatin through rat duodenum (–♦–) for SMEDDS (F5) and (–■–) for tablet



Pseudoternary phase diagram

A pseudoternary phase diagram of the investigated quaternary system water/oleic acid/cremophore RH 40/Transcutol P is presented in Figure 2. Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, one-phase low-viscous self-microemulsion system was formed. The phase study revealed that the maximum proportion of oil was incorporated in self-microemulsion systems when the surfactant-to-cosurfactant ratio was 1:1. From a formulation viewpoint, the increased oil content in self-microemulsions may provide a greater opportunity for the solubilization of simvastatin. Moreover, when the composition (% w/w) of surfactant mixture (Smix) in a self-microemulsion preparation was <50%, the formulation was less viscous. The optimum formulation of self-microemulsion contained simvastatin (9.04% w/w), Oleic acid (24.12% w/w), CremophoreRH-40 (52.38% w/w), and Transcutol-p (14.46% w/w).

Preparation of SMEDDS for Simvastatin

Several SMEDDS systems with the ability to dissolve 10 mg of Simvastatin were prepared and compared. During preliminary study, some SMEDDS were eliminated due to detection of oil droplets on the surface of the diluted SMEDDS, which translates to an incomplete emulsification. SMEDDS that were not able to self-emulsify upon mixing with water under mild-agitation or yielded an unstable emulsions were rejected. A few SMEDDS formulations were eliminated due to the formation of milky emulsions upon dilution. The transparency of the diluted SMEDDS reflects the proximity of the droplet size to that of the nanoemulsion range. Formulations, F1-F5 which were obtained transparent were given in Table-1, and they were subjected to test for self-emulsification and precipitation assessment.

Self-emulsification and precipitation assessment

Evaluation of self-microemulsifying properties of SMEDDS formulations was performed by visual assessment as reported. [20] These studies were carried out on various SMEDDS formulations. During the study, it was found that some formulations, F1 and F2 showed turbidity, precipitation and thus was not stable, due to the relative decrease in surfactant concentration and the presence of Transcutol-p. Hence, F3, F4, and F5 were prepared with increased concentrations of surfactant. Formulation F5 could be mixed with oleic acid, Cremophore RH-40, and Transcutol-p and hence was selected as good formulation and subjected to further investigation regarding droplet size, Zeta potential, etc.

Evaluation of SMEDDS for droplet size analysis, zeta potential, drug content determination and viscosity

Droplet size distribution following self-microemulsification is a critical factor to evaluate a self-microemulsion system. The mean globule size of selected SMEDDS formulation F5, of Simvastatin was 154.6 nm Table 2 is indicated the ability of the present technology to produce microemulsion that offers larger interfacial surface area required for drug absorption. [21,22] An increase in the ratio of the oily phase (Oleic acid) resulted in a proportional increase in particle size, because of the simultaneous decrease in the s/cos proportion. Increasing the s/cos (surfactant to cosurfactant) ratio led to decrease in mean droplet size. The optimized SMEDDS, with the highest proportion of surfactant (52.38% w/w Cremophore RH-40) at a fixed amount of oil (24.12% w/w), was produced lowest mean particle diameter of 154.6 nm. This could be attributed to an increased surfactant proportion relative to cosurfactant.

The optimized SMEDDS showed high absolute zeta potential value of -27.7 mv. The emulsion stability is directly related to the magnitude of the surface charge. [23,24,25] Generally, an increase of electrostatic repulsive forces between microemulsion droplets prevents the coalescence of droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. The results of zeta potential and drug content estimation are indicated in Table 3. The percent drug content (99.63 ± 5.32) of SMEDDS of simvastatin was found satisfactory.

FTIR studies

The compatibility of drug and excipients used in the SMEDDS were characterized by their FTIR spectra. The FTIR spectrum of pure simvastatin has three characteristic peaks at 3548, 2969, and 1698cm−1 for O–H stretching vibration, C-H vibration and ester stretching vibration and lactone carbonyl functional group respectively. The FTIR spectrum of pure Formulation has three characteristic peaks at 3417cm−1, 2925cm−1 and at 1732cm−1. The FTIR spectrum of pure Simvastatin and self-microemulsion formulation were almost similar because of the same functional groups. It indicates that there was no interaction between Simvastatin and exepients used in the formulation. Depicted on figure 5.

Thermodynamic stability

The developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. Table 3 gives the results of the evaluation test conducted on stability sample. The formulation was found to be stable for 3 months at intermediate and accelerated conditions and 6 months at long-term conditions. There was no significant change in the drug content, or particle size of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. Furthermore, the formulation was found to show no phase separation, drug precipitation, or capsule leaks. Thus, these studies confirmed the stability of the developed formulation and its compatibility with hard gelatin capsules.

***In-vitro* drug release studies**

The in-vitro drug release studies for marketed tablet (simvas 10mg) and SMEDDS was determined in USP dissolution medium pH 5.5. The results are shown in Figure 6. At the end of 1 h, the release of simvastatin from the microemulsion was significantly greater (98.62%) than that for marketed tablet (45.19%). This may be the result of surfactant molecules which leads to the enhancement of solubility of the drug in dissolution medium.

***In Vitro* Intestinal Permeability Study**

The drug concentration was determined by High performance liquid chromatography at maximum wavelength 238nm and the percent diffusion of drug was calculated against time and plotted on a graph. The *in-vitro* intestinal permeability results exhibits the drug diffused at a faster rate from the microemulsion system than from the tablet dosage form. After 1 hour of diffusion, 76.54% of drug was diffused from the microemulsion system, as compared with 34.23% diffused from the tablets.

CONCLUSION

An optimized SMEDDS formulation of Simvastatin consisting of simvastatin (9.04% w/w), Oleic acid (24.12% w/w), Cremophore RH-40 (52.38% w/w), and Transcutol-p (14.46% w/w) was successfully developed with an increased solubility and dissolution rate. The SMEDDS of simvastatin possessed mean microparticle size of 154.6 nm and other ideal characteristics required for enhanced dissolution rate. Thus, our study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulations of Simvastatin to improve its dissolution rate leading to enhanced bioavailability.

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