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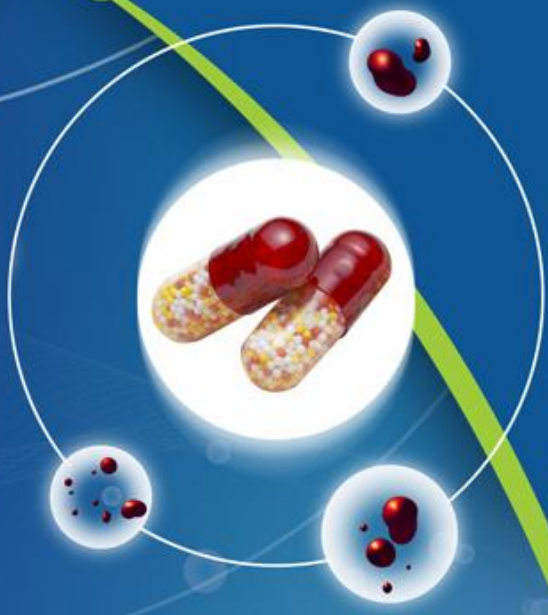

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Research Article

ADVANCED DELIVERY OF POORLY WATER SOLUBLE DRUG ATORVASTATIN BY LIPID BASED FORMULATION

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ABSTRACT

Atorvastatin Calcium used to lower cholesterol level in plasma of body by competitive inhibiting HMG-CoA reductase, the rate determining enzyme in cholesterol biosynthesis via the mevalonate pathway. However its effectiveness is limited by its poor water solubility and very low oral bioavailability. Therefore the aim of present investigation was to develop an optimal lipid based formulation for advanced delivery of poorly water soluble drug Atorvastatin Calcium, for enhancing its aqueous solubility and oral bioavailability. Oil and surfactants were screened based on drug solubility. Pseudo-ternary phase diagrams were constructed to determine suitable surfactant to cosurfactant ratio for the development of lipid based formulation as SMEDDS. Atorvastatin calcium loaded SMEDDS was developed and characterized. Solidification of SMEDDS was done by spray drying technique using aerosil 200 as solid carrier. The drug release study shows that the release of Atorvastatin will enhances in SMEDDS formulation as compared to plain drug.

KEYWORDS: *Atorvastatin Calcium, SMEDDS, Solubility enhancement, Oral bioavailabilty.*

INTRODUCTION

Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response.[1] Most of the BCS class II drugs has poor aqueous solubility and high permeability.[2,3] Poor aqueous solubility of lipophilic drugs creates problems in formulation as well as in oral administration. Various approaches have been developed to resolve poor aqueous solubility of lipophilic drugs. As oral route for drug administration is most commonly used among all the routes of administration due to its convenience non-invasiveness and cost effectiveness it become necessary that drug should have some aqueous as well as some lipid solubility for their absorption[4]. The most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles, such as oils, surfactant dispersions, self-emulsifying formulations, emulsions and liposomes, with every formulation approach having its special advantages and limitations. Efficacy of lipophilic drug is often hindered due to their poor aqueous solubility leading to low absorption after *in vivo* administration.[5] Self micro emulsifying drug delivery system (SMEDDS) are defined as an isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants that have a unique ability of forming fine oil-in-water (o/w) micro emulsions upon mild agitation followed by dilution in aqueous media, such as GI

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fluids.[6,7] These systems form homogeneous, transparent/translucent, isotropic and thermodynamically stable microemulsion upon dispersion in aqueous media with oil droplet sizes of less than 50 nm.[8] When the mixture of drug, oil and a surfactant comes in contact with the aqueous environment in GIT they form an emulsion under gentle agitation provided by digestive motility of stomach and intestine which is necessary for self-emulsification *in- vivo*. Once an emulsion is formed then the drug is quickly distributed throughout the GIT as fine droplets, due to this dispersion and large surface area of fine droplets the bioavailability of drug enhanced. Presence of surfactant also influences absorption due to membrane induced permeation changes. The mechanism of self-emulsification is specific for parameters like, pair of oil and surfactant, type and concentration of surfactant, oil/surfactant ratio, and temperature at which self emulsification occur. Since the drug delivery should be biocompatible so the selection of excipient used in formulation is very important.[9] Atorvastatin is a member of the drug class known as statins. It is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate determining enzyme in cholesterol biosynthesis via the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels.[10-14]

MATERIALS AND METHODS

Atorvastatin calcium was received as a gift sample from Atra Pharmaceuticals, Aurangabad. Soyabean oil use of the Gemini. Oils including Arachis oil, castor oil, olive oil, oleic acid and Isopropyl myristate; Surfactatnts including tween 20, tween 40, tween 60, tween 80, Span 20, span 40, span 60 and span 80; Co-surfactants including isopropyl alcohol, isobutyl alcohol, propylene glycol, polyethylene glycol 400, polyethylene glycol 600, transcutol P, methanol and ethanol, all are received from Research Lab Fine Chem Industries. All the chemicals and solvents used were of analytical reagent grade.

EXCIPIENTS SCREENING

Choosing the right combination of lipid (oil), surfactant, and co-surfactant is one of the important points in designing SMEDDS formulations. Selection of a good self micro emulsifying formulation depends on the (1) the solubility of the drug in oil/surfactant/co-surfactant (2) emulsion forming area as determined by phase diagram, and (3) the globule size distribution of the developed SMEDDS.[15]

Solubility study

Screening of excipient can be done by determining the equilibrium solubility of Atorvastatin in different oils, surfactants and co-surfactants. The solubility of Atorvastatin in different oils, surfactants and co-surfactants was determined using shake flask method. an excess amount of ATR was added to each vial containing 2ml of each excipient, and mixed by vortexing in order to facilitate proper mixing of ATR with the vehicles. Vials were then shaken for 48 hrs in a Thermostatically controlled shaking water bath at 37 ± 1°C followed by equilibrium for 24 h. In order to separate the undissolved drug, the supersaturated sample was centrifuged at 3000 rpm for 10 min. The supernatant was then filtered using a membrane filter (0.45 µm, Whatman) and suitably diluted with methanol. The drug concentration was obtained via UV validated method at 246 nm. The experiment was repeated in triplicates and results are shown in figure no. 1,2,3.[16,17]

Preliminary screening of oils, surfactants

The oil and surfactant having good solubilizing capacity for Atorvastatin were selected for the studying there emulsifying properties. Briefly, 3 ml of the surfactants were added to 3 ml of the oily phase. The mixture were gently heated at 40-50°C for homogenization of the components. Each mixture, 0.1 ml was then diluted with distilled water to 10 ml in a stopper conical flask. Ease of emulsification was judged by time required to yield homogeneous emulsion when it diluted 100 times with distilled water with

continuous stirring on magnetic stirrer. Emulsifying ability of the mixture was categorized in five classes on the basis of time required to form homogeneous emulsion (shown in Table no. 1). Emulsions were allowed to stand for 2 hr and emulsions were furthermore observed visually for any turbidity or phase separation.

Preliminary screening of co-surfactant

The selected oily phase and surfactant were used for further screening of the different co-surfactants for their emulsification ability. Mixtures of 3 ml of co-surfactant, 3 ml of surfactant and 3 ml of oil were prepared and evaluated in similar fashion as described in screening of surfactants.[18-23]

Table No. 1: Visual assessment of efficiency of Self Emulsification

Dispersibility and Appearance	Time of Self Emulsification (min)	Grade
Rapidly forming microemulsion, having a clear or slightly bluish appearance.	<1	A
Rapidly forming, slightly less clear microemulsion, having a bluish white.	<2	B
Fine milky microemulsion.	<2	C
Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify	2-3	D
Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.	>3	E

DRUG-EXCIPIENT COMPATIBILITY STUDY

After selection and screening of the oil, surfactant and co-surfactant for the formation of the SMEDDS formulation next step is the physicochemical compatibility study of Atorvastatin with excipients. The drug and excipient were equally distributed in glass ampoules. They were kept at room temperature 25°C and at 40°C/75% RH. The samples were drawn at intervals of 0, 2 and 4 weeks and analyzed for its physical appearance and drug stability by FT-IR.[24,25]

CONSTRUCTION OF TERNARY PHASE DIAGRAMS

The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram.[26] The methods are used to plot Ternary phase diagrams are namely Dilution method and Water Titration method.[6]

In this project work the water titration method is used. The four components consist of Oleic acid (Oil), Tween 20 (Surfactant), PEG 400 and Transcutol P (1:1) (co-surfactant) and distilled water (aqueous phase). In water titration method, the mixture of oil and surfactant/ co-surfactant (S/Cos) at certain volume ratios was diluted with water in a drop wise manner. The ratios of surfactant/ co-surfactant were prepared in specific manner, i.e. 1:1, 2:1, 3:1 and 4:1 (w/w). Each of these ratios was mixed with increasing percentage of oil, i.e. 10%, 20%, 30% up to 90% to get phase diagram. Then each mixture was titrated with water and agitation was provided by magnetic stirrer. The results obtained as described in above water titration method. These values of oil, surfactant and co-surfactant were used to determine the boundaries of emulsion region. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios, selection of microemulsion region from phase diagram was based on the fact that solution remains clear even on infinite dilution.[27,28]

PREPARATION OF LIQUID SELF MICRO-EMULSIFYING DRUG DELIVERY SYSTEM (LIQUID SMEDDS)

Based on the pilot studies (equilibrium solubility, phase diagram at different surfactant: co-surfactant ratio), the surfactant:

co-surfactant ratio at which maximum microemulsion region obtained was selected for formulation of liquid SMEDDS. Liquid SMEDDS formulation which shows maximum microemulsion region in the pseudoternary phase diagram is selected for the further study.

Drug Loading

The drug loading capacity of each mixture was determined by adding the excess drug to each prototype mixture till the clear solution was obtained. The solution was filtered, diluted and measured the absorbance at 246 nm using UV-Visible spectroscopy. As per drug loading capacity of prototype formulation, calculated quantity of drug was added to each prototype formulations. Phase diagram indicated that Surfactant : Co-surfactant (Smix) ratio 1:1 shows larger self emulsification region than Smix ratio 2:1, 3:1 and 4:1. Based on this, different formulations with Smix ratio 1:1 were prepared with the increasing concentration of drug to achieve the highest drug loading in to liquid SMEDDS. The liquid formulation containing Oleic acid (Oil), Tween 20 (Surfactant), PEG 400 : Transcutol P (1:1) (Co-surfactant) was prepared with the increasing amount of drug to achieve the highest drug loading in to liquid SMEDDS.[29,30]

Development of Liquid SMEDDS

The observation from liquid SMEDDS prepared with different drug loading shows that above the 5.25 % w/w drug loading tends to crystal out upon standing when liquid SMEDDS was diluted to 100 times with water. Hence for further study in all formulations, the level of Atorvastatin was kept constant (i.e. 5.25 % w/w).[19,27,30-32] The different liquid formulations that were developed are shown below in table no. 2.

Table No. 2: Liquid SMEDDS Formulation

Sr. No.	Ingredients	Quantity in % w/w		
		C1	C2	C3
1	Atorvastatin (Drug)	5.25	5.25	5.25
2	Oleic acid	27.13	28	28.87
3	Tween 20	33.81	33.37	32.94
4	PEG 400	16.90	16.69	16.47
5	Transcutol P	16.90	16.69	16.47
6	**% Total**	100	100	100

CHARACTERIZATION OF LIQUID SMEDDS

Emulsification efficiency

Various compositions were categorized on the basis of clarity and apparent stability of the resultant emulsion. 1 mL of Liquid SMEDDS was added drop wise to 200 ml of distilled water in the beaker during constant stirring on a magnetic stirrer at low speed, at temperature 37°C. SMEDDS assessed visually according to the rate of emulsification and final appearance of the emulsion.[27,32-34]

Precipitation assessment

Liquid SMEDDS formulation was diluted upto 100 times with distilled water with continuous stirring on magnetic stirrer to form emulsion. Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were then categorized as clear (transparent or transparent with bluish tinge), non-clear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours).[19,33]

Drug content determination

Amount of drug present in the liquid SMEDDS formulation was determined by UV Spectrometric method. Weighed accurate quantity of liquid SMEDDS formulation equivalent to 10 mg of drug (Atorvastatin) in 100 ml volumetric flask and diluted with methanol to make up volume upto 100 ml. Further 1 ml of the solution was diluted to 10 ml using methanol to make 10 µg/ml solution. The drug content was analyzed by taking UV absorbance at 246 nm.[35,36]

Self emulsification time

Few ml of prototype formulation (approximately 1 ml) was added to 250 ml of purified water, stirred gently and checked for clarity of solution. Self-emulsification time of formulation was determined using USP II dissolution apparatus. 1 ml of formulation was added drop wise to 250 ml of purified water at 37˚C, gentle agitation was provided by dissolution paddle rotating at 75 rpm. Time taken for formation of clear solution was noted as self-emulsification time.[29]

Refractive Index

Refractive Index proved the transparency of formulation. The refractive index of the system is measured by Abbe refractometer by placing drop of solution on slide and it compare with water (Refractive index of water1.333). If refractive index of system is similar to the refractive index of water, then formulation has transparent nature.[21]

Measurement of mean globule size

It has been reported that the smaller particle size of the micro emulsion droplets may lead to more rapid absorption and improve the bioavailability.[36] Prepared Atorvastatin SMEDDS (1 mL) was diluted 100 times with distilled water and 0.1N HCl in beaker with constant stirring on a magnetic stirrer to from a microemulsion. The droplet size of microemulsion was allowed to equilibrate for 1 h and distributions of resultant microemulsion were determined by laser scattering technique using Malvern zetasizer (Zetasizer Ver. 7.11, Serial Number: MAL 1036126 Malvern Ltd). All measurements were performed at a 25±2°C.[37]

Zeta potential measurement

SMEDDS formulation containing 10 mg of Atorvastatin was diluted to 20 mL with distilled water in a flask and was mixed gently by inverting the flask. The particle size so formed was determined by dynamic light scattering (DLS) technique using Zetasizer (Nano ZS, Malvern Instruments, UK).[36]

Drug release study

The *in-vitro* dissolution study of liquid SMEDDS and plain drug were carried out using dissolution test apparatus no. 1 as per IP. Quantity equivalent to 10 mg of liquid SMEDDS formulation was added to dissolution media. The dissolution media used was 900 ml of pH 6.8 Phosphate buffer. The paddle rotation speed was kept at 75 rpm. Samples of 5 ml at 5 min interval were with-drawn at regular time 5 min to 30 min and filtered using 0.45 µm filter. An equal volume of respective dissolution medium was added to maintain the volume constant. Drug content from sample was analyzed using UV-spectrophotometer at 246 nm. The parameters during study are given in table no. 3 below.[10,27,33]

Table No. 3: Parameters for Drug Release Study Liquid SMEDDS

Sr. No.	Parameter	Specification
1	Dissolution Apparatus	No. 1
2	Dissolution Medium	900 ml Phosphate buffer pH 6.8
3	Speed	75 rpm
4	Time	30 minutes

PREPARATION OF SOLID SELF MICROEMULSIFYING DRUG DELIVERY SYSTEM

Solid SMEDDS formulation was prepared from liquid SMEDDS by spary drying technique. Colloidal Silicon Dioxide (Aerosil 200) was used as the carrier for the conversion of liquid SMEDDS to solid SMEDDS. Aerosil 200 (5 gm) was dissolved in 100 ml distilled water by magnetic stirring. The liquid SMEDDS (10 gm) was then added with constant stirring, and the solution was kept at 50°C for 10 min to obtained a good o/w emulsion. Table no. 4 indicates the conditions

for the Spraymate spray dryer instrument, in that the emulsion was spray dried.

Table No. 4: Process Parameters for Spray Drying

Sr. No.	Parameter	Optimized Value
1	Inlet Temperature	120°C
2	Outlet Temperature	60°C
3	Aspiration Speed	1400 rpm
4	Compressed air flow rate	2.5 Bar
5	Feeding Rate	10 ml/min

After the completion of drying process, fraction of dried S-SMEDDS were collected from different parts of spray dryer, i.e. drying chamber, first cyclone separator and collector attached to it. These fractions were then mixed in polybag for 15 min to ensure the uniform mixing of blend.[38]

CHARACTERIZATION OF SOLID SMEDDS

The characterization of solid SMEDDS was carried out for reconstitution properties (visual observation and particle size determination), yield of spray dried product, powder flow properties (including angle of repose, bulk density, tapped density, Carr's compressibility index and Hausner ratio), weight variation test for capsules, drug content determination and in-vitro dissolution test. Solid state characterization includes Differential scanning calorimetry, Scanning electron microscopy and Powder X-ray diffraction.

STABILITY STUDY

The purpose of stability testing is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions, re-test periods and shelf lives to be established.

RESULTS AND DISCUSSION

EXCIPIENTS SCREENING

Solubility study

SMEDDS, an emulsion based formulation is a blend of oils and surfactants in suitable proportion that rapidly forms an oil in water (o/w) microemulsion with moderate gastric motility when exposed to the aqueous media present in the g.i.t. Co-surfactant and organic solvent can also be added sometime to improve the emulsification and solubility respectively. In order to select a best combination of oils, surfactants, and co-surfactants for SMEDDS formulation, component which shown a maximum solubility for Atorvastatin was selected.[47]

The solubility of Atorvastatin in different oils and surfactant and co-surfactant are shown in figure 1, 2 and 3. The solubility study of the Atorvastatin in different oils revels that Oleic acid, Olive oil and Soyabean oil shows very good solubility for Atorvastatin as compared with other oils as shown in figure 1. The surfactants such as Tween 80, Tween 60 and Tween 20 shows very good solubility for Atorvastatin as compared with other surfactants as shown in figure 2. The co-surfactant PEG 400, PEG 600 and transcutol P shows good solubility than other co-surfactants as shown in figure 3.

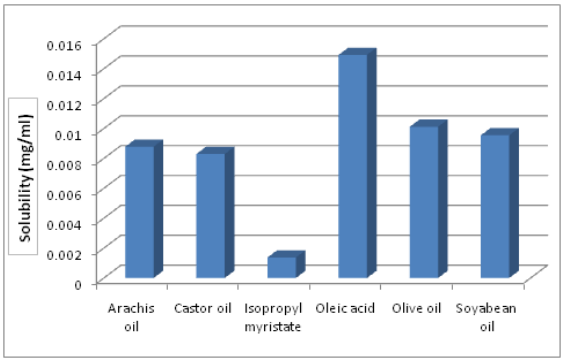

Figure No. 1: Solubility of Atorvastatin Calcium in OILS

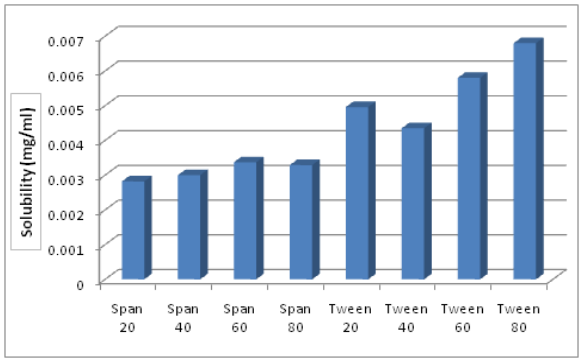

Figure No. 2: Solubility of Atorvastatin Calcium in SURFACTANTS

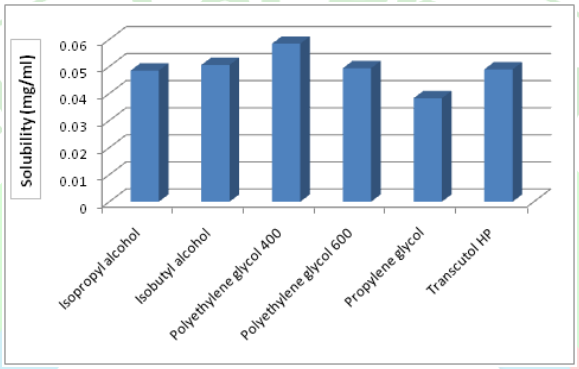

Figure No. 3: Solubility of Atorvastatin Calcium in CO-SURFACTANTS

Preliminary screening of oils, surfactants and cosurfactants concluded that finally selected components for the SMEDDS formulation of Atorvastatin were given in the following table no 5.

Table No. 5: Finally selected components for the SMEDDS formulation

Drug	Oil	Surfactant	Co-surfactant
Atorvastatin Calcium	Oleic acid	Tween 20	PEG 400 & Transcutol P

Drug-Excipient Compatibility Study

Visual method

Drug-excipient compatibility study was done for four week at 25°C (RT) and 40°C and samples are visually observed initially, after 2 weeks, after 4 weeks for any color change. The visual observation shows that there is no color change observed during storage for four week.

FT-IR Spectroscopic method

After four week FT-IR of all samples were taken to determine the any functional group change during the storage. All the FT-IR of sample placed for compatibility shows the functional peaks of Atorvastatin (drug) at 3542, 3487, 3418, 2926, 1735, 1649, 1389, 1156, 843 cm^{-1} and no change in functional peaks of drug observed after 4 week.

Construction of Pseudo-Ternary Phase Diagram

Pseudoternary phase diagrams were constructed in order to obtain the concentration range of components for the existing region of microemulsions using the water titration method. For each pseudoternary phase diagram at a specific S/CoS ratio, the oily mixtures of oil, surfactant, and cosurfactant were prepared with the ratio of oil to the mixture of surfactant and cosurfactant at 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10. Water was added in a dropwise manner to each oily mixture under proper magnetic stirring at 37°C until the

mixture became clear at a certain point. The concentrations of the components were recorded in order to complete the pseudoternary phase diagrams, and then the contents of oil, surfactant, cosurfactant, and water at appropriate ratios were selected based on these results. The boundaries of the self-microemulsification regions in the phase diagrams were determined by connecting the points representing formation of the microemulsion.[39-40] A pseudo ternary phase diagrams of the investigated quaternary systems of oil Oleic acid, surfactant Tween 20, co-surfactant PEG 400 : Transcutol P (1:1) and water are presented in figure 4. The figure 4: A, B, C and D has different Smix ratio as 1:1, 2:1, 3:1 and 4:1 respectively.

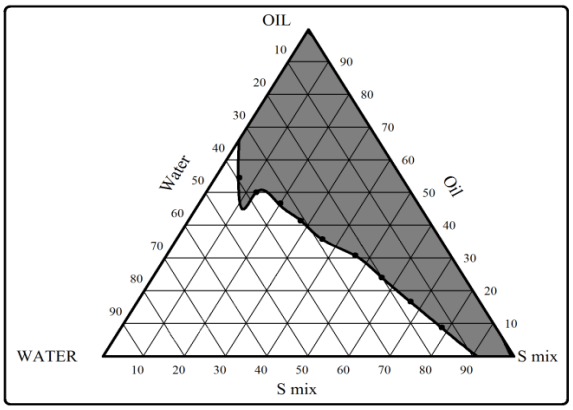

Figure No. 4: (A) Phase diagram Smix (1:1)

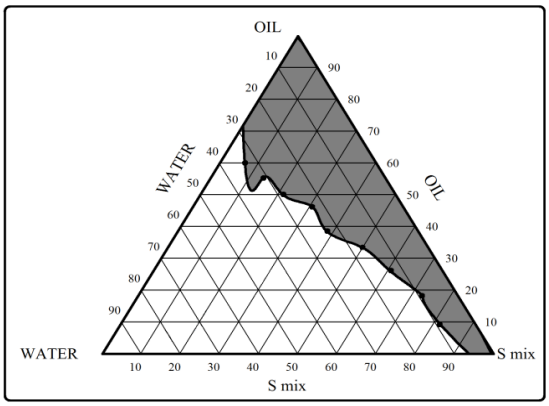

Figure No. 4: (B) Phase diagram Smix (2:1)

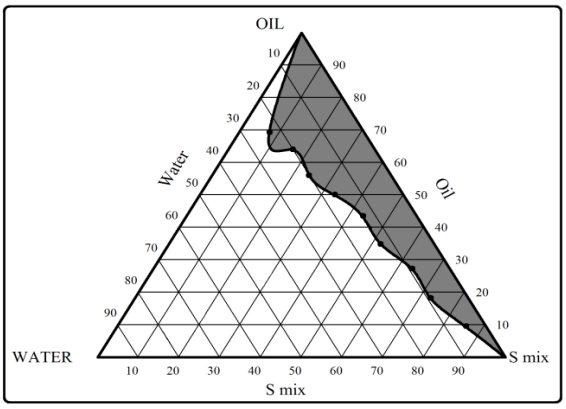

Figure No. 4: (C) Phase diagram Smix (3:1)

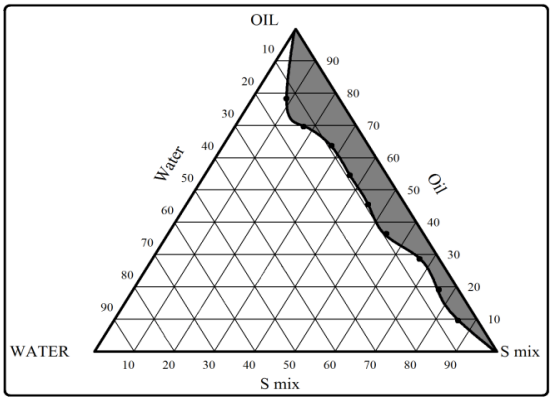

Figure No. 4: (D) Phase diagram Smix (4:1)

Phase diagram indicated that the Smix ratio 3:1 and 4:1 shows very less self emulsification region than the others therefore these ratios will be rejected. In case of the Phase diagram indicating the Smix ratio 1:1 and 2:1 there is slight difference in the self emulsification region but the Smix ratio 1:1 shows larger self emulsification region than the Smix ratio 2:1. Therefore the Smix ratio 1:1 will be selected for further study.

DEVELOPMENT OF LIQUID SMEDDS FORMULATION

Drug loading in liquid SMEDDS formulation shows that maximum 5.25 %w/w drug can be loaded in to the SMEDDS formulation. Hence for further study different formulations containing 5.25 %w/w drug were selected with the varying concentration of oil, surfactant: co-surfactant (1:1). System with highest water absorption capacity was selected for further formulation and also system showing larger micro emulsion region.

CHARACTERIZATION OF LIQUID SMEDDS

Emulsification efficiency

The study of emulsifying property of different liquid formulation revels that all three formulation shows good emulsifying property, C3 formulation forms whitish emulsion so, it may be rejected.

Precipitation assessment

From precipitation assessment C1 and C2 formulation found to be transparent, clear emulsion with no precipitation and found to be stable. While C3 formulation get whitish emulsion after 24 hrs, and forms whitish precipitate after 48 hrs. Therefore C3 formulation will be rejected. C1 and C2 formulation will be selected for further study.

Drug content determination

The total drug content of Liquid SMEDDS formulation C1 and C2 were found to be 98.06±0.79 and 99.23±0.35 respectively.

Self emulsification time

The C2 formulation requires less time for emulsification than the C1 formulation. And both formulations have good tendency for self emulsification.

Refractive Index

The refractive index of the liquid SMEDDS formulation was found to be 1.499, this was nearly equal to the refractive index of water 1.33. From this result it will concluded that the liquid SMEDDS formulation must be a transparent in nature.

Measurement of mean globule size

The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The globule size of SMEDDS formulation was shown in figure 5 and Table no. 6.

Table No. 6: Globule size distribution and Polydispersity index of Liquid SMEDDS

Formulation Code	Globule Size (nm)	Polydispersity Index (PDI)
C2	45.71	1.00

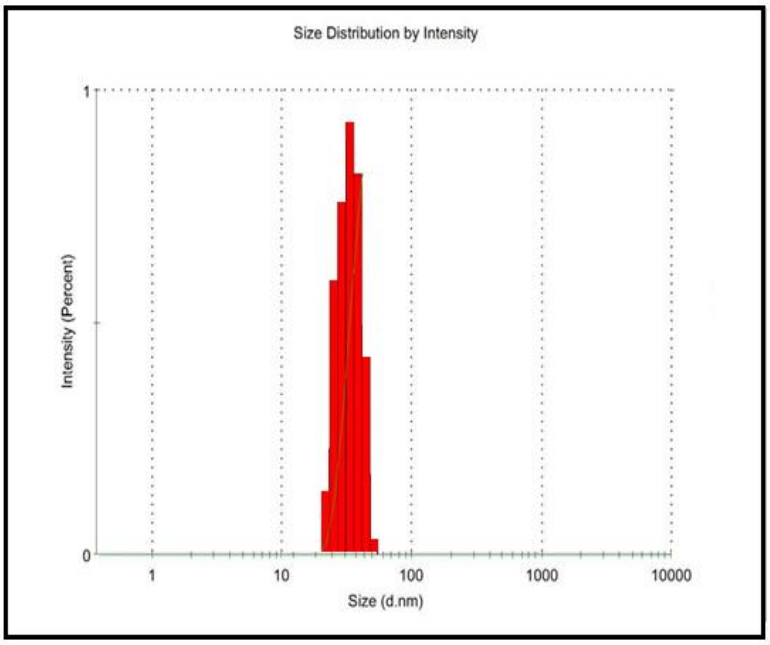

Figure No. 5: Histograms of globule size distribution of liquid SMEDDS C2

Zeta Potential Measurement

The zeta potential for optimized formulation was found to be -17.78 mV (Table No. 7 and figure no. 5a) which indicates high negative surface charge on particle which in turn indicates higher stability because of the anticipated surface repulsion between similar charged particles, hence inhibiting aggregation of the colloidal particles.

Table No. 7: Zeta Potential Measurement Parameters

Sr. No.	Parameters	Observations
1	Mean zeta potential	-17.78 mv
2	Zeta potential model	Huckel
3	Mean mobility	-0.93 (µs)/ (V/cm)
4	pH	7.0
5	Conductance	342 µs
6	Concentration	0.5 mg/ml
7	Liquid	Water
8	Temperature	25°C
9	Viscosity	0.890 cP
10	Refractive index	1.330
11	Dielectric Constant	78.54
12	Particle size	50.0 nm

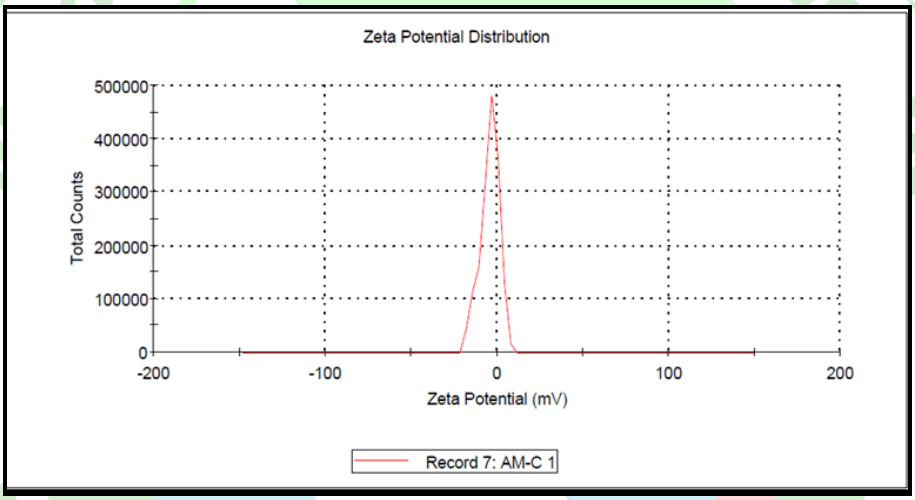

Figure No. 5a: Zeta potential measurement

Drug release study (In-vitro dissolution test)

The in-vitro drug release of C2 SMEDDS formulation was studied and obtained results were noted in the table no. 10 and figure 7.

In-vitro release study results revels that only 54.70% drug was released within 30 min from plain Atorvastatin filled in capsule, while 99.38% drug was released within 30 min from the liquid SMEDDS formulation.

PREPARATION OF SOLID SELF MICROEMULSIFYING DRUG DELIVERY SYSTEM

As C2 SMEDDS formulation passes all the evaluation parameter and also shows very good emulsifying property, it was selected as final formulation and it was converted into solid SMEDDS, so it can administered easily in the form of self emulsifying capsules. Liquid SMEDDS C2 formulation was converted in to solid SMEDDS by spray drying technique using Aerosil 200 (Colloidal Silicone Dioxide) as a carrier. 10 g of liquid SMEDDS contains 525 mg of drug Atorvastatin. After addition of 5 g of Aerosil 200 as carrier for spray drying the total weight 15 g contains 525 mg of drug Atorvastatin. Therefore by calculation 285.71 mg of spray dried powder contains 10 mg of Atorvastatin as a dose of drug.

CHARACTERIZATION OF SOLID SMEDDS

Reconstitution properties of solid SMEDDS

Visual Observation

Reconstitution property of S-SMEDDS was determined and observed visually. S-SMEDDS showed rapid dispersion without

any lump or agglomeration. This observed a good emulsification property within 1 minute without any phase separation.

Particle size determination

Globule size of emulsion form by dilution S-SMEDDS is shown in figure 6 and Table no. 8.

Table No. 8: Particle size distribution and Polydispersity index of Solid SMEDDS.

Formulation	Globule Size (nm)	Polydispersity index (PDI)
S-SMEDDS	82.46	0.158

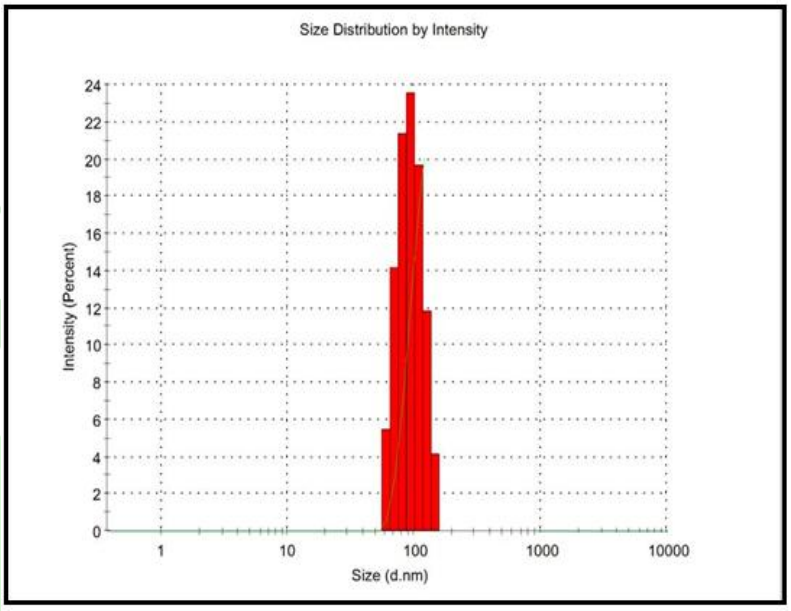

Figure No. 6: Histograms of globule size distribution of solid SMEDDS C2

Yield of Spray Dried Product

The percentage yield of spray dried S-SMEDDS product was found to be 91 %w/w.

Spray dried product was evaluated for bulk density, tapped density, Carr's compressibility index, Hausner ratio, Angle of repose and results are shown in table no. 9.

Powder flow properties

Table No. 9: Flow properties of Spray dried product

Sr. No.	Parameter	Result	Inference
1	Bulk Density	0.54 g/ml	--------
2	Tapped Density	0.63 g/ml	--------
3	Compressibility Index	13.59	Good
4	Hausner Ratio	1.16	Good
5	Angle of Repose	34.33	Passable

Weight Variation test for Capsules

The weight variation test defined by USP XX sequential test for weight variation of capsules. The test requirement met because none of individual capsule weights are less than 90% and none of individual weights are more than 110% of the average. In this study the average weight was found to be 449.95 mg.

Drug content determination

From this study the percentage drug content was found to be 98.15±0.98.

In-Vitro Dissolution Study

In vitro drug release studies were performed for liquid SMEDDS, Solid SMEDDS and plain drug Atorvastatin. Also the comparison of these with the marketed capsule

formulations. During this study the five samples were taken as-

D1 - Plain drug

D2 - Liquid SMEDDS

D3 - Solid SMEDDS

D4 - Ecosprin AV 75 Capsule (Marketed Capsule)

D5 - Aztor tablet (Marketed Tablet)

All the five formulations were subjected for in vitro dissolution studies using USP dissolution test apparatus II (basket). The dissolution medium pH 6.8 phosphate buffer was used to study the drug release.

In vitro release study reveals that only 54.70 %w/w drug was released from plain atorvastatin filled in capsule. While 99.38 %w/w, 98.23 %w/w, 59.29 %w/w and 61.58 %w/w drug released from the liquid SMEDDS, solid SMEDDS, marketed capsule and marketed tablet respectively. It also observed that both liquid and solid SMEDDS formulations will released completely within 30 min, while plain drug and other formulations requires 45 min and more. The drug release for all the five samples were shown in table no. 10 and figure 7.

From the dissolution study it will reveals that dissolution profile of Atorvastatin increases by formulating the SMEDDS formulation.

Table No. 10: In-vitro Dissolution data of Atorvastatin in different formulations

Sr. No.	Time (min)	Percentage Drug Release (%w/w)				
		D1	D2	D3	D4	D5
1	5	24.92	18.05	16.90	10.04	18.05
2	10	32.94	43.25	38.67	15.76	26.07
3	15	37.52	59.29	51.27	22.63	36.38
4	20	43.25	76.47	71.89	31.80	44.40
5	25	52.41	93.65	90.21	42.10	51.27
6	30	54.70	99.38	98.23	59.29	61.58
7	35	57.0	-	-	69.60	79.90
8	40	60.43	-	-	75.32	87.92
9	45	78.76	-	-	91.36	92.50

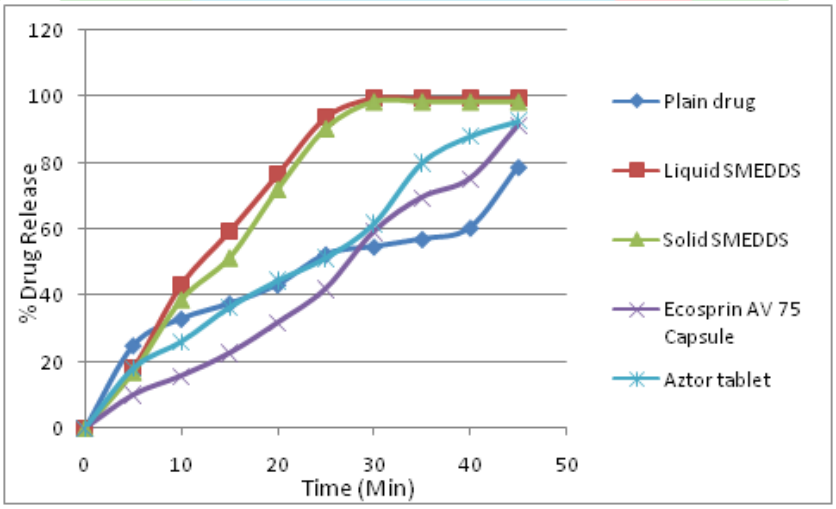

Figure No. 7: In-vitro Dissolution data of Atorvastatin in different formulations

SOLID STATE CHARACTERIZATION OF S-SMEDDS POWDER

Differential Scanning Calorimetry

DSC of Atorvastatin, Aerosil 200 and solid SMEDDS was performed and results are shown in figure 8 below.

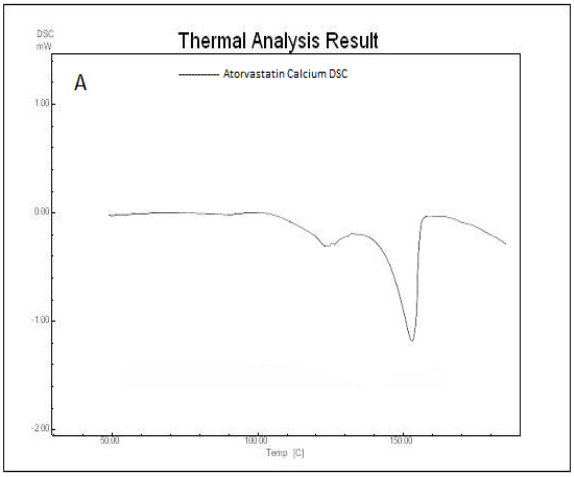

Figure (A)

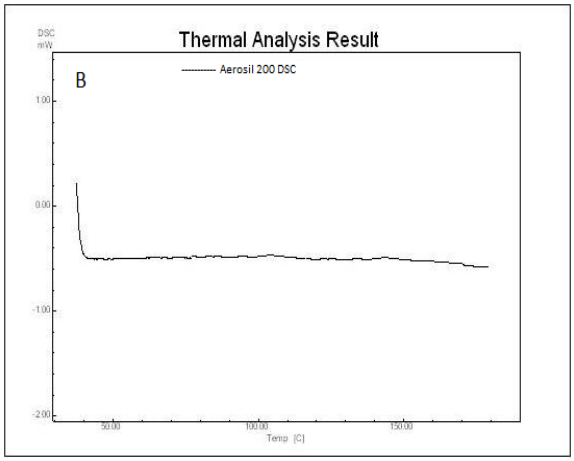

Figure (B)

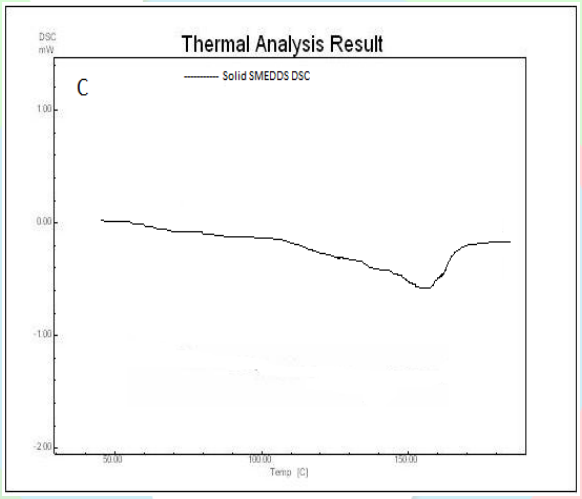

Figure (C)

Figure No. 8: Differential Scanning Calorimetry of (A) Atorvastatin, (B) Aerosil 200, (C) Solid SMEDDS.

The DSC thermogram of pure Atorvastatin exhibited a sharp endothermic peak at 154.2°C. The DSC of S-SMEDDS not shows any sharp melting peak of Atorvastatin. The absence of sharp melting peak of Atorvastatin in the range of 154-159°C in the DSC of S-SMEDDS indicate that the lipids and aerosil 200 inhibited the crystallization of Atorvastatin i.e. atorvastatin is in amorphous form or in solubilized form in S-SMEDDS.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to determine the particle morphology of pure drug and optimized SMEDDS. The SEM of Atorvastatin, Aerosil 200 and solid SMEDDS was done and results are shown in figure 9.

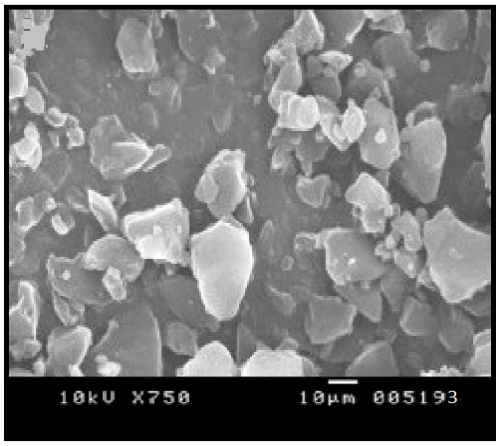

Figure (A)

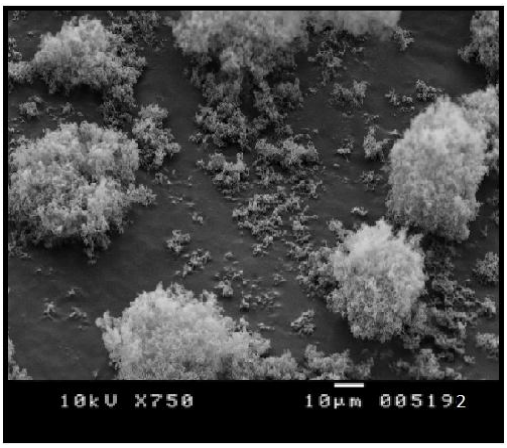

Figure (B)

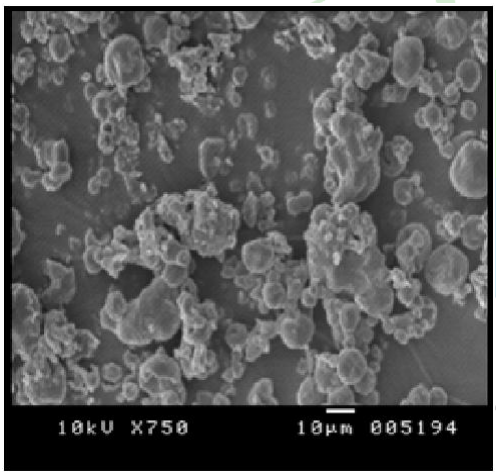

Figure (C1)

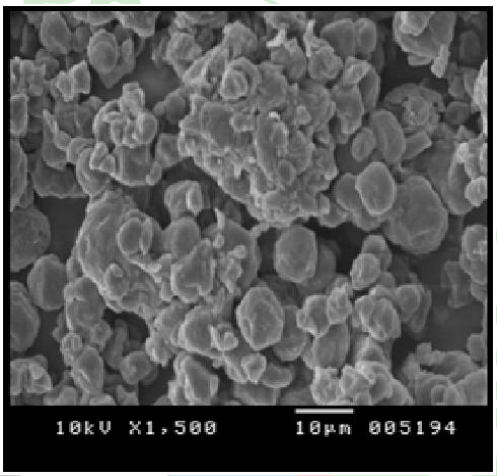

Figure (C2)

Figure No. 9: Scanning electron microscopy of (A) Atorvastatin, (B) Aerosil 200, (C) Solid SMEDDS.

Figure 9 revealed that Atorvastatin present as crystalline powder with rectangular plate shaped crystals. Aerosil 200 (Colloidal Silicone Dioxide) was detected as aggregates of amorphous particles. The solid-SMEDDS shows irregular shaped granular particle. SEM of the Solid-SMEDDS does not show any rectangular crystals of drug (Atorvastatin) on the surface of aerosil 200, the shape of formulation is spherical and somewhat smooth. It indicates that drug is present in the soluble form in lipid (SMEDDS formulation), which is adsorbed on the surface of aerosol 200.

Powder X-ray Diffraction

The X-ray diffraction pattern of Atorvastatin, colloidal silicone dioxide, physical mixture of atorvastatin and colloidal silicone dioxide and Solid SMEDDS was done and shown in figure 10.

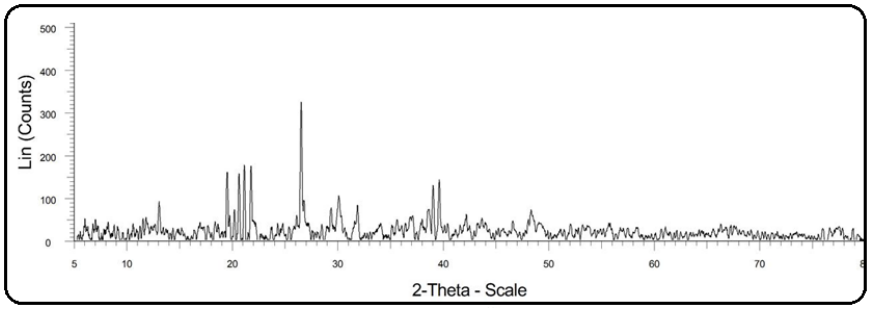

Figure (A)

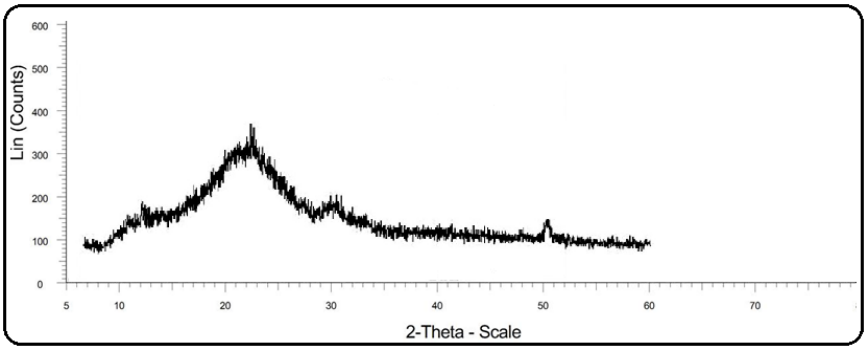

Figure (B)

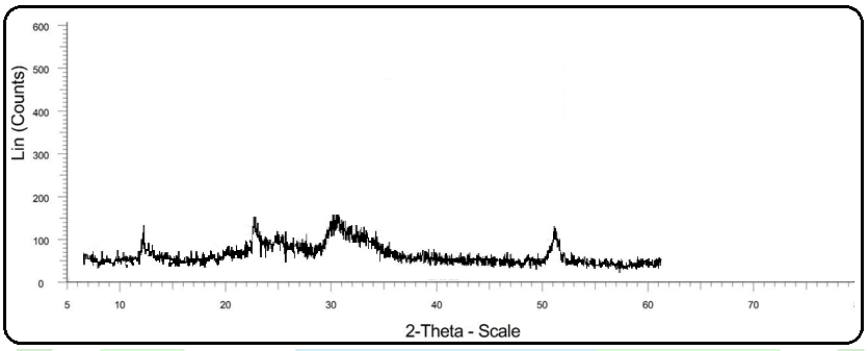

Figure (C)

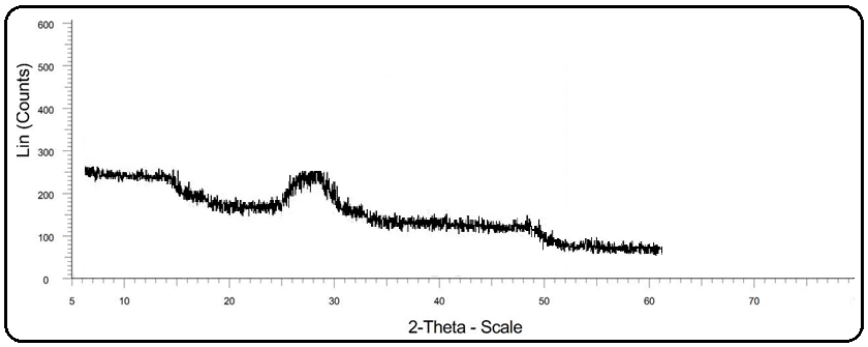

Figure (D)

Figure No. 10: X- ray Powder Diffraction of (A) Atorvastatin, (B) Aerosil 200, (C) Atorvastatin and

Aerosil 200, (D) Solid SMEDDS.

In the X-ray diffraction pattern of Atorvastatin, the sharp peaks at a diffraction angle 2Ɵ of 13.1°, 19.5°, 20.6°, 21.2°, 21.8°, 26.7°, 29.3°, 30.1°, 31.9°, 39.1° and 39.5° are present. The sharp diffraction peaks of atorvastatin were still detectable in physical mixture with Aerosil 200. The presence of sharp X-ray diffraction peaks of atorvastatin in physical mixture of atorvastatin and aerosol 200 and absence of sharp X-ray diffraction peaks of atorvastatin in the solid-SMEDDS formulation reveals that drug (Atorvastatin) either present in the amorphous form or present in solubilized form in solid-SMEDDS.

STABILITY STUDY
Liquid SMEDDS

The thermodynamic stability study was carried out for Liquid-SMEDDS formulation.

Heating cooling cycle: The liquid SMEDDS was subjected to the six cycles between refrigerator temperatures 4ºC and 45ºC with storage at each temperature for not less than 48 hours was studied. There is no change found in the formulation that means the formulation found stable.

Centrifugation test: Passed SMEDDS was centrifuged at 3500 rpm for 30 min using centrifuge (Remi motors Ltd.), there was no phase separation found.

Freeze thaw cycle: Three freeze thaw cycles between -21ºC and +25ºC with storage at each temperature for not less than 48 hours was done for SMEDDS, and it was found stable.

Solid SMEDDS

Stability study of Solid-SMEDDS at Freeze temperature (-20°C), Room temperature (25°C) and High temperature (40°C) was done for three month and evaluated for following parameters.

Visual Observation

Visual observation study reveals that there is no change in color observed during stability study for 3 month.

FT-IR

FT-IR of S-SMEDDS sample placed at different temperature (-20°C, 25°C, 40°C) was done after 3 month to determine any change in drug and result is shown in figure 11. The FT-IR of S-SMEDDS shows that there is no change in the functional peaks of drug (Atorvastatin) at all temperature after 3 months.

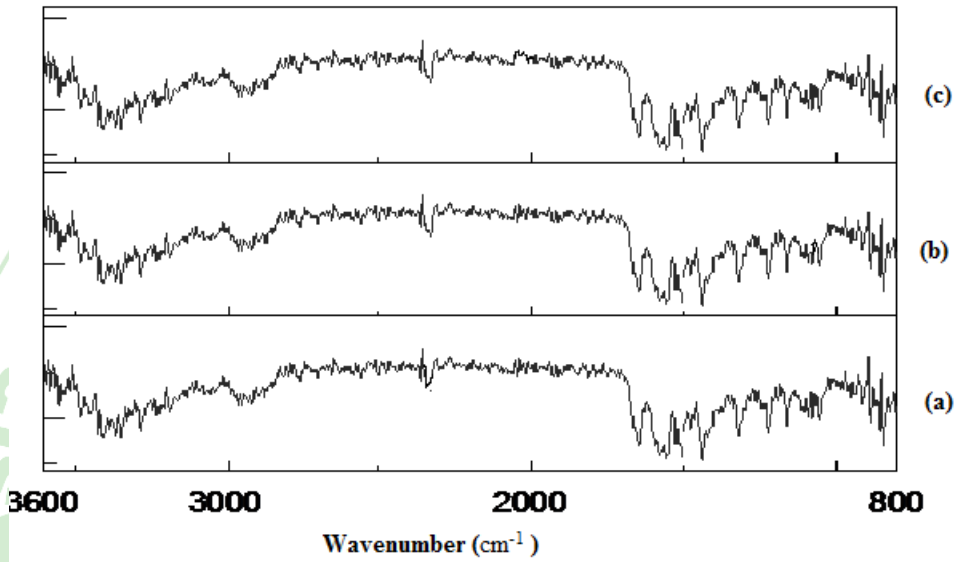

Figure No. 11: FT-IR of S-SMEDDS sample placed at different temperature (-20°C, 25°C, 40°C)
(Where, a, b and c are FT-IR of S-SMEDDS sample placed at different temperature -20°C, 25°C, 40°C respectively)

Drug Content and Emulsifying Property

Drug content and emulsifying property of S-SMEDDS was done after each month for 3 months. The drug content data shows that there is no change in drug content of S-SMEDDS. Also no change in emulsifying property of S-SMEDDS was found.

In-vitro drug release study

In vitro drug release study of Solid SMEDDS was done after 3 month in pH 6.8 phosphate buffer solution. And results were noted in table no. 11 and figure 12.

Table No. 11: In vitro dissolution data of S-SMEDDS after 3 months.

Sr. No.	Time (Min)	Percentage drug release (%w/w)		
		-20°C	25°C	40°C
1	5	15.76	16.90	27.21
2	10	40.96	34.09	47.83
3	15	57	54.70	61.58
4	20	70.74	75.32	74.18
5	25	87.92	83.34	81.05
6	30	97.09	99.38	94.80

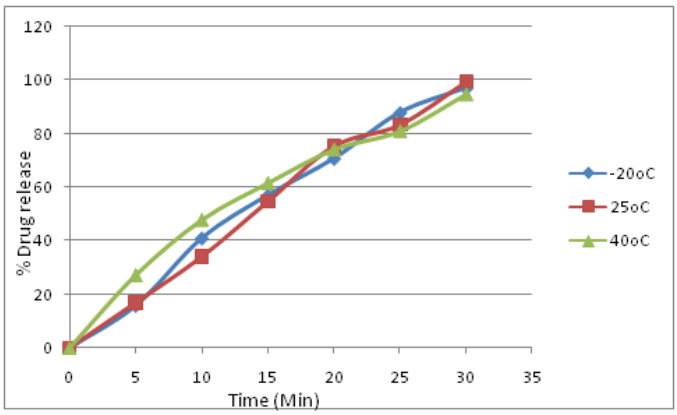

Figure No. 12: In vitro dissolution profile of S-SMEDDS at different temperatures after 3 month

The visual observation study, FT-IR study, Drug content determination, Emulsifying property and in vitro drug release study evidence that S-SMEDDS is stable at -20°C, 25°C, 40°C.

CONCLUSION

The optimized formulation was found to be the better formulation on the basis 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 SMEDDS in the improvement of the dissolution rate and there by oral bioavailability of poorly water soluble drug Atorvastatin without incompatibility between the ingredients.

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