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Self-emulsifying Delivery System in Solubility and Dissolution Enhancement of Cardiovascular Drug

Akiladevi D[*1], Nappinnai M[2], Jerad Suresh A[3], Amudha P[4], Vetrichelvan T[5]

[1]Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319, Tamilnadu, India
[2]Department of Pharmaceutics, Surya Group of Institutions, School of Pharmacy, Vikravandi, Villupuram-605 652, Tamilnadu, India
[3]Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-600 003, Tamilnadu, India
[4]Department of Pharmacology, CL Baid Mehtha College of Pharmacy, Thorapakkam, Chennai, Tamilnadu, India
[5]Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamilnadu, India

[*]**Corresponding author:** Akiladevi D, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319, Tamilnadu, India, E-mail: akilaajcp@gmail.com

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Abstract

Objective: *The objective of the present study was to formulate and develop a self-emulsifying drug delivery system for poorly water soluble cardiovascular drug of atorvastatin calcium (atc) (SEDDS) by improving its solubility and dissolution characteristics, thereby enhancing its relative bioavailability.*

Methods: *Atorvastatin calcium was identified by Fourier transform infrared (FT-IR) spectroscopic study. The SEDDS was prepared using sunflower oil, labrasol and transcutol HP as an oil phase, surfactant, and co-surfactant respectively. Initially, the solubility of atc was examined in different oils, surfactants and co-surfactants, and ternary phase diagrams were constructed subsequently to optimize the ratio of the excipients having greater microemulsion region. The self-emulsifying batches of atc were developed with the optimized excipients and evaluated for droplet size, polydispersity index, drug loading, zeta potential, optical clarity, turbidity, cloud point, viscosity determination, self-emulsification time assessment and in vitro drug release.*

Results: *The in vitro dissolution studies revealed that the optimized formulation of atorvastatin calcium showed more than 90% of drug release within 30 minutes when compared to that of marketed tablet.*

Keywords: *Cardiovascular drug, In vitro drug release, Solubility, Ternary phase diagram, Self-emulsifying system*

Introduction

Oral drug bioavailability of a chemically stable drug is limited by its solubility and its

permeability. The poor drug absorption, therefore, can be caused by inadequate rate and extent of drug dissolution and or low permeation. As per the biopharmaceutical system of classification, a drug is classified into four possible categories, class I to IV on the basis of these solubility and permeability characteristics. The bioavailability of poorly soluble class II drugs, on the contrary, is dependent on their aqueous solubility and dissolution rate [1]. As these drugs tend to exhibit dissolution limited bioavailability, the *in vivo* physiological response is well correlated with the *in vitro* dissolution, resulting eventually in good *in vitro/in vivo* correlations.

Self-Emulsifying Drug Delivery Systems (SEDDS) are promising approaches for enhancing bioavailability of low soluble drugs of biopharmaceutical classification. The main benefit of this approach is that dissolving the compound by overcoming the initial rate-limiting step of dissolution in the aqueous environment within the gastrointestinal tract [2]. SEDDS are isotropic mixtures of oils and surfactants, sometimes containing co-solvents, and it can be used for the design of formulations in order to improve the oral absorption of highly lipophilic compounds. Their dispersion in gastro intestinal (GI) fluid after administration forms micro or nano-emulsified drug which gets easily absorbed through lymphatic pathways by passing the hepatic first pass metabolism. These systems have the advantage that the drug in dissolved form and the small droplet size provides a large interfacial area for the drug absorption [3]. SEDDS typically produce emulsions with a droplet size between few nanometers (100–300 nm) to several microns (<5 µm) while self-micro-emulsifying drug delivery systems (SMEDDSs) form transparent micro-emulsions with a droplet size of less than 50 nm. SEDDS are physically stable formulations that are easy to manufacture, but when compared with emulsions, which are metastable dispersed forms. Thus, for lipophilic drug compounds that exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profiles [4]. The potential drug candidates for the formulation of SEDDS are BCS class II and class IV drugs. Atorvastatin calcium a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor is a plasma lipid regulating agent. The oral bioavailability of atorvastatin calcium is only 14% and poor bioavailability has been attributed to its poor solubility in water and high presystemic clearance (>80%). Atorvastatin calcium a BCS class II drug of log p (octanol /water) of 5.39 was selected for the formulation of SEDDS owing due to poor water solubility and poor permeability.

In the present research work for development of SEDDS it was planned to use a variety of modified long chain triglycerides natural oils (corn oil, olive oil, sesame oil, sunflower oil) long chain monoglycerides (Peceol) and medium chain triglycerides (coconut oil) with varying degree of saturation of hydrolysis because these oils offer distinct formulation and physiological advantages as their degradation products resemble that of natural end products of intestinal digestion [5]. The long chain triglyceride offers many other advantages such as easy availability in large quantities from a natural source, toxicologically safe, completely biocompatible and cost-effective replacement for commercial triglycerides and modified oils. In a comparative research study of Cornaire G *et al.* in the evaluation of P-gp inhibition of ten excipients labrasol was identified as the most effective excipient [6] and it improved the transcellular transport of BCS class II drugs through intestinal cell membranes. The excipients containing some esters of unsaturated fatty acid proved the enhancement of bioavailability of a poorly water soluble drug of ontazolast with peceol [7] through the lymphatic transport system. The newer cosurfactant transcutol HP was utilized in this research work for its better stability and less volatility.

Initially, solubility studies of atc in different excipients were performed, and ternary phase diagrams were constructed to obtain optimum emulsification region. The SEDDS were formulated with the optimized ratios of excipients and evaluated for droplet size, drug loading, zeta potential, polydispersity index optical clarity, turbidity, cloud point, viscosity determination, self-emulsification time assessment and *in vitro* drug release in and phosphate buffer (pH 6.8).

Materials and Methods

Materials

Atorvastatin calcium was generously gifted by Goodman Pharmaceuticals, Pondicherry. Capryol PGMC transcutol HP, peceol, labrasol, labrafil M 1944 CS and labrafil M 2125 CS were gift samples from Gattefosse, Mumbai. Virgin sesame oil, virgin coconut oil and sunflower oil from Vama oil industries, Coimbatore, Olive oil from Shaah Enterprises, Chennai, Mustard oil from Green spice products, Coimbatore, Rice bran oil from Jupiter Manufacturing industry, Chennai, Corn oil from Arumuga group of industries, Tamilnadu were purchased for the research work. All other chemicals were of analytical grade.

Fourier Transform Infrared (FT-IR) Spectroscopy of Atorvastatin Calcium

The drug sample was mixed with anhydrous potassium bromide (KBr) in 1:4 ratios. Briefly about 100 mg of this mixture was made into fine powder using mortar and pestle followed by compression to form transparent KBr pellet using Techno search hydraulic press set at 15-ton pressure. Each KBr pellet was scanned at 4mm/s at a resolution of 2 cm over a wave number region from 4000 to 400 cm^{-1} in an FTIR spectrophotometer (8400S Shimadzu, Japan).

Solubility Studies

The solubility for atorvastatin calcium were determined in aqueous solutions of various pH (pH 4 and 7.4), distilled water, organic solvents such as dimethylsulphoxide and dimethylformamide. Aqueous solution of pH 4.0 and 7.4 was obtained by adding suitable amount of dilute hydrochloric acid and dilute sodium hydroxide. About 2 ml of each solvent was transferred into 5 ml glass vial and an excess quantity of drug (150 mg) was added to the vial. The solubility of the drug samples was also analyzed by adding excess amount (150 mg) of the drug to 2 ml of various oils, surfactants, and co-surfactants in screw capped glass vials followed by vortex mixing for 30 sec using vortex mixer (Sphinx, Japan). The mixtures were shaken for 48 h at 30^0C in a thermostatically controlled shaking water bath, followed by equilibrium for 24 hr. The sample mixtures were then centrifuged at 3000 rpm [8] for 10 min and the supernatant liquid was filtered through a millipore membrane filter (0.45 μ). Samples were suitably diluted with methanol followed by sonication for 10 min and finally diluted with the same solvent. The final drug concentration was quantified by UV-visible spectrophotometer at 247 nm for atorvastatin calcium. The experiment was repeated in triplicates. The results are represented as mean value (mg/ml) ± SD.

Construction of Ternary Phase Diagram

Based on the results of saturation solubility studies in Table 1, sunflower oil, labrasol and transcutol HP for atorvastatin calcium were selected as oil, surfactant and co-surfactant respectively. A ternary phase diagram was constructed for the system containing oil-surfactant-co-surfactant by Chemix School software version 3.51. The grading method reported by Craig et al. [9] was modified and adopted in this study. A series of self-emulsifying systems were prepared with varying weight percentage of oil, surfactant, and co-surfactant. Since the drug incorporated in the SEDDS may have some effect on self-emulsion boundary, every system in the series also consisted of 10% w/w for atorvastatin calcium. The extreme and middle level of the independent variables consisting of oil, surfactant and co-surfactant were selected for further study. 0.2 ml of each formulation was introduced into 200 ml of water in a glass beaker maintained at 37°C and was mixed gently about 200 rpm with a magnetic stir bar. The tendency to emulsify spontaneously and the progress of emulsion droplets spread were observed. The tendency to form an emulsion was judged as ‘good’ when droplets spread easily in water and formed a fine milky or slightly bluish emulsion within 1 min. It was judged ‘bad’ when there was poor, slow or no emulsion formation or when oil droplets coalesce when stirring was stopped or when dull, grayish white emulsion was formed. All studies were repeated thrice.

Preparation of SEDDS

Optimum ratios of oil and Smix (surfactant and cosurfactant mixture) were

selected from the phase diagrams. SEDDS formulations were prepared by dissolving the drug in Smix mixtures along with gentle vortexing and sonicating and then by adding oil [10]. Nine different batches were prepared with each batch of SEDDS formulation containing single dose of atorvastatin with varying amounts of oil and Smix of nine formulations as illustrated in Table 2. Then the final formulation was equilibrated in water bath at 37°C for 48 h before carrying out the droplet size, polydispersity index and dissolution.

Self-emulsification and Drug Precipitation Studies

The efficiency of self-emulsification of oral micro/nano-emulsion is assessed by dispersibility test using a standard USP dissolution apparatus II [11]. One ml of each formulation is added to 500 ml of water at 37 ± 0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm tends to provide gentle agitation. The *in vitro* performance of the formulations is visually assessed from such dispersion, using a suitable grading system after 24 h. The *in vitro* performance of the formulations is visually assessed using the following grading system:

Grade I: Rapidly forming (within 1 min) nano emulsion, having a clear or bluish appearance. (Micro emulsion)

Grade II: Rapidly forming, slightly less clear emulsion, having a bluish white appearance. (Micro emulsion gel)

Grade III: Fine milky emulsion that formed within 2 min. (Emulsion)

Grade IV: A dull greyish white emulsion having slightly oily appearance that is slow to emulsify is formed (longer than 2 min). (Emulgel)

Grade V: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface with phase separation is observed.

Grade VI: The drug is precipitated.

Grade I and Grade II formulation will remain as nano-emulsion when dispersed in GIT. The formulation falling in Grade III is recommended for SEDDS formulation. The primary means of self-emulsification is a visual evaluation. The efficiency of self-emulsification could be estimated by determining the rate of emulsification, droplet size distribution, and turbidity measurements.

Phase Separation Study

The self-emulsifying formulation was diluted with distilled water up to 5 times and the temperature was maintained at 25°C. The mixture was then mixed for 2 min, stored for about 2 h and visually observed for any phase separation.

Determination of Emulsification Time

The emulsification time (the time for a preconcentrate to form a homogeneous mixture upon dilution) was monitored by visually observing the disappearance of SEDDS and the final appearance of the emulsion in triplicate. A dissolution apparatus USP II (Electrolab) was employed with 500 ml water and with a paddle speed of 50 rpm at 37°C. The SEDDS (1 ml) was added drop wise to the medium by dropping the pipette and time required for the disappearance of SEDDS was recorded.

Spectroscopic Characterization of Optical Clarity

SEDDS formulations disperse in aqueous phase forming the emulsion or micro emulsions and can be detected by the final appearance and droplet size. In practice, the key difference between the emulsion and micro emulsions concerns with their appearance. Emulsions are cloudy while micro emulsions are clear or translucent and the reason for their transparency appearance is due to very small droplet size. The optical clarity may be checked visually. But in order to measure it quantitatively, a UV-visible spectrophotometer was used to measure the amount of light of a given wavelength absorbed by the solution. The cloudier solutions will absorb more of the incident light, resulting in higher absorbance values and lower absorbance is obtained with optically clear solutions.

The optical clarity of aqueous dispersions of SEDDS formulations was measured spectroscopically. About l ml of SEDDS formulations were diluted to 50 times with double distilled water. The absorbance values of each formulation were measured by a UV-visible spectrophotometer (Shimadzu) at 400 nm [12].

Turbidity Measurement

The measurement of turbidity is to analyse whether the dispersion reaches equilibrium rapidly and in a reproducible time. The growth of emulsification is done by nepheloturbidimetric evaluation. The turbidity measurements in nephelometric turbidity unit (NTU) were performed on the resultant emulsion stored in a screw capped sample vials using a turbid meter (Elico D-10-model 331). 0.5 ml of the SEDDS formulation was introduced into 250 ml of distilled water in 500 ml conical flask under an action of magnetic stirrer rotating at constant speed. The emulsification was done at room temperature [13].

Viscosity Determination

The viscosity studies are necessary for SEDDS to characterize the system physically and to control its stability. If the system has low viscosity then, it is o/w type of the system and if a high viscosity then it is w/o type of the system. SEDDS preconcentrate (10 ml) was taken and its viscosity was measured by using Brookfield viscometer (Brookfield engineering Laboratories, USA) using spindle C 16-1 at 25 ± 0.5 ˚C with a shear rate of 50 rpm.

Cloud Point Measurement

Cloud point temperatures (Tc) was determined by visual observation. 0.5 ml of preconcentrate was diluted to 50 ml with distilling water in a glass beaker. The sample was heated at the rate of about 0.5°C/min. A close observation was made at the appearance of the dispersion with the increase in temperature. The temperature at which the dispersion became turbid was taken as Tc. After the temperature exceeds the cloud point, the sample was cooled below Tc, and then it was heated again to check the reproducibility of the measurements. It mainly insists about the stability of micro emulsion at body temperature.

Determination of Refractive Index

The refractive index, n, of a medium is defined as the ratio of the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium represented by n=c/vp. It was determined using an Abbes type refractometer. The clarity of micro emulsion could be estimated by measuring the refractive index of the formulations. The SEDDS formulations were diluted 100 times with water. The refractive index of the system was measured by an Abbe refractometer by placing 1 drop of solution on the slide and it compares with distilled water.

Droplet Size and Polydispersity Index (PDI) Analysis

The droplet size of the micro/nano emulsions is determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to a Brownian motion of the particles) using a Zetasizer which can measure sizes between 10 and 5000 nm. PDI is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PDI value the more homogenous are the particles. The mean droplet size and polydispersity index of formulations were determined by using Malvern Nano Zeta sizer-90. The resultant SEDDS 0.5 ml, was diluted to 100 ml with double distilled water. The samples were loaded into a cuvette placed in a thermostatic chamber and light scattering was monitored at 25^{o}C at a 90^{o} angle [14] after external standardization with spherical polystyrene beads.

Each determination was done in triplicate. The nanometric size range of the particle is retained even after 100 times dilution with water which proves the systems compatibility with excess water.

Zeta Potential Measurement

The zeta potential of prepared SEDDS formulations was determined using a Zeta sizer ZS 90 (Malvern Instruments UK) by using laser Doppler micro-electrophoresis. A suitable amount of the sample (50-100 µl) was diluted with 5 ml of distilled water and after sonicating in a bath sonicator to achieve a homogeneous state. Measurements were carried out at 25ºC using disposable polystyrene cuvette with a zeta dip cell. All the measurements were performed in triplicate and the data presented is mean ± SD.

Drug Loading Efficiency [15]

The drug efficiency was done to investigate the effect of drugs on a self-emulsifying performance of SEDDS. Approximately 10 mg of atorvastatin calcium was added to 1 ml of boundary formulations of SEDDS and checked for a formation of the clear solution.

Prototype Formulation for Atorvastatin Calcium

Prototype formulations of atorvastatin calcium were prepared by varying sunflower oil in 3:1 ratios of the mixture of labrasol and transcutol HP as per the formula composition mentioned in Table 2. In the first trial, the oil was used at 40% and increased up to 80%. The ratio of surfactant to co-surfactant was maintained at 3:1. Then drug of one dose equivalent of 10 mg atorvastatin calcium was added and stirred for 15 min. The mixture was heated to 30-40°C till the drug was solubilized. The drug loading capacity of each mixture was determined by adding the excess of atorvastatin calcium to each prototype mixture till the clear solution was obtained. The solution was filtered.

The drug content of the SEDDS formulation was determined by diluting the solution in methanol and the volume was made up to 10 ml with methanol (1mg/ml). From the above stock solution, 0.2 ml (200µg/ml) was withdrawn and diluted up to 10 ml with methanol (20 µg /ml). From the above solution 0.2ml (20 µg/ml) diluted up to 10 ml with methanol (2µg/ml) Samples were prepared in triplicate and absorbance was measured at 247 nm using UV-visible Spectrophotometer (Shimadzu UV-1700) using methanol as a reference solution.

Drug loading efficiency was calculated by equation:

Drug loading efficiency = Amount of drug in known amount of formulation X 100 / Initial drug load

***In Vitro* Dissolution Studies for Atorvastatin Calcium**

The *in vitro* studies were performed to find out the dissolution rate of SEDDS. The *in vitro* drug release [16] profiles of optimized atorvastatin of SEDDS, API atorvastatin calcium and marketed atorvastatin calcium tablet (Storvas 10 mg Ranbaxy Laboratories Ltd) were carried out using USP type II dissolution test apparatus (Electrolab) in 900 ml of Phosphate buffer (pH 6.8). The temperature was maintained at 37 ± 0.5°C and the speed of the paddle was set at 100 rpm. About 120 mg of each optimized SEDDS formulation (AF4) were filled into soft gelatin capsules (size '3') and used for dissolution studies. The capsules were held to the bottom of the vessel using copper sinkers. At predetermined time intervals of 5, 10, 20, 30, 40, 50, 60, 75 and 90 min, an aliquot (5ml) of a sample were collected and filtered through the membrane filter (0.45 µm, Whatman). The withdrawn samples were diluted suitably with phosphate buffer (pH 6.8) and analyzed for the drug content by standard calibration curve method by UV-visible spectrophotometer (Shimadzu UV-1700) at 247 nm. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain the sink condition. The dissolution profile of the atorvastatin calcium (API) and marketed tablet (Storvas 10 mg) were assessed by the same method.

Discussion

FT-IR Studies for Atorvastatin Calcium

From Figure 1 it was illustrated that the IR spectrum of atorvastatin calcium showed the characteristic peaks of aromatic N-H stretching at 3364.93 cm^{-1} and the asymmetric stretching of C=O of amide group at 1651.12 cm^{1}. However, similar peaks of symmetric C=O stretching were observed at 1579.75 cm^{-1} and O-H stretching at 3566.50 cm^{-1}. The characteristic peaks were observed at the wave numbers 1510.31 cm^{-1} and 1424.48 cm^{-1} due to the C=C ring stretching. The peak found at 1317.43 cm^{-1} was due to CH_3/CH_3 deformation bending vibration at the plane. The two characteristic bands were observed at 3735.28 cm^{-1} and 3055.35 cm^{-1} due the O-H stretching associated with the hydrogen bond. From the above study, it was inferred that the drug sample was identified as atorvastatin calcium.

Solubility Study

The atorvastatin calcium was found to have maximum solubilizing capacity in sunflower oil which is a long chain triglyceride, capable of solubilizing the drug in a specific amount facilitate self-emulsification and increase the fraction of atorvastatin calcium through the intestinal lymphatic system. The labrasol which showed the highest solubilization capacity was selected as surfactant followed by capryol PGMC, labrafil 1944 CS, Labrafil 2125 for formulation of atorvastatin calcium SEDDS as shown in Table 1. Transcutol HP showed maximum solubility for atorvastatin calcium and it was selected as the cosurfactant for the formulation of SEDDS.

Construction of Phase Diagram

As observed from the ternary plot in Figure 2 sunflower oil gave a wider micro emulsion region at 3:1 Smix ratio for atorvastatin calcium. However, it was observed that increasing the surfactant ratio resulted in a loss of flowability and increase in surfactant toxicity. The percentage of oil, surfactant and cosurfactant selected for both the drugs were selected from the phase diagram and only those formulations which used the minimum and maximum concentration of Smix were taken for the formulation of SEDDS. Based on the feasibility of micro emulsion formation at extreme values, the range for each component was selected as follows: oil (40-80%), Smix (30-70%) for atorvastatin calcium.

Self-emulsification, Drug Precipitation, Phase Separation and Assessment of Emulsification Time Studies

The self-emulsification was visually assessed to measure the apparent spontaneity of nano-emulsion formation. SEDDS when diluted in water were found to be non-turbid and bluish transparent in appearance indicating spontaneous emulsification. All the resulting nanoemulsions were transparent with some opalescence in appearance and did not show any sign of phase separation. In the study formulations AF4 does not show any signs drug/excipient precipitation or phase separation were found and the results are shown in Table 3.

All the results of the nano-emulsion formulations were transparent and their absorbance's were below 1 which showed good optical clarity as illustrated in Table 4. The selection of surfactants and cosurfactants are determined by emulsification ability which depends on the physicochemical properties such as globule size, Zeta potential, turbidity measurement and PDI of the resulting nanoemulsion. All the formulations of atorvastatin calcium showed rapid emulsification time within a minute as indicated in Table 4 which proves the performance of the formulations for enhancing the dissolution profile. Thus, it can be concluded that the absorption of the drug can be increased *in vivo* if the formulations have low emulsification time. The results are correlated with the findings of Warisnoicharoen et al. [17] which concluded that emulsification is also influenced by the structure and chain length of the surfactant. Labrasol a hydrophilic surfactant having HLB value of 12 rendered very good nanoemulsions that required a short emulsification period.

Turbidity Measurements

The turbidity of SEDDS was performed determined as per procedure and turbidity of AF4 was found to below 100NTU which shows the stability of SEDDS and the results were shown in Table 4.

In the formulation AF3 of atorvastatin calcium the turbidity value was high of 210 NTU due to the larger droplet size of the emulsion formed of 290 nm which was shown in Table 4.

Refractive Index and Viscosity Measurement

There was no significant difference in the refractive index values of the formulations tested. The refractive index values close to that of the water (1.333) prove the isotropicity of the formulations as indicated in Table 4.

Droplet Size

The globule size observed for all the formulation was less than 500 nm which were shown in Table 2. The drug loading did not show significant difference in the polydispersity values. The droplet size distribution is one of the most important characteristics of nano-emulsion for stability

evaluation and is a critical step in the pathway of enhancing drug bioavailability. The smaller nano-emulsion particle size leads to larger interfacial surface area, thus promoting rapid absorption and improved bioavailability.

Zeta Potential

The zeta potential of the optimized formulation of AF4 of atorvastatin calcium was found to be -31.8 mV which were nearer the limits with good separation. The zeta potential value was found to carry negative charges due to the presence of free fatty acids. Significant increase in the value of zeta potential was observed after drug loading, higher absolute values of zeta potential generally, indicated an increase of electrostatic repulsive forces between emulsion droplets preventing the coalescence droplets and increases in the stability. Among all the vehicles tested Labrasol (surfactant) and Transcutol HP (co-surfactant) proved to be the most promising vehicles for SEDDS formulation.

Polydispersity Index

The PDI for all the formulations were less than 0.5, formulation with combination of Smix showed lower PDI values as illustrated with the results given in Table 4 thus indicating the uniform size distribution improving the performance of the spontaneous emulsification.

Cloud Point

The cloud point is an essential factor in the SEDDS consisting of non-ionic surfactants, and it is responsible for the successful formation of a stable microemulsion. When the temperature is higher than the cloud point, an irreversible phase separation will occur and the cloudiness of the preparation would have a bad effect on drug absorption, because of the dehydration of the polyethylene oxide moiety. Hence, the cloud point for SMEDDS should be above 37°C, which will avoid phase separation occurring in the gastrointestinal tract. The cloud point for all the formulation as shown in Table 4 tested was above 37°C. Therefore, it would suggest a stable micro emulsion can be formed at physiological temperature *in-vivo*.

Drug Loading

The drug loading was calculated from the standard calibration curve of atorvastatin calcium in methanol using the linear regression equation y=0.045x+0.003 with the correlation co efficient (r^2) of 0.999. The maximum drug loading for atorvastatin calcium SEDDS has been obtained ranging from 7.1 mg to 9.15 mg. The maximum drug loading of 8.72 mg for atorvastatin calcium can be incorporated safely in to the optimized formulation of AF4.

***In Vitro* Dissolution Study**

The drug concentration was calculated from the linear regression equation for atorvastatin calcium in phosphate buffer pH 6.8 is y=0.012x+0.001 with the correlation co efficient of 0.999. The dissolution study for atorvastatin calcium API, marketed formulation and optimized formulation of AF4 were performed in 6.8 pH phosphate buffer. The comparison results in Figure 3 and Table 5 depicted that the optimized formulation AF4 showed more than 90% drug release in 30 min of atorvastatin from SEDDS formulations. The rapid release of atorvastatin from SMEDDS formulations could be attributed to the spontaneous formation of micro emulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain atorvastatin. Thus, this greater availability of dissolved atorvastatin from the SEDDS formulation could lead to higher absorption and higher oral bioavailability.

Conclusion

The optimal atorvastatin calcium SEDDS containing sunflower oil as oil phase, labrasol as a surfactant and transcutol HP as cosurfactant (Smix) formulates SEDDS with lower droplet size (169.7 nm) and percentage drug load (87.2%) values. The *in vitro* drug release from optimized atorvastatin SEDDS formulation were found to be 99.75% after 90 min was higher in comparison to the marketed formulation and API suspension. It could be concluded that increase in solubility profile of atorvastatin calcium by formulating through SEDDS as a potential carrier could be employed successfully as an alternative approach for improved dissolution profile for atorvastatin calcium.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1: Solubility of Atorvastatin calcium in various excipients

S.No.	Excipients	Solubility (mg/ml)
1.	Virgin sesame oil	15.36 ± 0.006
2.	Virgin coconut oil	25.37 ± 0.015
3.	Sunflower oil	30.13 ± 0.02
4.	Corn oil	4.86 ± 0.030
5.	Mustard oil	10.35 ± 0.01
6.	Rice bran oil	12.29 ± 0.040
7.	Olive oil	17.62 ± 0.010
8.	Peceol	12.84 ± 0.021
9.	Labrasol	89.23 ± 0.015
10.	Labrafil 1944CS	1.78 ± 0.011
11.	Labrafil 2125	1.62 ± 0.012
12	Capryol PGMC	2.22 ± 0.006
13.	Transcutol HP	38.62 ± 0.28
	Solvents	
14.	Distilled water	0.0096 ± 0.012
15..	Methanol	0.666 ± 0.002
16.	pH Phosphate buffer 7.4	0.0095 ± 0.013
17.	Acetonitrile	0.0092 ± 0.003
18.	Ethanol	0.0089 ± 0.014
19.	Dimethyl sulphoxide	0.0793 ± 0.022
20.	Dimethyl formamide	0.0757 ± 0.003
21.	Aqueous solution of pH 4	0.02 ± 0.005

** Values are mean ± SD (n=3)*

Table 2: Composition of various formulations of atorvastatin calcium

Formulation Code (FC)	Oil (mg)	Smix (mg)	Particle size (nm)	Drug Loading (%)
AF1	40	70	106.8 ± 4.08	81.8 ± 6.63
AF2	40	50	172 ± 7.5	83.1 ± 4.54
AF3	80	50	290 ± 4.9	91.5 ± 2.78
AF4*	60	50	169.7 ± 3.23	87.2 ± 1.23
AF5	40	30	415 ± 8.7	70.1 ± 2.25
AF6	80	70	285 ± 8.6	87.6 ± 1.65

AF7	80	30	229.7 ± 4.98	89.1 ± 4.53
AF8	60	30	197.6 ± 5.65	75.1 ± 2.75
AF9	60	70	233.1 ± 3.44	86.1 ± 4.37

Table 3: Self-emulsifying and drug precipitation of atorvastatin calcium SEDDS

Formulation Code	Visibility grade	Phase separation	Precipitation
AF1	IV	+	++
AF2	III	+	++
AF3	IV	+	++
AF4*	I	X	XX
AF5	II	X	XX
AF6	III	+	++
AF7	IV	X	++
AF8	V	+	++
AF9	III	+	++

X- No phase separation, XX-No precipitation, +-phase separation and ++-precipitation

Table 4: Refractive index, Turbidity, Optical clarity, Polydispersity Index, Viscosity, Cloud point measurement and Emulsification time of SEDDS formulations of atorvastatin calcium

FC	Refractive Index ± SD (n=3)	Turbidity (NTU)	Absorbance	Polydispersity Index ± SD (n=3)	Viscosity (cps) ± SD (n=3)	Cloud point measurement (°C) ± SD (n=3)	Emulsification time (sec)
AF1	1.3343 ± 0.0006	132	0.402	0.171 ± 0.01	253±2.65	78±3.46	132
AF2	1.3352 ± 0.0003	146	0.487	0.244 ± 0.005	262 ± 2.66	73 ± 3.61	119
AF3	1.3366 ± 0.0005	210	0.529	1.097 ± 0.2	264 ± 1.73	75 ± 5.57	121
AF4*	1.3331 ± 0.0002	90	0.455	0.381 ± 0.03	280 ± 2.31	77 ± 3.46	138
AF5	1.3334 ± 0.0002	94	0.432	0.377 ± 0.06	291 ± 3.51	74±3.46	126

AF6	1.3345 ± 0.0003	168	0.517	0.148 ± 0.012	272 ± 4.58	78 ± 5.20	112
AF7	1.3363 ± 0.0006	320	0.456	0.379 ± 0.06	269 ± 2.89	75 ± 3.61	95
AF8	1.3358 ± 0.0004	357	0.493	0.292 ± 0.03	254 ± 2.66	75 ± 4.36	82
AF9	1.3349 ± 0.0004	92	0.501	0.128 ± 0.04	249 ± 2.08	79 ± 4.58	75

Table 5: Cumulative percent release of atorvastatin calcium from various formulations

Time in min	AF4 SEDDS	API	Marketed Tablet
0	0	0	0
5	26.21 ± 0.74	38.69 ± 1.24	33.21 ± 2.03
10	39.3 ± 0.23	47.56 ± 0.75	45.23 ± 1.12
20	58.36 ± 0.45	65.22 ± 1.12	60.33 ± 2.21
30	72.66 ± 0.32	80.45 ± 1.23	79.54 ± 1.64
40	79.5 ± 0.18	86.23 ± 1.56	85.62 ± 0.54
50	86.72 ± 0.16	89.21 ± 2.73	86.74 ± 2.21
60	91.3 ± 0.55	92.34 ± 1.23	90.69 ± 1.72
75	94.5 ± 0.49	93.86 ± 0.62	92.66 ± 1.54
90	99.75 ± 0.31	95.64 ± 1.26	93.31 ± 1.18

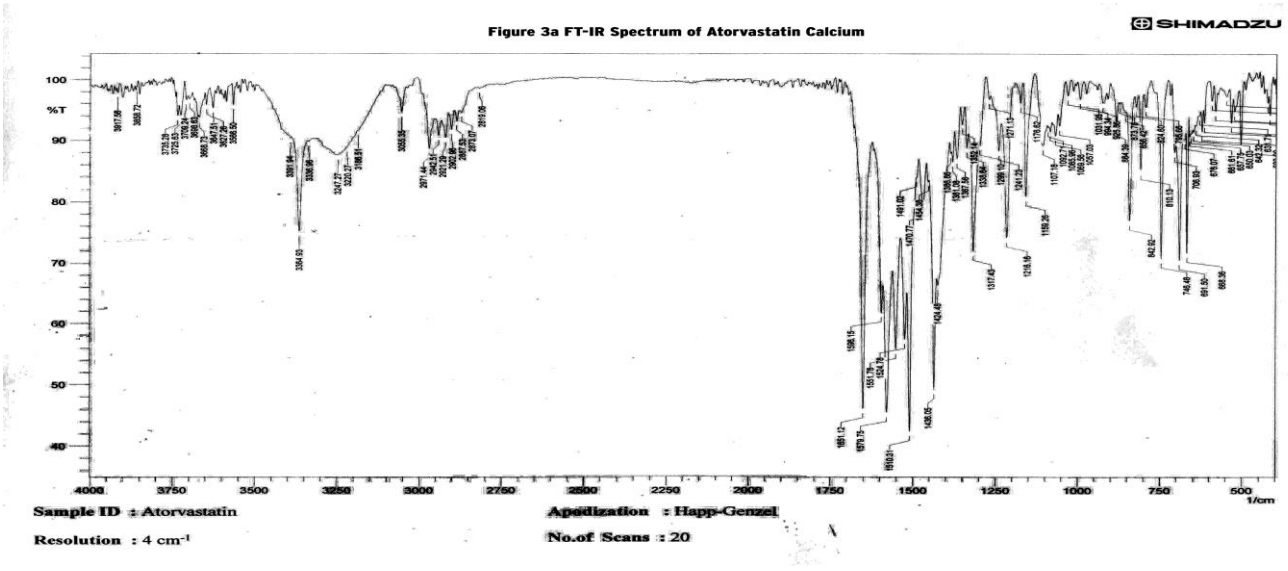

Figure 1: FT-IR spectrum of atorvastatin calcium

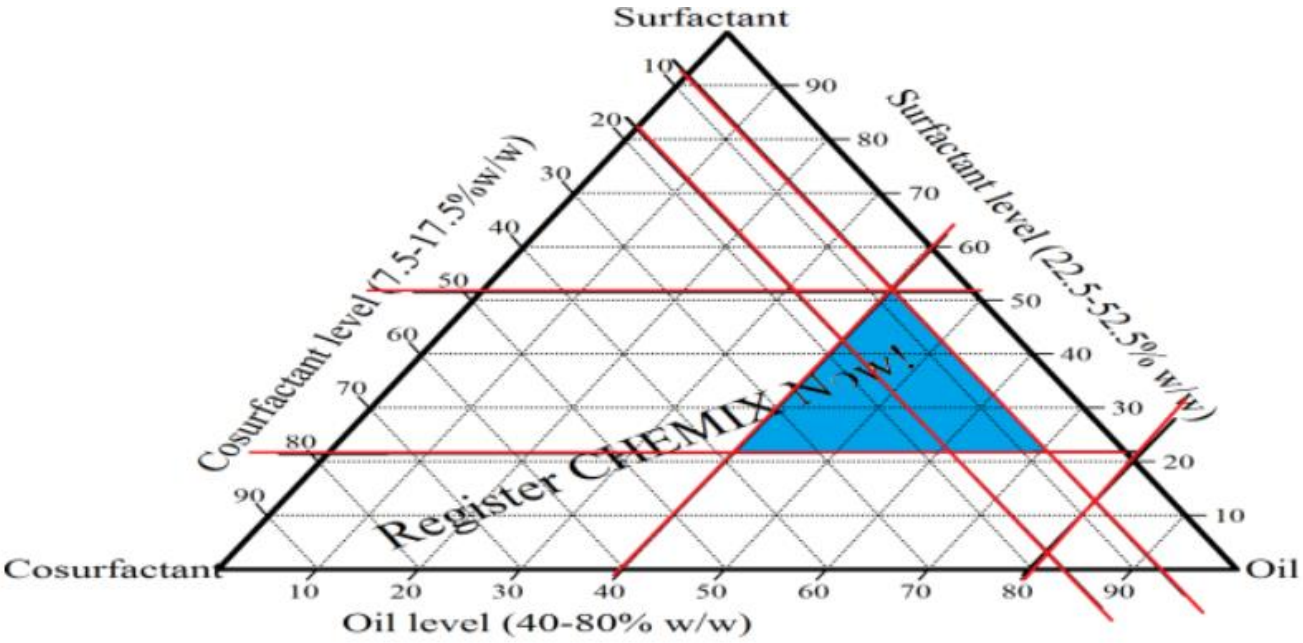

Figure 2: Ternary phase diagram of atorvastatin calcium SEDDS

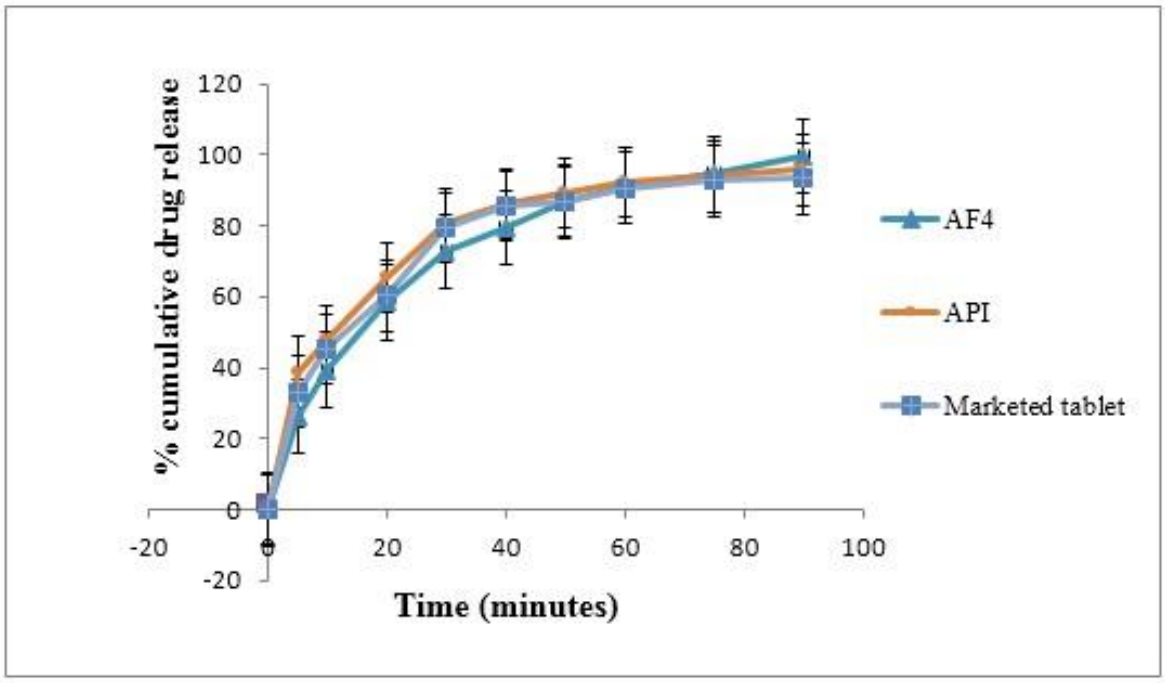

Figure 3: Dissolution comparison graph of API, marketed and optimized formulation AF4 of atorvastatin calcium SEDDS

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