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Design, Development and Formulation of Self Emulsifying Drug Delivery System for Atorvastatin

Rahul Gupta*, Praveen Kumar, Pratyush Jain

RKDF College of Pharmacy, Bhopal

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Address for Correspondence: Rahul Gupta
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Abstract

The aim of the present study is to develop self-micro-emulsifying drug delivery system (SMEDDS) of atorvastatin, a poorly water soluble anti-hyperlipidemic drug to enhance its oral bio-availability. Solubility of atorvastatin in various oils, surfactants and co-surfactants was determined. On the basis of solubility studies, Oleic acid, Labrasol and Transcutol were selected as oil, surfactant and co-surfactant, respectively. Ternary phase diagrams were constructed at different ratios using CHEMIX ® software to determine microemulsion region. The prepared SMEDDS were evaluated. The optimized formulation showed drug release of 93.51% in 0.1N HCl in 120 mins, droplet size of 180.1nm and zeta potential of -29.9mV. Drug release from all SMEDDS formulations was found to be higher compared to pure drug. The optimized liquid self micro-emulsifying drug delivery system formulation (F3) was converted into solid SMEDDS by adsorbing onto solid carriers like Aerosil 200, Fugicalin, and Neusilin US2 at various liquid SMEDDS to carrier ratios (1:1, 1:2 and 2:1). Prepared S-SMEDDS was evaluated. The optimized S-SMEDDS (A2) showed drug release of 91.07%, droplet size of258.1nm and zeta potential of -34.40mV. Compatibility study of drug and excipients was done by using FTIR. Solid state characterization was done by DSC and SEM. DSC thermo gram showed that there was no crystalline drug in S-SMEDDS. SEM photograph showed smooth surface of S- SMEDDS with less aggregation. Drug release was found to be higher as compared with that of pure drug and was comparable to liquid SMEDDS.

Keywords: SMEDDS, Atorvastatin, Self-emulsifying drug delivery system.

Introduction:

The majority of new drugs exhibit poor aqueous solubility, which affects their low bioavailability after oral delivery. Many strategies have been described to increase the dissolution rate of drugs by reducing their particle size and salt formation, using surfactants, cyclodextrins, liposomes or nanoparticles [1–4]. A relatively new approach for poorly soluble drugs is lipid-based formulations, particularly self-emulsifying drug delivery systems (SEDDS) [5,6]. SEDDS are isotropic mixtures of oils and surfactants with or without co-surfactants, which act as lipid-based formulations after oral application in aqueous gastrointestinal fluid and upon gentle agitation can form an oil-in-water emulsion [7–10]. SEDDS technology was employed to increase solubility and consequently the bioavailability of many poorly water soluble drugs such as phyllanthin, celastrol, ketoprofen, indomethacin and hydrocortisone [11–14].

The fact that a majority of the newly discovered chemical entities and many existing drugs molecules are poorly water soluble and present a serious challenge to the successful formulation and marketing of new drugs in the pharmaceutical industry. Since in many cases the dissolution step is the rate limiting step,

formulation design can be a useful approach to improve the absorption and thus the oral bioavailability of such drug candidates. As oral route has always been preferred and has dominated over other routes of administration due to its convenience, non- invasiveness and cost effectiveness thus it become necessary that drug should have some aqueous as well as some lipid solubility for better absorption through this route. The oral route is not suitable for those chemical entities which exhibit poor aqueous solubility. To overcome these problems, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins, nanoparticles and solid dispersions. Recently, much attention has been paid to lipid-based formulations with particular emphasis on Self-Dispersing Lipid Formulations (SDLF's) to develop the oral bioavailability of lipophilic drugs.

The Self-Dispersing Lipid Formulations (SDLFs) is one of the promising approaches to overcome the formulation difficulties of various hydrophobic/lipophilic drugs and to improve the oral bioavailability of poorly absorbed drugs. The SDLF's contain oil and a surfactant mixture into which the drug is incorporated. They emulsify when mixed with aqueous environment [4]. The self emulsification process is specific to the particular pair of oil and surfactant, surfactant concentration, oil/surfactant ratio, and the temperature at which self-emulsification occurs. After self dispersion, the drug is rapidly distributed throughout the gastrointestinal tract as fine droplets. Bioavailability enhancement results from the finely dispersed state of the drug containing lipid globules. The large surface area enhances the dissolution.[15].

The SDLF's are of two kinds namely, Self-Emulsifying Drug Delivery Systems (*SEDDS) formed using surfactants of HLB < 12 and Self-Micro Emulsifying Drug Delivery Systems (SMEDDS) formed with surfactants of HLB > 12. Both SEDDS and SMEDDS are stable preparations and improve the dissolution of the drug due to increased surface area on dispersion. Therefore, they are not dependent on bile secretion for absorption. The emulsified form itself is readily absorbable. This ensures a rapid transport of poorly soluble drugs to the blood. Many researchers have reported applications of SEDDS for delivering and targeting lipophilic drugs[16]. Self emulsifying formulations comprises of isotropic mixtures of natural or synthetic oils, with lipophilic surfactants and co surfactants which spontaneously emulsify when exposed to the fluids in GIT to form emulsions. SEDDS are formulations which produces milky crude emulsions when dispersed in water with a droplet size ranging from few nanometers to several microns [9]. Self Micro-emulsifying drug delivery system (SMEDDS) are formulations which produces clear, transparent, micro emulsions with a droplet size ranging from 100-250 nm. Self-Nano emulsifying drug delivery systems (SNEDDS) produces Nano emulsions when dispersed in water with a droplet size less than 100nm. [17-18]. S-SMEDDS, one of the lipid-based drug delivery systems prepared by the incorporation of liquid excipients into powders by solidification, is a promising drug delivery system for poorly water soluble compounds as it combines the advantages of liquid SMEDDS (solubility and bioavailability enhancement) with those of solid dosage forms (high stability with various dosage forms options)[18].*

Role of SEDDS

SEDDS are promising approach for oral delivery of poorly water-soluble compounds. It can be achieved by pre-dissolving the compound in a suitable solvent and fill the formulation into capsules. The oral drug delivery of hydrophobic drugs can be made possible by SEDDS. The main benefit of this approach is that pre-dissolving the compound overcomes the initial rate limiting step of particulate dissolution in the aqueous environment within the GI tract. However, a potential problem is that the drug may precipitate out of solution when the formulation disperses in the GI tract, particularly if a hydrophilic solvent is used (e.g. polyethylene glycol). If the drug can be dissolved in a lipid vehicle there is less potential for precipitation on dilution in the GI tract, as partitioning kinetics will favor the drug remaining in the lipid droplets.[19]

Atorvastatin, an Anti-hyperlipidemic , used in the belongs to class II in biochemical classification system i.e. low solubility and high permeability. One of the major problems with this drug is its low solubility in biological fluids, which results in poor oral bioavailability. Poor solubility of Atorvastatin leads to poor dissolution and hence variation in bioavailability. Thus increasing the aqueous solubility and dissolution of Atorvastatin is of therapeutic importance. Aqueous solubility and dissolution of Atorvastatin can be increased by formulating in SEDDS. Hence main objective of the study was to develop and evaluate an optimal S- SEDDS formulation of the drug[20].

MATERIALS AND METHODS

Atorvastatin (Gift sample from Ajanta Pharmaceticals Pvt. Ltd., Mumbai) Oleic acid, Soya bean oil, Sunflower oil, Sesame oil, Maisine, Labrafil, Labrasol (Matrix Laboratories, Hyderabad), Olive oil, Corn oil, Tween 20, Tween 80, PEG 300, PEG 400, Transcutol P, Propylene glycol, HCl (SD Fine Chem. Limited, Mumbai).

Selection of self emulsified drug delivery system components Based on solubility studies

Oils, Surfactants and Co-surfactants

The solubility of Atorvastatin in each of various oil phases, surfactants, co-surfactants and co-solvents was determined by adding an excess amount of drug to 5 ml of each selected vehicle contained in 25 ml volumetric flask. The liquids were mixed using a vortex mixer and then were shaken using orbital shaker at 25°C±1°C for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged 5000 rpm for 30 min. The supernatant was taken out, suitably diluted with distilled water and the concentration of Atorvastatin in various vehicles was determined by UV spectrophotometer at λ max of drug, 243 nm [11]

Construction of the Pseudo Ternary Phase Diagram

The oil, surfactant and co-surfactant selected from the solubility studies were used to construct the pseudo-ternary phase diagrams employing water titration method. The pseudo ternary phase diagrams were prepared to identify micro-emulsion region. Surfactant and co-surfactant was mixed in weight different ratios 1:1, 1:2, 1:3, 2:1, 3:1, 4:1 and 5:1. Oil and surfactant/co-surfactant mixture (Smix) were mixed thoroughly in different weight ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The mixture of oil and Smix at different weight ratios was titrated with water by drop wise addition under gentle agitation. Resulting mixtures were evaluated visually for transparency and flow properties. Endpoint of titration was the point, where mixture became turbid or phase separation was observed. At this point the amount of water, oil, surfactant and co-surfactant added was noted and were used to construct phase diagrams. The ternary phase diagrams were constructed using CHEMIX ® software [13].

FORMULATION

Preparation of liquid SMEDDS:

Various formulations were prepared with a constant amount of Atorvastatin (10 mg) loaded into 200 mg of liquid SMEDDS prepared in varying ratios of oil, surfactant to co-surfactant. Surfactant and co-surfactant were blended in different weight ratios. To the above mixture, required amount of oil phase was added and blended using vortex mixer to obtain good blend of Oil/Smix mixture (SMEDDS) at a liquid state. To 200 mg of above liquid concentrate, 10 mg of drug was added and mixed properly using vortex mixer [22].

PREPARATION OF SOLID SMEDDS:

Different solid carriers like Aerosil 200, micro crystalline cellulose and Neusilin US2, and at various carriers to SMEDDS ratios (1:2, 2:1, 1:1) were used for solidification. The SMEDDS formulation was added drop wise over the solid adsorbent contained in a porcelain dish. After each addition the mixture was homogenized using glass rod to ensure uniform distribution of the formulation. Resultant mass was passed through sieve no.80 and stored until further use [21].

Table 1: Formulation of solid SMEDDS

Formulation code	Ratio of carrier to liquid SMEDDS	Carrier (gm)	SMEDDS (gm)
A1	1:2	1	2
A2	2:1	2	1
A3	1:1	1	1
F1	1:2	1	2
F2	2:1	2	1
F3	1:1	1	1
N1	1:2	1	2
N2	2:1	2	1
N3	1:1	1	1

A-Aerosil 200; F-Fugicalin; N-Neusilin US2

CHARACTERIZATION AND EVALUATION OF SEDDS

Assessment of self-emulsification time: The emulsification time (the time for a pre concentrate to form a homogeneous mixture upon dilution) was monitored by visually observing the disappearance of SMEDDS and the final appearance of the micro-emulsion. In this method, a predetermined volume of formulation (100 µl) was introduced into 20 ml and 300 ml of distilled water and 0.1 N hydrochloric acid solution in separate glass beakers maintained at 37°C and the contents were mixed gently using a magnetic stirrer. The time to emulsify spontaneously and progress of emulsion droplets were observed. The tendency to form an emulsion was judged as **"good"** when droplets spread easily in water and formed a fine emulsion that was clear or transparent in appearance and it was judged as **"bad"** when the corresponding performance was poor or there was less clear emulsion formation [28].

Phase separation and stability study: 100 µl of each SMEDDS formulation was added to 300 ml of distilled water and 0.1 N hydrochloric acid solution in a beaker at room temperature and the contents were gently stirred magnetically. Diluted emulsion was stored for a period of 24 hrs and observed for any phase separation or precipitation of the drug. The observations were made after 2, 4, 6, 8, 12 and 24 hrs. 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) [20].

Robustness to dilution: Dilution study was done to access the effect of dilution on SMEDDS pre concentrate. Robustness to dilution was studied by diluting SMEDDS to 50, 100 and 1000 times with various dissolution media like distilled water, 0.1N hydrochloric acid and phosphate buffer pH 6.4. The diluted micro-emulsions were stored for 24 hr and observed for any signs of phase separation or drug precipitation [22].

Thermodynamic stability: The physical stability of a formulation is very important for its performance as it can be adversely affected by precipitation of the drug in excipient matrix. Poor physical stability of formulation can lead to phase separation of excipients which affects bioavailability as well as therapeutic efficacy. Also the incompatibilities between formulation and gelatin shell of capsule (if formulation filled in capsule) may cause brittleness, softness and delayed disintegration or incomplete release of drug. The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SMEDDS formulations. The thermodynamic stability studies were performed on prepared micro-emulsion in three main steps:

Heating cooling cycle: Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 hr is studied. Those formulations which were stable at these temperatures were subjected to centrifugation test.

Centrifugation: Passed formulations were centrifuged at 3500 rpm for 30 minutes. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

Freeze thaw cycle*:* Formulations were subjected to three freeze thaw cycles (-20°C for 2 days followed by 25°C for 2 days). Those formulations which passed this test showed good stability with no phase separation, creaming, or cracking [17].

***In vitro* drug release studies:** The release of drug from liquid SMEDDS formulations filled in capsules and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. A hard gelatin capsule size '0' filled with pre concentrate (equivalent to 10 mg Atorvastatin) and pure drug (10 mg) were separately placed into 900 ml of 0.1N hydrochloric acid. The temperature of the dissolution medium was maintained at 37°C ± 0.5°C and operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals of 5, 10, 15, 20, 30, 45,60 min and 120 min and replaced with equal volume of fresh medium. The samples were filtered through whattman filter paper and were analyzed using UV spectrophotometer at 243 nm. All measurements were performed in triplicate from the independent samples [19].

Droplet size analysis: This is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release, as well as the stability of the emulsion. The average droplet size and polydispersity index of SMEDDS formulation was measured by photon correlation spectroscopy that analyzes the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a Malvern Zetasizer (Nano ZS 90, Malvern instrument ltd., U.K.). Light scattering was monitored at 25°C at 90° angle. 100µl of formulation was dispersed into 100 ml of distilled water under gentle stirring in a glass beaker. Then 1ml aliquot was withdrawn and added into sample cell (1 cm^2 cuvette). Each sample was analyzed in triplicate [23].

Polydispersity index (PI): The polydispersity index is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PI value the more homogenous are the particles. An ideal SMEDDS should be widely distributed with particles less than 100 nm and so PDI should be less than 0.3 or in other words particles having size more than 100 nm should be maximum up to 23 % [28].

Zeta potential measurement: Zeta potential helps to predict the stability of the emulsion system. If the zeta potential value falls below a certain level, colloids will aggregate due to attractive forces. Conversely a high zeta potential maintains a stable system. Zeta potential was measured by Laser Doppler velocimetry technique using a Malvern Zetasizer (Nano ZS 90, Malvern instrument ltd., U.K.).

Percentage transmittance: Percentage transmittance is made to denote the reconstitution property of the formed liquid SMEDDS. The SMEDDS equivalent to 15 mg of drug was accurately weighed and diluted with distilled water to 100 ml and its percentage transmittance was measured at 593 nm by UV spectrophotometer using distilled water as blank [19].

RESULTS AND DISSCUSION

ANALYTICAL METHOD

Suitable analytical method was established for atorvastatin using UV-Spectrophotometer. The λmax was found to be 243 nm in 0.1 N hydrochloric acid and R^2 value was found to be 0.998. The UV method was further used for solubility studies, drug content and dissolution studies.

SOLUBILITY STUDIES

Screening of Oils: The solubility of the drug was tested in different oil phases and maximum solubility was found to be in Oleic acid (93.3 ±0.102 mg/ml) and was selected as oily phase for SMEDDS formulation.

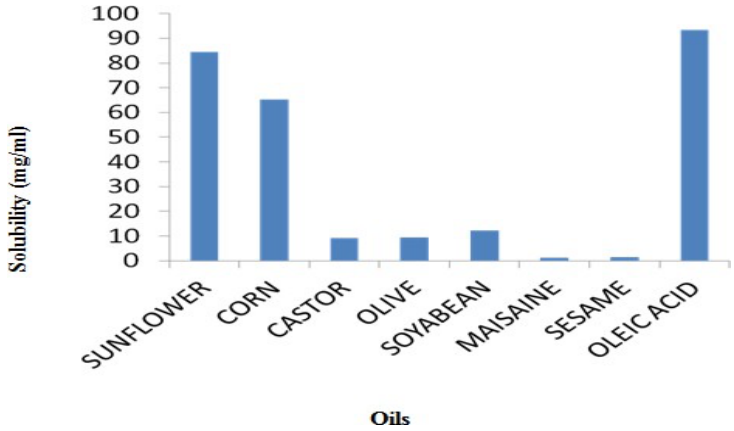

Fig 1: Solubility of atorvastatin in various oils

Screening of Surfactants: The solubility of the drug was tested in different surfactants and maximum solubility was found to be in Labrasol (49.93 ±0.009 mg/ml) and was selected as surfactant for SMEDDS formulation

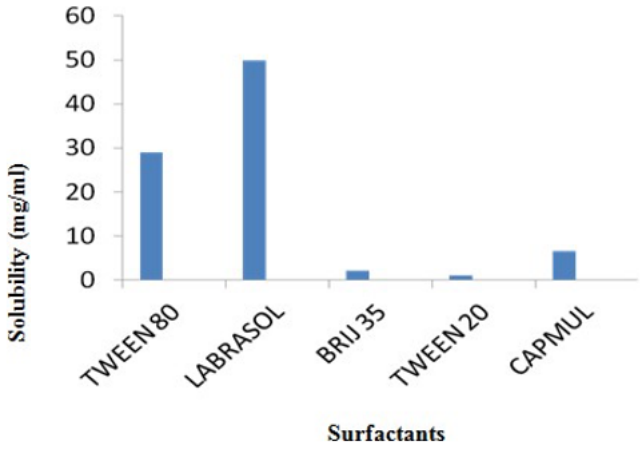

Fig 2: Solubility of atorvastatin in various surfactants

Screening of Co-surfactants: The solubility of the drug was tested in different co-surfactants and maximum solubility was found to be in Transcutol P (21.1 ± 0.018 mg/ml) and was selected as co-surfactant for SMEDDS Formulation.

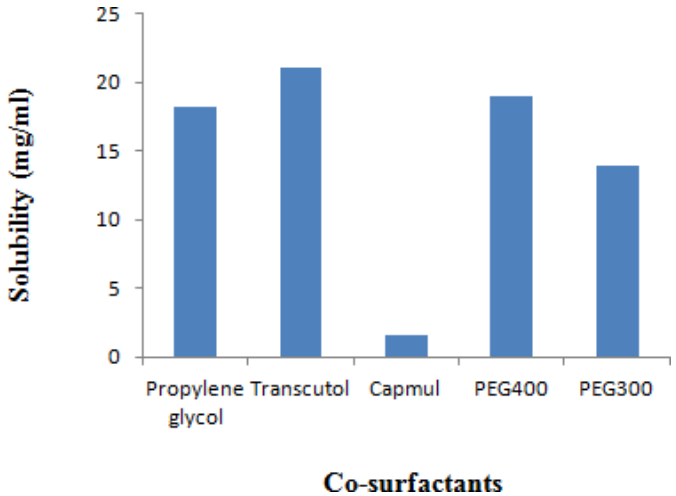

Fig 3: Solubility of atorvastatin in various co-surfactants

PSEUDO TERNARY PHASE DIAGRAM

Pseudo ternary phase diagrams are used to identify the micro-emulsion region. The micro-emulsion phase was identified as the area where clear and transparent formulations were obtained on dilutions based on visual inspection of samples. Phase diagram also help to study the micro-emulsifying capacity and effect of drug on phase structure. Based on solubility studies, oleic acid was selected as oil phase, Labrasol as surfactant and Transcutol P as co-surfactant which was used to construct pseudo ternary phase diagrams. Nine different combination of oil to Smix at different ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) were used for construction of pseudo ternary phase diagram using aqueous titration method. At the endpoint, the amount of water, oil and Smix added was noted to give phase diagram data which is given in Table 2. The ternary phase diagrams were constructed using CHEMIX ® software.

Table 2: Percentage composition of oil, S_{mix} and water consumed-Ternary phase diagram data

Oil: S_{mix}	Surfactant :Cosurfactant (S_{mix}) →																	
	5:1		4:1		3:1		2:1		1:1		1:2		1:3		1:4		1:5	
	%of oil	% of water	%of oil	% of water	%of oil	% of water	% of oil	% of water	% of oil	% of water	% of oil	% of water	% of oil	% of water	% of oil	% of water	% of oil	% of water
9:1	78.95	13.38	70.31	21.88	75	16.67	65.21	27.59	43.77	18.04	63.23	23.08	72.58	19.36	78.95	12.28	75	16.67
8:2	68.98	15.20	68.97	13.79	59.70	25.38	58.82	26.48	60.60	24.25	60.62	24.25	57.14	28.57	71.43	10.71	68.97	13.79
7:3	61.40	12.28	60.34	13.80	54.63	21.89	57.37	26.48	56.45	19.35	51.03	24.28	61.40	12.28	60.34	13.8	54.69	21.87
6:4	52.63	12.28	52.63	12.28	48.38	19.37	50	16.67	50	16.67	46.15	23.09	42.23	29.58	47.62	20.63	46.88	21.87
5:5	43.10	13.8	45.45	90.90	40.32	19.36	40.32	19.56	39.06	21.88	41.66	16.68	39.06	21.88	42.37	15.26	39.68	20.64
4:6	34.48	13.80	35.21	10.72	31.74	20.65	33.09	18.27	31.74	20.65	29.85	25.38	29.85	25.37	30.73	22.08	31.75	20.63
3:7	23.81	20.64	23.81	20.64	21.42	28.58	22.72	23.98	22.72	24.25	23.43	21.89	23.81	20.64	23.06	23.82	22.06	33.82
2:8	15.63	21.87	15.38	33.08	15.62	45.32	15.87	29.64	15.15	24.25	13.88	30.57	12.19	60.97	13.33	33.34	12.35	38.27
1:9	60.41	35.9	6.10	39.02	8.92	10.73	13.54	45.20	12.20	28.58	12.72	38.58	6.49	35.07	6.94	30.16	6.25	37.50

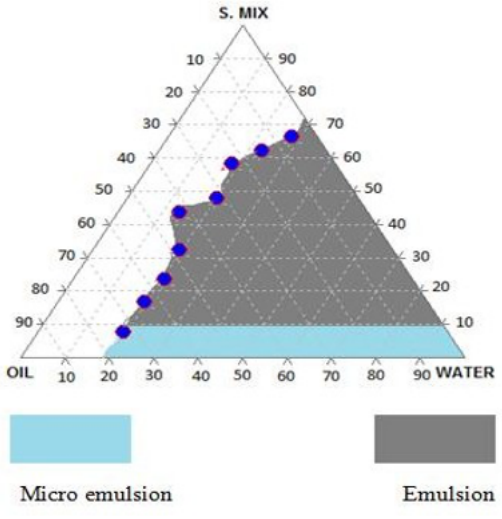

Fig 4: Pseudo ternary phase for S_{mix} 1:2

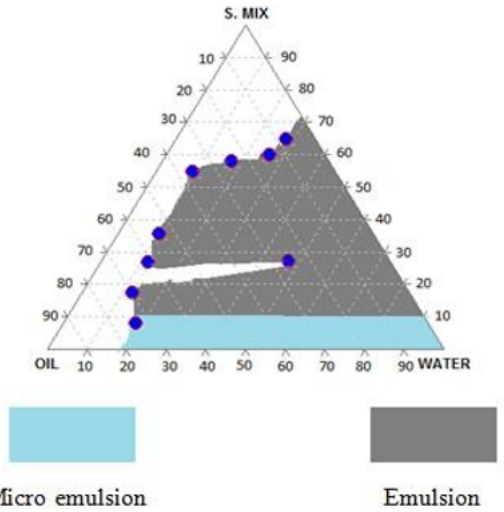

Fig 5: Pseudo ternary phase for S_{mix} 1:3

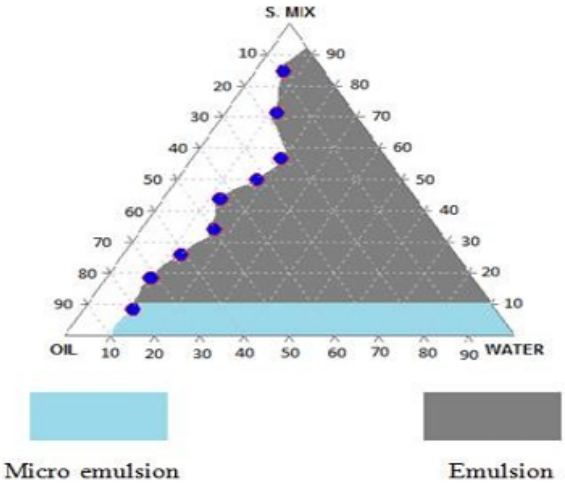

Fig 8.6: Pseudo ternary phase for S_{mix} 1:4

From the results it was observed that, as the amount of surfactant increased the emulsifying effect was good and emulsifying region was maximum at Oil: Smix ratio 1:9. Further increase When the concentration of S_{mix} was increased compared to oil phase, the micro emulsion region also expanded. An increase in the ratio of oil phase resulted in formation of less clear emulsion. For Oil:S_{mix} ratios 9:1, 8:2, 7:3 less clear emulsion was formed. Hence SMEDDS of atorvastatin were formulated using oil to S_{mix} ratios of 1:2, 1:3, 1:4, 4:1 and 5:1 as they showed highest micro-emulsion region in surfactant concentration decreased the micro- emulsion region. Co-surfactant aided in further emulsification but high amount of co-surfactant than surfactant contracted micro-emulsion region.

SELECTION OF FORMULATIONS

On the basis of visual observation after water titration, 5 formulations were selected, as these formulations produced micro-emulsion upon dilution. The composition data is given in Table 8.2. Further the compositions were reported in terms of percentage for ready comprehension (Table 8.3). These optimized formulations were subjected to further characterization.

Table 8.2: Formulation codes for the optimized formulations

S mix ratio	Oil: S mix ratio	Formulation code
1:2	1:9	F1
1:3	1:9	F2
1:4	1:9	F3
4:1	1:9	F4
5:1	1:9	F5

Table 8.3: Percentage composition of ingredients for the optimized formulations

S. No.	Formulation code	Oil (% w/w)	Surfactant (% w/w)	Co- surfactant (% w/w)
1	F1	30	23.3	46.6
2	F2	20	26.6	53.3
3	F3	10	29.9	60.1
4	F4	10	22.5	67.5
5	F5	60	26.6	13.3

EVALUATION OF LIQUID SMEDDS

Assessment of self-emulsification time:

The emulsification time of the formulations was in the range of 3.09 to 5.41 sec. It was observed that higher concentrations of the S mix increased the spontaneity of the self micro-emulsification process. When a co-surfactant was added to the system, it further lowered the interfacial tension between the o/w interfaces and also influenced the interfacial film curvature. The results of emulsification time studies are shown in Table 8.4

Table 8.4: Self-micro emulsification time in seconds (AM ± SD)*

Formulation Code	In distilled water		In 0.1N hydrochloric acid	
	Self-micro emulsification time (sec)	Performance of emulsion	Self-micro emulsification time (sec)	Performance of emulsion
F1	4.26 ±1.24	Good	7.66 ± 2.05	Good
F2	5±1.63	Good	8.33 ± 1.24	Good
F3	5.41± 1.63	Good	9.33 ± 1.24	Good
F4	3.09 ± 0.94	Good	6.6 ± 0.47	Good
F5	3.10± 2.05	Good	7.56 ± 0.81	Good

* Each value is an average of 3 determinations

Phase separation and stability study of micro emulsion:

Phase separation studies revealed that the designed SMEDDS formulation did not show any separation in 0.1 N hydrochloric acid and distilled water for the period of 24 hrs, which confirmed the ability of formation of stable micro emulsion.

Table 8.5: Phase separation and stability study of resultant micro emulsion

Formulation	Phase separation	Precipitation
F1	Nil	Nil
F2	Nil	Nil
F3	Nil	Nil
F4	Nil	Nil
F5	Nil	Nil

Robustness to dilution:

SMEDDS formulation was diluted with different dilution media to observe the effect of degree of dilution and pH on micro-emulsion. Robustness to dilution was performed with distilled water, 0.1 N hydrochloric acid and phosphate buffer pH 6.4. Micro-emulsions resulting from dilution of SMEDDS with various dissolution media were robust to all dilutions and did not show any separation even after 24 hrs of storage [24].

Table 8.6: Dilution study of optimized SMEDDS formulations

Formulation code	Distilled water	0.1 N hydrochloric acid	Phosphate buffer (pH 6.4)
F1	Pass	Pass	Pass
F2	Pass	Pass	Pass
F3	Pass	Pass	Pass
F4	Pass	Pass	Pass
F5	Pass	Pass	Pass

Thermodynamic stability:

Thermodynamic stability studies were performed to observe the ability of the formulation to withstand different stress conditions. The results of thermodynamic stability studies are reported in Table 8.7. Stability studies of the SMEDDS samples were carried out by subjecting them to temperature stability and centrifugation.

Table 8.7: Thermodynamic stability studies of optimized SMEDDS

Formulation	Heating and cooling cycle (45 °C and 4 °C)	Centrifugation (3500 RPM)	Freeze thaw cycle (-20°C and 25°C)
F1	Pass	Pass	Pass
F2	Pass	Pass	Pass
F3	Pass	Pass	Pass
F4	Pass	Pass	Pass
F5	Pass	Pass	Pass

The temperature stability study was carried out by keeping the resultant micro emulsion sample at different temperatures. No evidence of phase separation or any flocculation or precipitation was observed in SMEDDS formulations. No formulation showed any sign of phase separation when subjected to centrifugation at 3500 rpm. Thus, it was concluded that SMEDDS formulation were stable thermally as well as under stressful conditions [16].

In vitro drug release studies:

Dissolution studies were performed for the optimized SMEDDS formulations and the pure drug in 0.1 N hydrochloric acid solution and the results were compared with the pure drug. As the emulsification time is below 10 sec, about 100% percentage of the drug was released within 120 min in case of SMEDDS, while plain drug showed only 24.58 % release. The in vitro dissolution studies indicate that formulation of atorvastatin in the form of SMEDDS enhances the dissolution properties [13].

Table 8.8: Cumulative percentage drug release from optimized Formulations in 0.1N HCl

Formulation code	mulative % release of atorvastatin in 0.1 N hydrochloric acid in minutes (AM*±SD)				
	10	20	30	60	120
Pure drug	11.22 ± 0.26	15.89 ± 1.12	16.82 ± 0.15	23.86 ± 0.36	24.58 ± 1.38
F1	56.98± 0.42	73.12 ± 1.41	78.39 ± 1.14	88.11 ± 1.12	**92.78 ± 2.21**
F2	28.57± 0.37	52.73 ± 2.19	66.51 ± 2.02	84.51 ± 1.03	**88.39 ± 1.73**
F3	25.50± 0.58	48.39 ± 2.71	53.35 ± 1.71	73.53 ± 1.39	**93.51 ± 2.25**
F4	18.41± 0.89	38.76 ± 1.25	52.29 ± 1.69	72.85 ± 0.66	81.94 ± 0.74
F5	29.51± 1.85	58.81 ± 1.2	63.55 ± 0.81	79.83 ± 1.59	**89.51 ± 1.49**

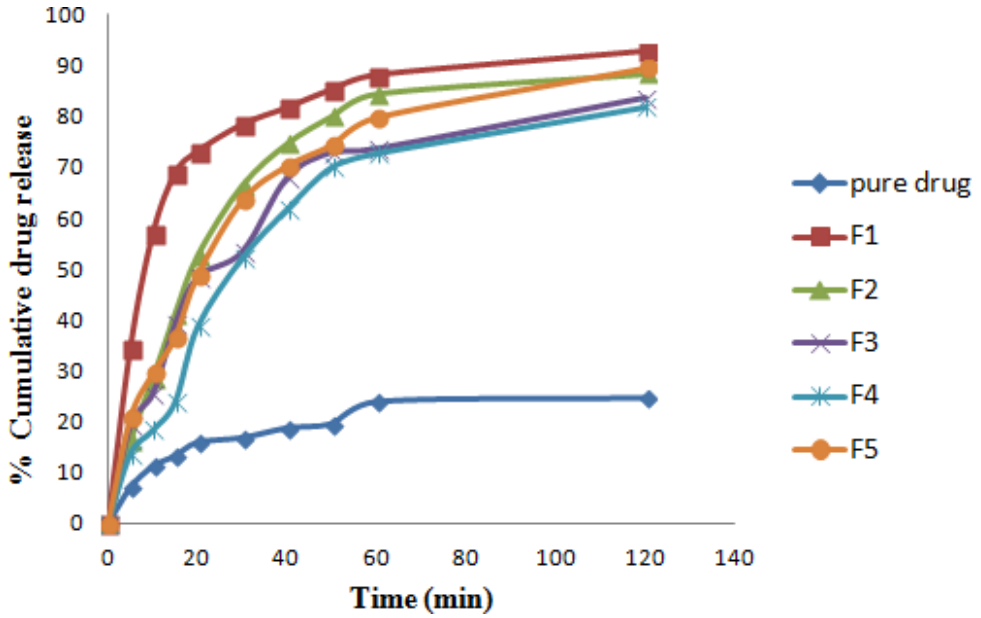

Fig 8.9: In-vitro dissolution profile of optimized SMEDDS formulations in 0.1N hydrochloric acid

Form the above dissolution results five formulations F1, F2, F3, F4, and F5 were selected for further characterization studies i.e. the droplet size analysis and zeta potential measurement as these formulations showed highest percentage (more than 80 %) of cumulative drug release.

Droplet size analysis:

Droplet size analysis of all tested five formulations showed resultant droplet size of micro emulsion between 180.1 to 1611 nm in distilled water media. Formulation F3 showed smaller droplet size (180.1 nm) compared to other formulations as it contains higher concentration of surfactant that promotes faster emulsification process and results into finer droplet formation.

Zeta potential determination:

The zeta potential of the formulations was from -15.5 to -37.36 mV. Negative charge indicates the presence of free fatty acids on the droplets. In general, the zeta potential value of ±40 mV is sufficient for the stability of a micro emulsion. All formulations comply with the requirement of the zeta potential for stability.

Table 8.10: Droplet size, polydispersity index and zeta potential of optimized SMEDDS

Formulation code	Droplet size (nm) AM*± S.D	Polydispersity index	Zeta potential (mV) AM*± S.D
F1	522.0 ± 6.99	0.31	-22.60 ± 0.14
F2	1611 ± 6.51	0.46	-37.36 ± 4.24
F3	**180.1 ± 3.57**	**0.155**	**-29.9 ± 0.78**
F4	576.5 ± 17.21	0.18	-22.5 ± 0.28
F5	805.2 ± 5.29	0.26	-15.5 ± 0.21

*Each value is average of three determinations

*Each value is average of three determinations

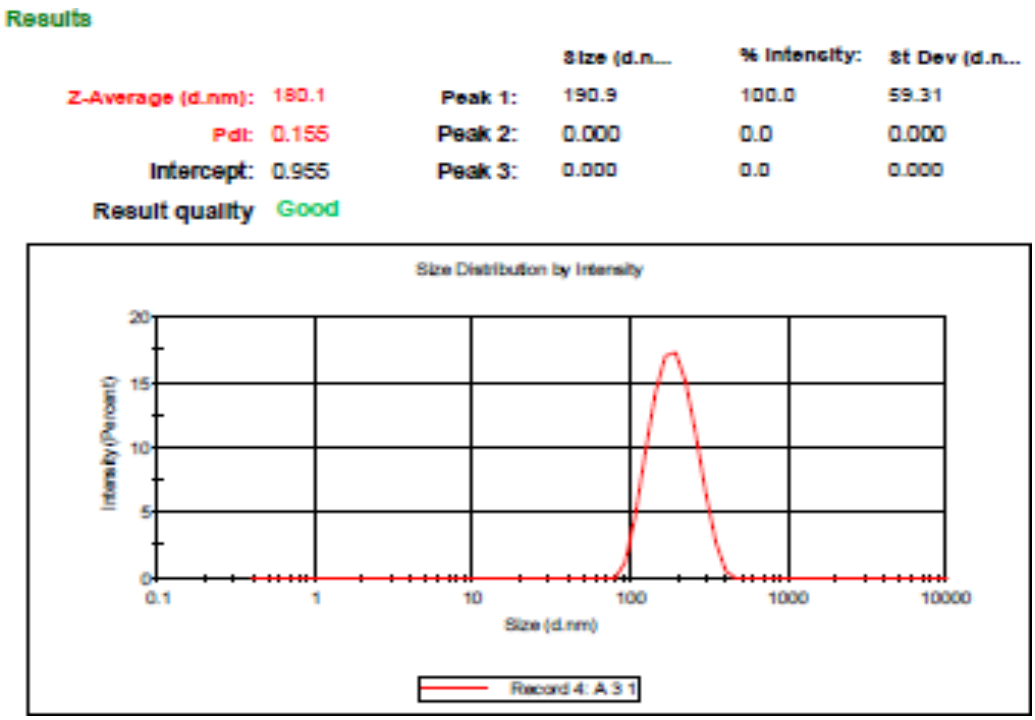

Fig.8.10: Droplet size of formulation F3

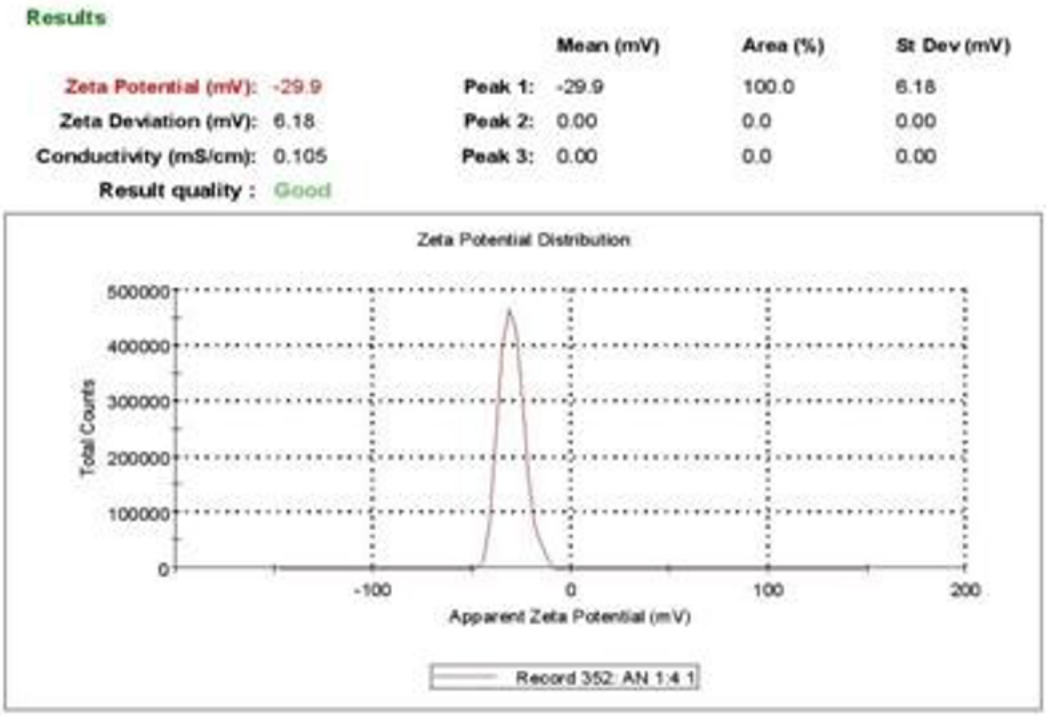

Fig 8.11: Zeta potential of formulation F3

Based on results of dissolution studies, droplet size analysis and zeta potential measurement, formulation F3 was selected as the best formulation and was used for further solidification. The optimized formulation contained 30 % Oleic acid, 56 % Labrasol and 14 % Transcutol.

Percentage transmittance: The optimized formulation F20 was tested for percentage transmittance (reconstitution property). Liquid SMEDDS equivalent to 15 mg of drug was accurately weighed and diluted with distilled water to 100 ml and its % transmittance was measured at 593 nm by UV visible spectrophotometer using distilled water as blank and the value was found to be 98.7.

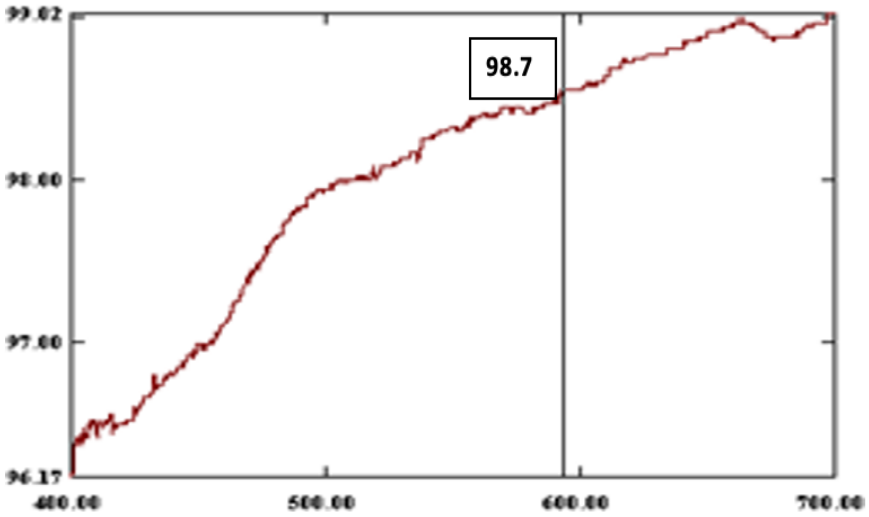

Fig 8.12: Percentage transmittance graph of F3

CONCLUSIONS

Based on solubility data, Oleic acid, Labrasol and Transcutol were selected as oil, surfactant and co- surfactant, respectively, as they solubilized relatively high amount of atorvastatin. Micro- emulsion region was observed in 5 formulations based on ternary phase diagrams. All 5 formulations exhibited emulsification time as less than 10 seconds.

None of these exhibited phase separation and drug precipitation. Thermodynamic stability studies were satisfactory. Robustness to dilution did not exhibit phase separation in resultant micro-emulsion. The *in-vitro* release profile of all formulations showed a significant increase rate of dissolution (more than 80%) when compared with the pure drug (24.58%) in 120 mins.Droplet size was found to be in the range of 180.1 to 1611nm and zeta potential results indicated the range -15.5 to 37.36mV. By considering all the parameters such as droplet size(180.1nm), zeta potential(-29.9 mV) and *in vitro* drug release in 0.1N HCl in 120mins (93.51%), formulation F3 was considered superior and selected for solidification All the solid formulations were subjected to powder flow property studies (angle of repose, Carr's index and Hausner's ratios). Among them, 8 formulations showed excellent to passable flow properties. The *in vitro* drug release of 8 solid formulations was comparable to liquid formulations and was higher than the pure drug. Droplet size of solid SMEDDS ranged from 258.1 to 311.4 nm and zeta potential results indicated the range -21.10 to -34.40mV.Based on flow properties, *in vitro* drug release in 0.1HCl in 120mins (91.07%), droplet size analysis (180.1nm) and zeta potential (-34.40 mV) formulation A2 was selected characterization.DSC thermo gram showed the solubilization of drug in SMEDDS and SEM photograph showed smooth surface of S- SMEDDS with less aggregation indicating the complete adsorption of liquid SMEDDS on solid carrier. FTIR studies showed no interaction between drug and excipients.Thus, the objectives envisaged in this work are achieved. Further, the *in vitro* studies on the developed SMEDDS are needed to be investigated to verify its correlation with *in vitro* release data and to confirm the enhancement of bioavailability.

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