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Research Article

PREPARATION AND EVALUATION OF THE SELF EMULSIFYING DRUG DELIVERY SYSTEM CONTAINING ATORVASTATIN HMG-COA INHIBITER

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ABSTRACT

As a consequence of modern drug discovery techniques, there has been a steady increase in the number of new pharmacologically active lipophilic compounds that are poorly water-soluble. It is a great challenge for pharmaceutical scientists to convert those molecules into orally administered formulations with sufficient bioavailability. Among the approaches to improve the oral bioavailability of these molecules, the use of self-emulsified drug delivery systems (SEDDS) has been shown to be reasonably successful in improving the oral bioavailability of poorly water-soluble and lipophilic drugs. In the present study we formulate self-emulsified drug delivery systems (SEDDS) through the nanoemulsion for the effective delivery of Atorvastatin which is a HMG-COA Inhibiter.

Keywords: Atorvastatin, Self emulsifying drug delivery systems, Oral drug delivery, Bioavailability.

INTRODUCTION

The oral route is one the most commonly used method for administration of drugs and drug delivery. The only disadvantages of this method are sluggish onset time, the possibility of erratic absorption, degradation of some specific drugs by digestive enzymes. The poorly water-soluble drugs such as HIV protease inhibitors, glycoprotein inhibitors and anticancer drugs have problems to produce and retain a good solubility in gastrointestinal tract. The drug delivery industry scientists are used a wide range of methods to improve the dissolution rate of poorly water-soluble drugs, including formulations containing nanoparticles, a solid solution formulation or self emulsifying drug delivery system (SEDDS), and stable amorphous form of the drug [1-5]. SEDDS is an isotropic mixture of oil, surfactant and/or co-surfactant can be used for formulations to improve the absorption of drugs in gastrointestinal tract and solve the solubility problems. SEDDS can produce fine oil/water emulsion after dilution in gastrointestinal fluids and provide large interfacial area for drug partitioning between oil and water phases and so increase in solubility rate and extent of absorption [6-10]. Atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (a statin), is a lipid regulating drug with actions on plasma lipids similar to those of simvastatin. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides, and to increase HDL-cholesterol in the treatment of hyperlipidaemias , including hypercholesterolaemias and combined (mixed) hyperlipidaemia (type IIa or IIb hyperlipoproteinaemias), hypertriglyceridaemia (type IV), and dysbetalipoproteinaemia (type III). Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL, its also reduces LDL production and the number of LDL particles. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30% this low systemic availability is ascribed to presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism.

The food decreases the rate and extent of drug absorption by approximately 25% and also responsible for reduction in C_{max} and AUC, LDL-C level. A blood/plasma ratio of approximately 0.25 indicates poor drug penetration into red blood cells. Based on observations in rats, atorvastatin calcium is likely to be secreted in human milk. So it is aimed to formulate a SEDDS containing a lipophilic drug, atorvastatin and to explore the potential of this carrier for improvement of the oral bioavailability of atrovastatin.[11]

MATERIALS AND METHODS

Materials

Atorvastatin was obtained from Lupin Laboratory, Aurangabad as a gift sample.

Formulation of self emulsifying drug delivery system (SEDDS)

The drug stock solutions in oil mixture were prepared in such a way that 10 mg dose is present in each formulation complying the oil percentage for each formulae selected from the phase diagram. This was prepared by dissolving the 1000 mg of drug individually in the 10, 15, 20 and 25 mL of oily mixture, which complies the 10%, 15%, 20% and 25% oil compositions respectively in the formulae. The drug stock is shown in Table **no 1**.

Table 1: Preparation of drug stock for each formula selected in phase diagram

S.NO	Oil percentage in formulations	Amount of drug (mg)	Volume of oil (mL)	Final concentration (mg/µL)
1	10%	1000	10	10 mg/100 µL
2	15%	1000	15	10 mg/150 µL
3	20%	1000	20	10 mg/200 µL
4	25%	1000	25	10 mg/250 µL

Construction of pseudo-ternary phase diagrams [15-16]

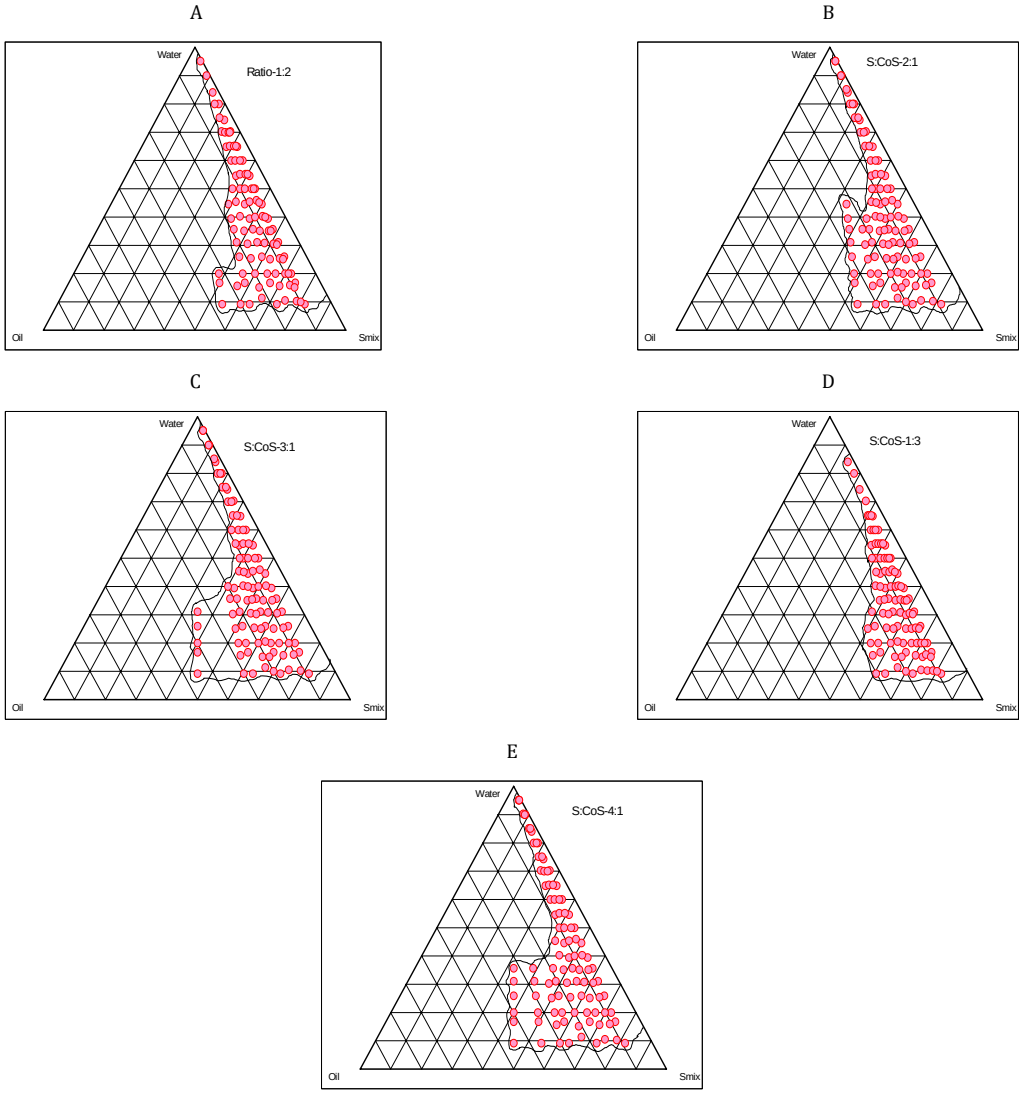
Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1) and the stock of 100 mL of each groups was prepared (**Table 2**). These Smix ratios were chosen in increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams for the nanoemulsions formation.

Table 2: Different volumes of surfactant and co-surfactant taken to make a stock Smix ratio

S.No	Volume of Surfactant (mL)	Volume of Cosurfactant (mL)	Ratio of Smix
1	100	0	1:0
2	50	50	1:1
3	33.3	66.7	0.5:1 or 1:2
4	25	75	1:3
5	66.7	33.3	2:1 or 1:0.5
6	75	25	3:1
7	80	20	4:1

For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratios from 1:9 to 9:1 in different small glass test tubes. Sixteen different combinations of oil and each Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

For the determination of existence zone of microemulsion, pseudoternary phase diagrams were constructed using water titration method [17-19]. To construct pseudoternary phase diagrams, the oil phase (oleic acid: Sefsol, 1:1) was mixed with different ratio of surfactant and cosurfactant (Tween 20 and Carbitol® respectively) and mixture was titrated with distilled water until it turned turbid. Examine each and every point I detailed and note it down. Pseudo ternary phase diagrams were drawn by using data obtained in aqueous titration method as shown in **Figure.1** (A-G). The amount of water added to give water concentration in the range of 5-95% of total volume at 5% intervals. After every 5% addition of the water to the oil and Smix mixture, visual observations were made as shown in **Figure 1**. The ratio of surfactant and co surfactant (Tween 20 and Carbitol®) were used for the titration



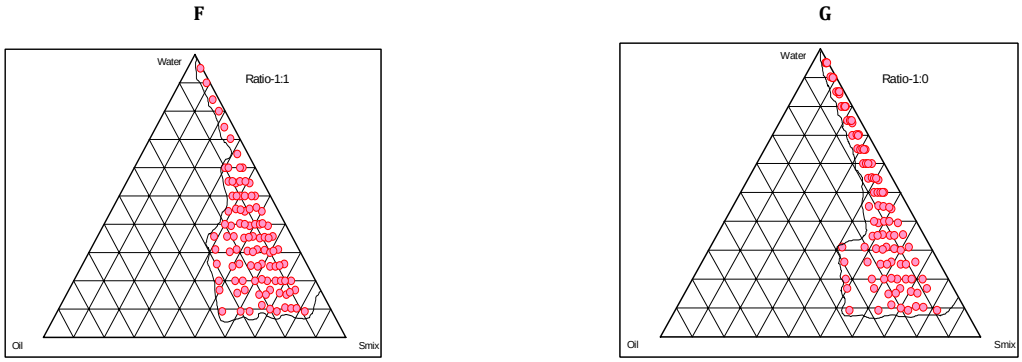


Fig. 1: A-G Phase diagram of Smix containing the surfactant and co surfactant in ratio 1:0, 1:1, 1:2, 1:3, 2:1 3:1and 4:1 respectively

Self emulsifying capacity

For evaluation of self-emulsification properties of formulations, 1 ml of each formulation was added to 0.1N hydrochloric acid (50 mL) under continuous stirring (60 rpm) at 37°C and then spreadability, tendency to emulsifying and progress of emulsion droplets were observed. Formulation were categorized as clear, non clear, stable or unstable. In the other hand, refractive index of different formulations were measured and compared with 0.1N hydrochloric acid.

Solubility determination in the various oils, surfactants and co-surfactants

2 ml of different oils was taken in small vials separately and excess amount of the drug was added to each vial. The vials were tightly stopper and were continuously stirred for 72 hrs in mechanical shaker at 25^{0}C and after that, oils were centrifuged. The supernatant was separated and dissolved in methanol and solubility was quantified by UV-Spectroscopy (Shimadzu 1701, Japan) method at 247 nm after appropriate dilution with methanol.

Screening of formulations on the basis of thermodynamic stability studies

The thermodynamic stability studies were performed on the basis of following tests.

Centrifugation study

The selected formulations were centrifuged (REMI, India) at the 5000 rpm for 30 mins and observed for phase separation, creaming or cracking. The formulations which showed maximum stability (no creaming, cracking, phase seperation) were selected and studied for heating-cooling cycle, freeze-thaw cycles and Dispersibility tests.

Heating cooling cycles

It is used to see the stressed effect of heating and cooling on the nanoemulsion`s stability. In this study the formulations were kept at 45^0 c and at 0 ^{0}C temperature for not less then 48 hrs for each temperature cycle.

Freeeze –thaw cycles (Accelerated ageing)

This test was performed for accelerated stability testing of nanoemulsion formulations. In this study the formulations were exposed at two different temperatures i.e -21^{0}C and 21^{0}C for each temperature cycles not than 24 hrs. For the better estimation of accelerated stability studies three such cycles should be run for each batch of formulation .The formulations which showed the maximum stability were selected for further study.

Dispersibility tests

The efficiency of self-emulsification of oral nanoemulsion was assessed using a standard USP XXII dissolution apparatus 2. [20]**.** One ml of each formulation was added to 500 mL of distilled water and in 0.1N HCl respectively at 37 ± 0.5 ^{o}C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The *in vitro* performance of the formulations was visually assessed using the following grading system (Table 5). Those formulations that passed the thermodynamic stability and also dispersibility test in Grade A were taken for the further studies. Further from each Smix Group one formulation is selected, having the least Smix concentration irrespective of Smix ratio used, but passing dispersibility test in Grade A in distilled water as well as in 0.1N HCl.

Table 3: Observation table of dispersibility study

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

Viscosity

The viscosity of the prepared nanoemulsion formulations were determined as such without dilution by Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) using spindle # CPE40 at 25 ±1.0°C. The software used for the viscosity calculations was Rheocalc V2.6. The results are shown in Table 6.4.

Table 4: Observation table of viscosity measurements

Code	Zero time	After 120 sec
	Mean viscosity ± SD (cps)	Mean viscosity ± SD (cps)
GM1	27.51 ± 1.01	27.21 ± 0.93
GM2	21.32 ± 1.1	20.85 ± 0.64
GM3	10.3 ± 0.91	10.02 ± 0.63
GM4	28.22 ± 0.91	27.76 ± 1.75

Droplet size analysis (Particle size distribution)

Droplet size of the prepared nanoemulsion was determined by using photon correlation spectroscopy, which analyzes the fluctuations in light scattering due to Brownian movement of the particles . The formulation (0.1 mL) was dispersed in 50 mL (500 dilution) of distilled water in a volumetric flask and gently mixed by inverting the flask and measurement done using a Zetasizer (Nano ZS-90, UK). Light scattering was monitored at 25°C at a 90° angle.

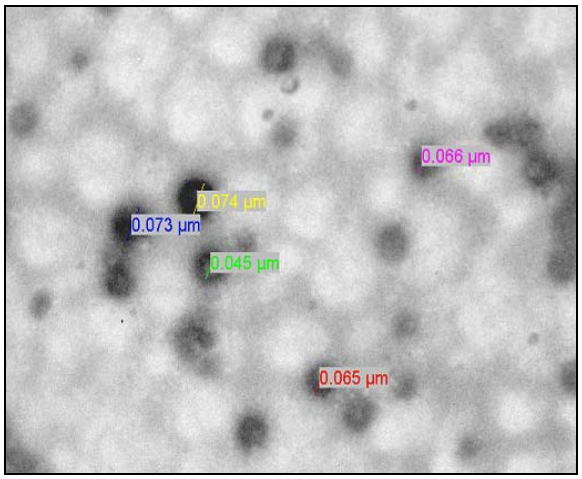


Fig. 2: TEM of formulation GM1

Transmission electron microscopy

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM) TOPCON 002B operating at 200 KV and of a 0.18 nm capable of point to point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. In order to perform the TEM an observation, the nanoemulsion formulation was diluted with distilled water (1/100). A drop of the diluted nanoemulsion was directly deposited on the Copper holey film grid and observed after putting fixing agent and drying it in the filtered air.

Table 5: Droplet size of the various emulsion formulations

S. No	Formulation Code	Particle Size
1	GM1	42.8±5.2 nm
2	GM2	195±10.5 nm
3	GM3	107±9.7 nm
4	GM4	81.6±6.1 nm

In vitro drug release study

In vitro release test was performed using Dialysis bag method and release study was carried out in 250 ml of distilled water, 1 ml of emulsion formulation (Single dose containing 10 mg of AT Calcium) was placed in treated dialysis bag (MWCO 12,000 g/mole; Sigma, USA) and 1 mL samples was withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2,2.5,3,3.5, 4,4.5,5.5, 6,6.5,7, 8,9, 10, 12, 22 and 24 h) and same amount of distilled water was replaced.[21-22]. The withdrawn 1 ml samples were diluted with 3 ml methanol and analyzed for the drug content by using developed UV-spectroscopy at 247 nm. The same method was used for the suspension containing 10 mg of AT Calcium in 1 ml distilled water. The release of drug from different selected nano emulsion formulations was compared with drug suspension and finally selected formulation was used for the further study i.e. for preparation of solid self-nano emulsifying drug delivery system (SSNEDDS).

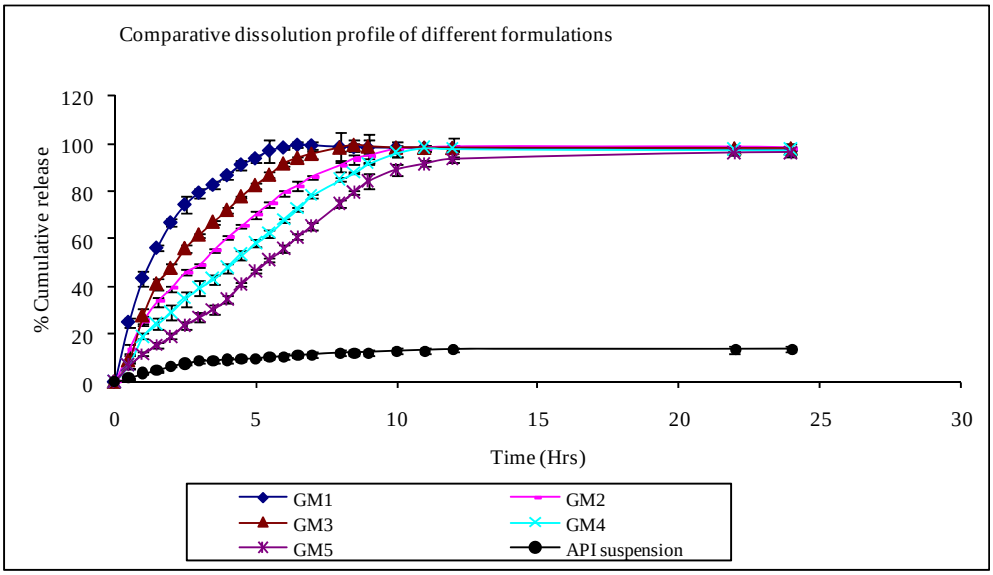


Fig. 3: In-vitro Drug Release of AT nano emulsion by dialysis bag method in distilled water

RESULTS AND DISCUSSION

The SEDDS of the drug Atorvastatin were prepared by emulsification method. Constructing a phase diagram is one of the primary steps and makes a backbone for the SEEDS, particularly when the aim is to accurately delineate a phase boundary. Observations are made carefully to separate metastable systems from phase boundary, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous.

The relationship between the phase behaviour of a mixture and its composition can be selected with the aid of a phase diagram. Sefsol 218 (oil) and oleic acid (oil), Tween 20, Tween 80 (surfactants) and Carbitol (co surfactants), were used to study the phase diagrams in

detail. The systems were observed for visual clarity and flowability caracteristics. Those which did not show a change in the miniscus after tilting to an angle of 90° were classified as nanogels a metastable system were not selected. After taking observation, pseudoternary phase diagrams were constructed based on the observations marked during titration. Phase diagrams were constructed separately for each ratio of Smix prepared so that o/w nanoemulsion regions could be identified. In the phase diagrams (Figure. 6.1, A-G) only o/w nanoemulsion region is shown. After building the backbone of the nanoemulsion delivery system, different formulations were selected at different point from the phase diagram justifying the drug dose.

Vigorous studies were done for the phase diagram construction, phase boundry were plotted on the ternary phase diagram. After building the backbone of the nanoemulsion delivery system, different formulations were selected at different point from the pseudophase diagram which justified the drug dose considering the drug solubility in the oils phase. As per saturation solubility studies of AT Calcium in oily mixture, Sefsol 218: Oleic acid (1:1), around 130 mg of drug can be solubilized per mL.The concentration 10% (100 µL) of oil in 1 mL formulation is just able to solubilize 10 mg of AT Calcium. Therefore 10% was selected as the least oil concentration to be taken for one mL formulation from the phase diagram. The selected formulations shown were screened on the basis of the thermodynamic stability studies.

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano or micro emulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for dispersibility test. Those formulations, which survived thermodynamic stress tests, were taken for dispersibility test to see the visual clarity after infinite dilution (**Table 3**). A number of formulations passed the thermodyanamic stability (Accelerated stability) tests. So it was not possible to go for the further study by selected all the formulations. Finally five formulations were selected on the basis of the dispersibility test in distilled water as well as in 0.1N HCl considering the minimum surfactant concentrations from the entire phase diagram (**Table 3**).

The viscosities of the various formulations were determined. It was observed that the viscosity of all the formulations is ranges between 10-43 cps **(Table 4)**. Formulation GM3, has the minimum viscosity (10 ± 0.91 cps), which shows that the amount of co-surfactants is directly proportional to the film flexibility. The viscosity of finally selected formulations, GM1 was found to be 27.51±1.01 cps.

The size and size distribution analysis was performed on selected formulatios by using Malvern Zetasizer (Nano ZS-90 U.K). Droplet size analyses of the selected formulations showed that the size increased with the increase in concentration of oil in the formulations GM1, GM2 (**Table 5**) and increase in the concentration of co-surfactants in formulation GM3. This may be due to the increase in the oil concentration form 10% to 15% v/v although having the Smix concentration same which shows enability to disperse the increased amount of oils. The mean droplet size of the formulation GM1, GM2, GM3 and GM4 were 42.8 nm, 195 nm, 107 nm and 81.6 nm respectively. In GM1 formulation 82.5% droplets were found to be 14.4 nm range .The droplet size in the case of GM2 and GM3 were found to be i.e. 195 and 107 respectively, as compared to GM1, this attribute to the fact that formulation GM2 and GM3 containing higher concentrations of oil and co-surfactant respectively.The result also reveals that co-surfactant (Carbitol ®) doesn't play a vital role as compared to the surfactant (Tween 20®) for the nanoemulsifications of oily mixture (sefsol: oleic acid). The polydispersity was found to be minimum in the case of GM1 (0.237) and GM4 (0.397) as compared to GM2 (0.478) and GM3 (0.407).

The nanoemulsion appears dark and the surroundings was bright in TEM as it was clear from the image in Figure. 6.3, a "positive" image is seen using TEM. Some equally distributed droplet sizes are measured using TEM, as it is capable of point-to-point resolution. The droplet size is in agreement with the results obtained from droplet size analysis.

Dissolution study was performed by dialysis bag method in distilled water for the final selection of formulation for further development of solid self-nanoemulsifying dosage forms. The dissolution study was performed for 24 hrs. Drug dissolution from formulation GM1 was very fast as 99.65±1.29% of drug released in 6.5 hrs; while formulations GM2, GM3, GM4 and GM5 showed comparatively slow release i.e. 82.36±2.12, 93.97±0.56, 72.69±0.81 and 61.06±1.31 in 6.5 hrs. In contrast to this drug released from API suspension was found to be very low i.e. 13.77±0.98% in 6.4 hrs. This result was attributed to the fact that formulation GM1 is having comparatively smaller size (42.8 nm) of oil droplets and hence the larger surface area for dissolution as justified by droplet size distribution and by TEM photograph. The another possible reason was the oil concentration in formulation GM1 (10%) as compared to the GM2 (15%), although having the same concentrations of the Smix, which was not sufficient to emulsify the increased amount of oil in GM2.

CONCLUSION

The formulation was found to be the optimized formulation on the base of results of pseudo ternary phase diagram, in vitro drug release, droplet size and other parameters. The present study was clearly indicated that the usefulness of SEDDS in the improvement of the dissolution rate and there by oral bioavailability of poorly water soluble drugs like ATV without incompatibility between the ingredients.

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