FORMULATION AND EVALUATION OF A LIPID BASED DRUG DELIVERY SYSTEM FOR THE DELIVERY OF POORLY WATER SOLUBLE DRUG

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ABSTRACT
Poorly water-soluble drug candidates are becoming more prevalent. It has been estimated that approximately 60–70% of the drug molecules are insufficiently soluble in aqueous media and/or have very low permeability to allow for their adequate and reproducible absorption from the gastrointestinal tract (GIT) following oral administration. Formulation scientists have to adopt various strategies to enhance their absorption. One of the most popular approaches to combat these challenges is the utilization of lipid-based drug delivery systems (such as nanoemulsion, microemulsion, self-emulsifying drug delivery system, micellar system etc.). Their use in product development is growing due to the versatility of pharmaceutical lipid excipient and drug formulations, and their compatibility with liquid, semi-solid, and solid dosage forms. The aim of the present investigation was to formulate a self-emulsifying drug delivery system (SEDDS) of poorly water soluble drug Atorvastatin calcium to improve its solubility. Labrafil PG, Tween 80, and Transcutol were selected as oil, surfactant and co-surfactant, respectively for the development of liquid self-emulsifying system. Based on preliminary evaluation, the best 27 self-emulsifying region of different compositions were identified. Pseudo ternary phase diagrams were constructed to identify efficient self-emulsification region using oils, surfactants, and co-surfactants in aqueous environment. The pseudo ternary diagram of Smix at 2:1 showed the maximum emulsifying region. Thus, it was selected for the further study. Out of the nine formulations, best five combinations of oil and Smix were selected based on the physical appearance and % transmittance and drug was loaded in them and evaluated for their physicochemical parameters and dissolution profile (in vitro) in comparison to the marketed tablet. Optimal formulations containing 2:1 mixture of Tween-80/ Transcutol showed maximum transmittance, a minimum droplet size and higher in vitro drug release. This work provides an overview of the SEDDS of Atorvastatin calcium as a promising alternative to improve solubility and dissolution release profile. The results suggested that the SEDDS could be used as an effective oral dosage form to improve the oral delivery of poorly water soluble drug.

KEYWORDS: Atorvastatin calcium, self-emulsifying drug delivery system.

INTRODUCTION

LIPID BASED DRUG DELIVERY SYSTEM

In these modern days, many significant efforts have been applied to use the potentials of lipid-based drug delivery systems, as it provides the suitable means of site specific as well as time controlled delivery of drugs with different molecular weight, either small or large, and also the
bioactive agents [1, 2]. Poorly water-soluble drugs are challenging for the formulation scientists with regard to solubility and bioavailability. Lipid-based drug delivery systems (LBDDS) have shown the effective size dependent properties so they have attracted a lot of attention. Also LBDDS have taken the lead because of obvious advantages of higher degree of biocompatibility and versatility. These systems are commercially viable to formulate pharmaceuticals for topical, oral, pulmonary, or parenteral delivery. Lipid formulations can be modified in various ways to meet a wide range of product requirements as per the disease condition, route of administration, and also cost product stability, toxicity, and efficacy. Lipid-based carriers are safe and efficient hence they have been proved to be attractive candidates for the formulation of pharmaceuticals, as well as vaccines, diagnostics, and nutraceuticals [3]. Hence, lipid-based drug delivery (LBDD) systems have gained much importance in the recent years due to their ability to improve the solubility and bioavailability of drugs with poor water solubility.

**General Routes of LBDDS**

Routes like oral, parenteral, ocular, intranasal, dermal/transdermal, and vaginal can be for the administration of the lipid based drug delivery systems (LBDDS) [4, 5]. However, oral route is the most preferred route because of the properties like noninvasiveness, less expensive, and less prone to side effects, such as injection-site reactions. It is also considered as the easiest and the most convenient method of drug delivery for chronic therapies. But, at a very early stage of development, formulation strategies based on a rational and systematic approach need to be developed to avoid erratic and poor *In vitro/In vivo* correlations and thus increase the chances of success in formulation development.

**Lipid Formulation Classification System**

The lipid formulation classification system (LFC) was introduced as a working model in 2000 an formulation was added in 2006. In recent years the LFCs have been discussed more widely within the pharmaceutical industry to seek a consensus which can be adopted as a framework for comparing the performance of lipid-based formulations. The main purpose of the LFCs is to enable in vivo studies to be interpreted more readily and subsequently to facilitate the identification of the most appropriate formulations for specific drugs [6–9].
Table 1: The lipid formulation classification system: characteristic features, advantages, and disadvantages of the four essential types of “lipid” formulations.

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Materials</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Oils without surfactants (e.g., tri-, di-, and monoglycerides)</td>
<td>Non dispersing requires Digestion</td>
<td>Generally recognized as safe (GRAS) status; simple; and excellent capsule compatibility</td>
<td>Formulation has poor solvent capacity unless drug is highly lipophilic</td>
</tr>
<tr>
<td>Type II</td>
<td>Oils and water insoluble Surfactants</td>
<td>SEDDS formed without water-soluble components</td>
<td>Unlikely to lose solvent capacity on dispersion</td>
<td>Turbid o/w dispersion (particle size 0.25–2 ( \mu \text{m} ))</td>
</tr>
<tr>
<td>Type III</td>
<td>Oils, surfactants, and Co solvents (both water-insoluble and water-soluble excipient)</td>
<td>SEDDS/SMEDDS formed with water-soluble components</td>
<td>Clear or almost clear dispersion, drug absorption without digestion</td>
<td>Possible loss of solvent capacity on dispersion, less easily digested</td>
</tr>
<tr>
<td>Type IV</td>
<td>Water-soluble surfactants and Co solvents</td>
<td>Formulation disperses typically to form a micellar solution</td>
<td>Formulation has good solvent capacity for many drugs</td>
<td>Likely loss of solvent capacity on dispersion may not be digestible</td>
</tr>
</tbody>
</table>

Points to be considered for the Formulation

Main factors affecting the choice of excipient for lipid-based formulations are as follows:

(i) Solubility,
(ii) Dispersion,
(iii) Digestion,
(iv) Absorption.

Other factors are as follows:

(i) Regulatory issues-irritancy, toxicity, knowledge, and experience,
(ii) Solvent capacity,
(iii) Miscibility,
(iv) Morphology at room temperature (i.e., melting point),
(v) Self-dispensability and role in promoting self-dispersion of the formulation,
(vi) Digestibility and fate of digested products,
(vii) Capsule compatibility,
(viii) Purity, chemical stability,
(ix) Cost of goods.

**Solubility.** While the lipids (fatty acid derivatives) are the core ingredient of the formulation, one or more surfactants, as well as perhaps a hydrophilic Cosolvent, may be required to aid solubilization and to improve dispersion properties. Surfactants are categorized by their hydrophilic-lipophilic balance (HLB) number, with a low value (≤10) corresponding to greater lipophilicity and a higher value (≥10) corresponding to higher hydrophilicity. As a guideline as a starting point for formulation design, most of the lipids used in these oral formulations have a known “required HLB” value (generally available from the vendors), which corresponds to the optimal HLB for the surfactant blend necessary to emulsify the oil in water.

**Dispersion.** Formulations that exhibit sufficient solubility of the drug candidate should be examined for emulsification and dispersion properties in aqueous vehicles. A preliminary screening can be carried out by microscopic observation of the formulation when mixed with water. Vigorous mixing, accompanied by diffusion and stranding mechanisms, occurring at the water/formulation interface is indicative of an efficient emulsification. Absence of drug precipitate after complete mixing of the formulation with aqueous medium is another requirement. Particle size measurement of emulsion droplets by laser light scattering or other techniques is useful to select promising formulations. Construction of ternary phase diagrams is a method frequently used to determine the types of structures resulting from emulsification and to characterize behavior of a formulation along a dilution path.

**Digestion.** The actions of intestinal lipases can have a profound effect on the behavior of lipid-based formulations in the GI tract and must be considered in their design. It has long been recognized that non dispersible but digestible lipids such as triglycerides can be metabolized by lipases to mono-/diglycerides and fatty acids which will emulsify any remaining oil. Thus, the presence of high amounts of surfactants may be unnecessary to assure creation of the requisite small particle sizes and large surface areas for drug release.

**Absorption.** Efficient absorption of the drug by the intestinal mucosal cells is of course the ultimate goal of any oral lipid-based formulation [10]. First the components are dispersed to form lipid droplets (for type I formulations) or emulsion droplets (for types II-III), followed by
lipolysis and solubilization of the digestion products by bile acids, forming colloidal mixed micelles. It is believed that drug then partitions from the emulsion oil droplets and bile salt mixed micelles to be absorbed by the mucosal cells of the intestinal wall.

**Advantages of LBDDS [11]**

1. Drug release in controlled and targeted way.
2. Pharmaceutical stability.
3. High and enhanced drug content (compared to other carriers).
4. Feasibilities of carrying both lipophilic and hydrophilic drugs.
5. Biodegradable and biocompatible.
7. Formulation versatility.
8. Low risk profile.
9. Passive, noninvasive formation of vesicular system which is available for immediate commercialization.

**Formulation Approaches for LBDDS**

**Spray Congealing.** This is also referred to as spray cooling. In this method, molten lipid is sprayed into a cooling chamber and, on contact with the cool air, congeals into spherical solid particles. The solid particles are collected from the bottom of the chamber, which can be filled into hard gelatin capsules or compressed into tablets. Ultrasonic atomizers are frequently used to produce solid particles in this spray cooling process. The parameters to be considered are the melting point of the excipient, the viscosity of the formulation, and the cooling air temperature inside the chamber to allow instant solidification of the droplets.

**Spray Drying.** This method is somewhat similar to preceding one but differs in the temperature of the air inside the atomizing chamber. In this method, the drug solution (drug in organic solution/water) is sprayed into a hot air chamber, where the organic solvent or water evaporates giving rise to solid micro particles of drug. During this process, along with the lipid Excipients, solid carriers like silicon dioxide can be used. Gelucire (lipid excipient) enhances the drug release process by forming hydrogen bonds with the active substance, leading to the formation of stable solids of amorphous drug in micro particles[12, 13]

**Adsorption on to Solid Carrier.** This is a simple and economical process (in the context of equipment investment) in which a liquid-lipid formulation is adsorbed onto solid carrier like silicon dioxide, calcium silicate, or magnesium aluminometasilicate. The liquid-lipid formulation is added to the carrier by mixing in a blender. The carrier must be selected such that it must have
greater ability to absorb the liquid formulation and must have good flow property after adsorption. Gentamicin and erythropoietin with caprylocaproylpolyoxylglycerides (Labrasols) formulations were successfully converted into solid intermediates whose bioavailability was maintained even after adsorption on carriers.

**Melt Granulation.** This is also referred to as pelletization, which transforms a powder mix (with drug) into granules or pellets [14–16]. In this method a melt able binder (molten state) is sprayed onto the powder mix in presence of high shear mixing. This process can be referred to as a “pumpon” technique. Alternatively, the melt able binder is blended with powder mix and, due to the friction of particles(solid/semisolid) during the high-shear mixing, the binder melts. The melted binder forms liquid bridges between powder particles and forms small granules which transform into spheronized pellets under controlled conditions.

**Supercritical Fluid-Based Method.** This method uses lipids for coating drug particles to produce solid dispersions. In this method, the drug and lipid-based Excipients are dissolved in an organic solvent and supercritical fluid (carbon dioxide) by elevating the temperature and pressure [17,18]. The coating process is facilitated by a gradual reduction in pressure and temperature in order to reduce the solubility of the coating material in the fluid and hence precipitate onto the drug particles to form a coating [19,20].

**Other Formulation Tools.** Analysis of drug solubilization in bile salt-lecithin mixed micelles is an uncomplicated and effectual diagnostic test. Drug solubilization can be analyzed directly by spectrophotometry in some cases or alternatively by HPLC. This technique offers a rapid indication of whether a drug is likely to be solubilized in the gut lumen. The solubility enhancement ratio of steroids is a good illustration that solubilization cannot be predicted simply by octanol/water partition coefficient. Molecular dynamics modeling may become a useful formulation tool as available computing power increases. The structure of lipid formulations could be examined using similar techniques and studies of the partitioning [21].

**Characterization of Lipid-Based Drug Delivery Systems**

**Appearance.** The appearance can be checked in graduated glass cylinder or transparent glass container for its uniformity and color at equilibrium [22].

**Color, Odor, and Taste.** These characteristics are especially important in orally administered formulation. Variations in taste, especially of active constituents, can often be accredited to changes in particle size, crystal habit, and subsequent particle dissolution. Changes in color, odor, and taste can also indicate chemical instability [23].
Density. Specific gravity or density of the formulation is an essential parameter. A decrease in density often indicates the entrapment air within the structure of the formulation. Density measurements at a given temperature can be made using high precision hydrometers.

pH Value. The pH value of aqueous formulation should be taken at a given temperature using pH meter and only after settling equilibrium has been reached, to minimize “pH drift” and electrode surface coating with suspended particles. Electrolyte should not be added to the external phase of the formulation to stabilize the pH, because neutral electrolytes disturb the physical stability of the suspension.

Self-Dispersion and Sizing of Dispersions. Assessment of the dispersion rate and resultant particle size of lipid-based systems is desirable so attention has been given to measuring dispersion rate. The particle size measurement can be performed by optical microscope using a compound microscope for the particles with measurement within microns. Particle size analyzer can be used for the measurement of the particle size.

Applications
(i) So far, the design of successful lipid-based delivery systems has been based largely upon empirical experiences. Systematic physicochemical investigations of structure and stability do not only help to speed up the development of new and improved formulations, but may also aid in the understanding of the complex mechanisms governing the interaction between the lipid carriers and the living cells. Hence they sought to be safe, efficient, and specific carriers for gene and drug delivery.

(ii) LBDDS can be used to deliver various types of drugs from new chemical entities to more recent new developments for proteins and peptides, nucleic acids (DNA, siRNA), and cellular site specific delivery [24–26].

(iii) The utility of lipid-based formulations to enhance the absorption of poorly water-soluble, lipophilic drugs has been recognized for many years. Lipids are perhaps one of the most versatile excipient classes currently available, providing the formulator with many potential options for improving and controlling the absorption of poorly water-soluble drugs. These formulation options include lipid suspensions, solutions, and emulsions, micro emulsions, mixed micelles, SEDDS, SMEDDS, thixotropic vehicles, thermo softening matrices, and liposomes.

(iv) Lipid-based formulations, which are by no means a recent technological innovation, have not only proven their utility for mitigating the poor and variable gastrointestinal absorption of poorly soluble, lipophilic drugs, but also, in many cases, have shown the ability to reduce or eliminate the influence of food on the absorption of these drugs.
Self-emulsifying drug delivery system (SEDDS)

The drugs are most often administered by oral route, but approximately 40% of new drug candidates have poor-water solubility and the oral delivery of such drugs is difficult because of their low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality. To overcome these problems, various strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins, nanoparticles and solid dispersions [27]. There are a number of formulation strategies that could be used to improve the bioavailability of class II drugs, either by increasing the dissolution rate or by presenting the drug in solution and maintaining the drug in solution in the intestinal lumen. Absorption of a class II drug can be markedly improved by attention to the formulation. Formulation may improve the bioavailability of class IV drugs but they are likely to be compromised by their poor membrane permeability. If a class II drug can be maintained in a solubilized state in the lumen of the gut one can achieve an absorption profile more like that of a class I drug. Formulation strategies can do little to improve the absorption of classes I and III drugs which are limited by poor membrane permeability. Recently, due to good and reliable result, there is a great emphasis on self-emulsifying drug delivery systems (SEDDS) to improve the oral bioavailability of lipophilic drugs [28,29]. Self-emulsification is a phenomenon which has been exploited commercially for many years in formulations of emulsifiable concentrates of herbicides and pesticides. The most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles, surfactant dispersions [30, 31], self-emulsifying formulations [32], emulsions [33] and liposome [34] with every formulation approach having its special advantages and limitations. SEDDS or self emulsifying oil formulations (SEOF) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or, alternatively, one or more hydrophilic solvents and co-solvents/surfactants.

Advantages

Potential advantages of these systems (SEDDS) include [35]

1. Enhanced oral bioavailability enabling reduction in dose.
3. Selective targeting of drug(s) toward specific absorption window in GIT.
4. Protection of drug(s) from the hostile environment in gut.
5. Control of delivery profiles.
6. Reduced variability including food effects.
7. Protection of sensitive drug substances.
8. High drug payloads.
9. Liquid or solid dosage forms.

These formulations have attracted interest because they can improve the bioavailability of compounds that fall into Class II of the Biopharmaceutical Classification System (BCS). Class II compounds are poorly water soluble and highly permeable. This bioavailability enhancing property has been associated with a number of in vivo properties of lipidic formulation including:

1. The formation of fine dispersions and micellar suspensions to prevent precipitation and recrystallization of the drug compound.
2. The ability of certain lipid compounds and their metabolites to initiate changes in the gastrointestinal fluid to favour improved drug absorption.
3. The inhibition of cellular efflux mechanisms, which keep drugs out of the circulation.
4. Certain lipidic excipient are associated with selective drug uptake into the lymphatic transport system, thereby reducing the effect of first-pass drug metabolism in the liver.

**Composition of SEDDSs** - The self-emulsifying process depends on:

- The nature of the oil–surfactant
- The surfactant concentration
- The temperature at which self-emulsification occurs.

**Oils:** Oils can solubilize the lipophilic drug in a specific amount. It is the most important excipient because it can facilitate self emulsification and increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract. Long-chain triglyceride and medium-chain triglyceride oils with different degrees of saturation have been used in the design of SEDDSs. Modified or hydrolyzed vegetable oils have contributed widely to the success of SEDDSs owing to their formulation and physiological advantages. Novel semi synthetic medium-chain triglyceride oils have surfactant properties and are widely replacing the regular medium-chain triglyceride.

**Surfactant:** Nonionic surfactants with high hydrophilic–lipophilic balance (HLB) values are used in formulation of SEDDSs (e.g., Tween80, Labrasol, Labrafac CM 10, Cremophoreetc.). The usual surfactant strength ranges between 30–60% w/w of the formulation in order to form a stable SEDDS. Surfactants have a high HLB and hydrophilicity, which assists the immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous media. Surfactants are amphiphilic in nature and they can dissolve or solubilizere relatively high amounts...
of hydrophobic drug compounds. This can prevent precipitation of the drug within the GI lumen and for prolonged existence of drug molecules.

**Co surfactant/ Cosolvent:** Co-surfactant/Co-solvents like Spans, capryol 90, Capmul, lauroglycol, diethylene glycol monoethyl ether (Transcutol), propylene glycol, polyethylene glycol, polyoxyethylene, propylene carbonate, tetrahydrofurfuryl alcohol polyethylene glycol ether (Glycofurol), etc., may help to dissolve large amounts of hydrophilic surfactants or the hydrophobic drug in the lipid base. These solvents sometimes play the role of the co surfactants in the micro emulsion systems.

**Mechanism of self emulsification:** Self-emulsification occurs when the entropy change that favors dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional emulsion is a direct function of the energy required to create a new surface between the oil and water phases and can be described by the equation

\[
\Delta G = \sum_i N \pi r^2 \sigma
\]

Where, \(\Delta G\) is the free energy associated with the process (ignoring the free energy of mixing), \(N\) is the number of droplets of radius \(r\) and represents the interfacial energy. The two phases of emulsion tend to separate with time to reduce the interfacial area, and subsequently, the emulsion is stabilized by emulsifying agents, which form a monolayer of emulsion droplets, and hence reduces the interfacial energy, as well as providing a barrier to prevent coalescence. In the case of self-emulsifying systems, the free energy required to form the emulsion is either very low and positive, or negative (then, the emulsification process occurs spontaneously). Emulsification requiring very little input energy involves destabilization through contraction of local interfacial regions. For emulsification to occur, it is necessary for the interfacial structure to have no resistance to surface shearing [36].

**DRUG PROFILE**

**ATORVASTATIN CALCIUM [37]**

The Atorvastatin calcium is chemically described as \([R-(R^*,R^*)]-2-(4-fluorophenyl)-\beta,a-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Its empirical formula is \((C_{33}H_{34} FN_{2}O_{5})2Ca•3H2O. The profile of drug is described as follows:
Chemical Structure

![Chemical Structure of Atorvastatin](image)

Fig 1: Chemical structure of Atorvastatin

**Chemical Name:** Calcium (βR,δR)-2-(p-fluorophenyl)-β,δ-dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoic acid (1:2) Trihydrate

**Molecular Formula:** [C\textsubscript{33}H\textsubscript{35}FN\textsubscript{2}O\textsubscript{5}] 2 Ca .3H\textsubscript{2}O

**Generic Name:** Atorvastatin calcium

**Molecular Weight:** 1209.4 g/mol

**Category:** Cardiovascular Agents

**Sub-category:** HMG-CoA Reeducates Inhibitor

**Percentage Purity:** 98.0% - 101.0%

**Calcium percentage:** 3.3-3.6%

**Physical Properties:** White powder

**Appearance:** White to off white amorphous powder.

**Pharmacokinetics Profile [38]**

**Absorption**

After oral administration alone, Atorvastatin is rapidly absorbed; maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to Atorvastatin dose. The absolute bioavailability of Atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reeducates inhibitory activity is approximately 30%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism.

**Distribution**

Mean volume of distribution of Atorvastatin is approximately 381 liters. Atorvastatin is 98% bound to plasma proteins. A blood/plasma ratio of approximately 0.25 indicates poor drug penetration into red blood cells.

**Metabolism**
Atorvastatin is extensively metabolized to ortho- and parahydroxylated derivatives and various metabolites is equivalent to that of Atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reeducates is attributed to active metabolites.

**Elimination**

Atorvastatin and its metabolites are eliminated primarily in bile following hepatic and/or extrahepatic metabolism; however, the drug does not appear to undergo enterohepatic recirculation. Mean plasma elimination half-life of Atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reeducates is 20 to 30 hours due to the contribution of active metabolites. Less than 2% of a dose of Atorvastatin is recovered in urine following oral administration [39].

**Therapeutic Uses**

Atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor is a lipid regulating drug with actions on plasma lipids similar to those of simvastatin. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides, and to increase HDL-cholesterol in the treatment of hyperlipidaemias.

**MATERIALS AND METHODS**

**MATERIALS REQUIRED**

**Active pharmaceutical ingredient:**

**Active Pharmaceutical Ingredient.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Chemicals</th>
<th>Name of company/Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Atorvastatin Calcium</td>
<td>Voffin Biotech Pvt.Ltd. Himachal Pradesh</td>
</tr>
</tbody>
</table>

**Excipients/Chemicals used.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Chemicals</th>
<th>Name of company/Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tween 80</td>
<td>Procured from Sigma Aldrich, India</td>
</tr>
<tr>
<td>2.</td>
<td>Transcutol P</td>
<td>Loba chemie Pvt. Ltd. Mumbai 400005.India</td>
</tr>
<tr>
<td>3.</td>
<td>Labrafac PG</td>
<td>Gattefosse (France)</td>
</tr>
<tr>
<td>4.</td>
<td>Hydrochloric Acid</td>
<td>CDH New Delhi</td>
</tr>
<tr>
<td>5.</td>
<td>Sodium hydroxide</td>
<td>CDH New Delhi</td>
</tr>
</tbody>
</table>
equipment’s used.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Equipments</th>
<th>Model, Name of company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Digital weighing balance</td>
<td>Citizen CY-220</td>
</tr>
<tr>
<td>2</td>
<td>UV–Visible Spectrophotometer</td>
<td>Shimadzu-1800, Kyoto, Japan</td>
</tr>
<tr>
<td>3</td>
<td>FT-IR Spectrophotometer</td>
<td>Shimadzu-8400S</td>
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<tr>
<td>4</td>
<td>USP dissolution apparatus type II</td>
<td>Electrolab-08L, Goregaon, Mumbai</td>
</tr>
<tr>
<td>5</td>
<td>Deluxe pH meter</td>
<td>151-R</td>
</tr>
<tr>
<td>6</td>
<td>Dissolution Tester (USP)</td>
<td>Electrolab TDT-08L</td>
</tr>
<tr>
<td>7</td>
<td>Magnetic Stirrer with hot plate</td>
<td>The modern scientific industries (Meerut)</td>
</tr>
<tr>
<td>8</td>
<td>Heating Plate</td>
<td>The modern scientific industries (Meerut)</td>
</tr>
<tr>
<td>9</td>
<td>Vortex Shaker</td>
<td>Macro Scientific Works Jawahar Nagar, Delhi</td>
</tr>
</tbody>
</table>

Methods

Drug Selection

Atorvastatin calcium was selected as a model drug for incorporation into the nanoemulsions. The most important criterion for the selection of Atorvastatin as the model drug for the study was its low aqueous solubility. Atorvastatin calcium belongs to the class II drugs of BCS, having very low solubility but high permeability. Thus, Atorvastatin was a perfect candidate for the incorporation of drug into a lipid based drug delivery system.

Characterization of drug

Drug is characterized for the following properties:

Determination of absorption maxima

A UV absorption maxima was determined by scanning a 10 µg/ml solution of atorvastatin in 0.1M HCl pH 1.2 between 400nm - 200nm.

Media for Preparation of Standard Curve

Preparation of 0.1M HCl pH 1.2

8.5 ml of hydrochloric acid was dissolved in small amount of distilled water and finally the volume was made up to 1000 ml with distilled water (pH was adjusted if necessary using either base or acid).

Preparation of calibration curve of Atorvastatin in 0.1M HCl pH 1.2

50 mg of Atorvastatin was weighed accurately and dissolved in small quantity of 0.1M HCl (pH 1.2) in a 50 ml volumetric flask. Then the volume was made up to the mark using 0.1M HCl (pH 1.2).
The above prepared solution of Atorvastatin was subsequently diluted with 0.1M HCl of pH 1.2 to get 2, 4, 6, 8, 10µg/ml of the final solution. The absorbance’s of these solutions were measured at 246 nm against blank 0.1M HCl (pH 1.2) using UV spectrophotometer.

**FTIR Characterization of Atorvastatin Calcium**

The FTIR analysis of the drug carried out for qualitative compound identification. The KBr pellet of the drug was prepared with KBr (1:100) in pressure compression machine. The sample pellet was mounted in FTIR compartment and taken scan at wavelength 4000 cm$^{-1}$ - 400 cm$^{-1}$.

**Partition Coefficient**

The partition coefficient study was performed using n-octanol as the oil phase and 0.1M HCl (pH 1.2) as the aqueous phase. A drug solution of 1 mg/ml was prepared in 0.1M HCl (pH 1.2) and 25 ml of this solution was taken in a separating funnel and shaken with an equal volume of n-octanol until equilibrium was reached. Then, the aqueous phase was assayed after partitioning using a UV spectrophotometer to get the partition coefficient values.

The partition coefficient of drug K o/w was calculated using the following formula: $K_{o/w} = \frac{\text{Concentration in octanol}}{\text{Concentration in 0.1M HCl (pH 1.2)}}$

**Drug solubility determination:**

The solubility of drug in various excipient (surfactant, co-surfactant and oil) was determined. An excess amount of drug was added to each of 2 mL of excipient in a test tube and these mixtures were vortexed mixed for using vortex shaker while maintaining the temperature at 37°C. Each of the system was centrifuged using high speed centrifuge at 10,000 rpm for a period of 10 min. The supernatant of each system was separated and its drug content was estimated after suitable dilution with methanol using double beam UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against blank. The study was conducted in triplicate and their mean values were recorded [11-12].

**Construction of pseudo ternary phase diagram**

The pseudo-ternary phase diagram was constructed without drug to recognize the maximum self-emulsifying domain existence and to specify the optimal ratio of oil, surfactant and co surfactant for the SEDDS formulations. Pseudo ternary phase diagrams for all formulations were constructed by using CHEMIX School 3_60 version, to recognize the zone of nanoemulsion formation. Based on preliminary optimization, components like oil, surfactant and co surfactant were utilized as apex of ternary phase diagram. The procedure described by Craig et al. (1995) was used in this study. Twenty-seven formulations were prepared with varying percentage of selected excipient. Surfactant (Tween 80) and co-surfactant (Transcutol) were mixed in different
volume ratios (1:1, 1:2 and 2:1). For every phase diagram, oil and specific surfactant: co-
surfactant ratio (Smix) were mixed in ratios ranging from 1:9% to 9:1% (w/w) in nine ratios like
1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. The ternary mixture (0.3 ml) was mildly agitated with
300 ml of distilled water in a glass beaker using a magnetic stirrer at 37ºC. The emulsions were
allowed to stand for 2 h and their transmittance was assessed at 638.2 nm by double beam UV
spectrophotometer (Shimadzu, Japan) using distilled water as blank [15-17].

**Formulation development**

From the pseudo ternary diagram, it was observed that the maximum SEDDS zones were
exposed by 2:1 ratio of Tween-80 and Transcutol. Hence, different SEDDS formulations of
Atorvastatin were prepared using 2:1 Smix ratio. From 2:1 Smix ratio, different SEDDS
formulations with SEDDS codes of F1–F5 were precisely selected. 25mg of drug was solubilized
in Transcutol due to the highest solubility of drug in it, and rest of the components was added
spontaneously by vortexing. Atorvastatin-loaded SEDDS (F1-F5) were transferred to glass test
tubes, and the compositions of these SEDDSs are listed in table 2.

**Table 2: Composition Table-**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Oil (Labrafac PG)</th>
<th>Smix (Surfactant:Cosurfactant) (2:1)</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100 mg</td>
<td>900 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>F2</td>
<td>200 mg</td>
<td>800 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>F3</td>
<td>300 mg</td>
<td>700 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>F4</td>
<td>400 mg</td>
<td>600 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>F5</td>
<td>500 mg</td>
<td>500 mg</td>
<td>25 mg</td>
</tr>
</tbody>
</table>

**Characterization of SEDDS**

**Globule size analysis**

The droplet size and polydispersity index of the formulations were determined by Zetasizer Nano
ZS (Malvern Instruments, UK). Each formulation was diluted with distilled water before
analysis. The sample of formulation was diluted sufficiently with distilled water and around 3 ml
of each formulation was taken in disposable cuvette. These samples were subjected for
measurement of droplet diameter and PI. Size analysis was monitored at 25ºC at 90ºangle. The
mean droplet diameter, Polydispersity Index (PI) and zeta potential of formulation was
determined with the help of Malvern Zetasizer at room temperature.
**Zeta Potential Measurement**

Zeta potential of the formulations was measured by using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Zetasizer measures the potential ranged from −120 to 120 mV. For zeta potential measurement, the sample of formulation was diluted sufficiently with distilled water and around 3 ml of each formulation was taken in glass electrodes.

**Determination of emulsification time**

Self-emulsifying properties of SEDDS formulations (F1-F5) were determined by visual assessment. The USP XXIV type II dissolution apparatus was used to evaluate the efficiency of self-emulsification of different formulations. One gram of each formulation was added drop wise into 500 mL of is solution media (0.1M HCl, pH 1.2) maintained at 37°C±0.5°C with gentle agitation condition provided by rotating paddle at 50 rpm. Time required for the complete disappearance of SEDDS was recorded.

**Content uniformity**

Content uniformity was done on the different selected formulations, where 500 mg of each system was added in volumetric flask containing suitable amount of methanol and mixed well with shaking, sonicated for 10-15 min and then filtered through a filter. Aliquot of the filtrate was suitably diluted with methanol and drug content was determined using UV-spectrophotometer at λmax 241 nm using methanol as a blank.

**In-Vitro Drug Release Profile Studies:**

Each 500 mg SEDDS system, containing 20 mg drug, was filled into capsules size 00. Dissolution studies of Atorvastatin calcium from the prepared capsules were performed using the USP Dissolution Tester, Apparatus II (Rotating paddle) at a rotation of 50 rpm over a period of 190 min. Studies were carried out at 37°C±0.5°C in 500 mL of 0.1 M HCl (pH 1.2) which was chosen as dissolution medium since it provided better discrimination between the different chosen systems. At appropriate time intervals, 2 mL sample was taken, filtered through a 0.2 mm filter and analyzed for drug content by measuring the absorbance at the predetermined wavelength 241 nm against 0.1 M HCl as blank.

All the dissolution data of Atorvastatin after 5 min (Q5min) from the selected SEDDS filled into capsules were compared to that of the commercially available Atorva 20 mg tablets. In vitro dissolution was done in triplicates and the average values (±SD) were taken.

**Drug release kinetic study:**

The rate and the mechanism of release of drug through the prepared formulations were analyzed by fitting the drug released data into zero order (percentage of drug released vs time), first order
(log percentage of drug to be released vs. time) and Higuchi’s (percentage of drug released vs square root of time).

1. Zero-order equation: \( Q = Q_0 - k_0 t \), \hspace{1cm} (5.1)
   Where \( Q \) is the amount of drug released at time \( t \), and \( k_0 \) is the release rate.

2. First-order equation: \( \ln Q = \ln Q_0 - k_1 t \), \hspace{1cm} (5.2)
   Where \( k_1 \) is the release rate constant and

3. Higuchi's equation: \( Q = k_2 t^{1/2} \), \hspace{1cm} (5.3)
   Where \( Q \) is the amount of the drug released at time \( t \) and \( k_2 \) is the diffusion rate constant.

The drug release data was further analyzed to define the mechanism of release by applying the following empirical equation of Korsmeyer and Peppas
\[
\frac{M_t}{M_a} = K t^n, \hspace{1cm} (5.4)
\]
where \( \frac{M_t}{M_a} \) is the fraction of drug released at time \( t \), \( K \) is a constant and \( n \) characterizes the mechanism of drug release from the formulations during the diffusion process.

RESULTS AND DISCUSSION

Characterization of Drug

Quantitative Estimation of Drug

The absorption maxima of drug solution as determined by UV-Spectrophotometer were found to be 241 nm in 1.2 pH 0.1M HCl when scanned between 400-200 nm. The method of analysis using UV Spectrophotometer of Atorvastatin at a scanned wavelength range was found to be reproducible (241 nm) (Fig. 2).

Standard calibration curve of Atorvastatin was prepared in 0.1M HCl (pH 1.2) at 241 nm, using UV/Visible spectrophotometer. The standard curve is shown in Fig. 3. The data were found to have nearly perfect correlation coefficient 0.997 and hence it is linear in nature. The reproducibility of the method was tested by repeating the procedure.

Fig. 2: Spectra of \( \lambda_{\text{max}} \) scan of Atorvastatin calcium in 0.1M HCl.
FTIR characterization of drug

The FTIR spectrum of Atorvastatin Calcium (Fig.4) showed characteristic bands at 3666.43 cm\(^{-1}\) (O-H stretching), 3363.62 cm\(^{-1}\) (aromatic N-H stretching), 3247 cm\(^{-1}\) (asymmetric O-H stretching) and 3055 cm\(^{-1}\) (symmetric O–H stretching), 1650 cm\(^{-1}\) (asymmetric C=O stretching), 1577 cm\(^{-1}\) (symmetric C=O stretching), 1550-1469 cm\(^{-1}\) (four bands related to C–C ring stretching), 1317 cm\(^{-1}\) (CH\(_3\)/CH\(_2\) deformation), 1242 cm\(^{-1}\) (C–N stretching) and 1215 cm\(^{-1}\) (C–O stretching) and 1159 cm\(^{-1}\) (aryl fluorides stretching). These characteristic peaks are also observed elsewhere in literature which indicates the purity of drug and absence of any kind of impurity.

![Calibration curve of Atorvastatin in 0.1M HCl (pH 1.2).](image)

Fig. 3: Calibration curve of Atorvastatin in 0.1M HCl (pH 1.2).

![FTIR spectra of pure drug (Atorvastatin calcium).](image)

Fig. 4: FTIR spectra of pure drug (Atorvastatin calcium).
Partition coefficient

In this study the partition coefficient value of Atorvastatin calcium in n-octanol/0.1M HCl (pH 1.2) system was found to be 3.63. The log P value of Atorvastatin indicates that the drug possesses sufficient lipophilicity.

Selection of Oil (Solubility studies)

One of the critical steps in the formulation of SEDDS is selection of oil phase, since the oil is digested in the GI tract and may play a major role in determining rate and extent of dissolution. The higher solubility of the drug in the oil phase is important for the self-emulsification so as to maintain the drug in solubilised form and also to improve the intestinal absorption through lymphatic route. In the present study, selection of oil for the preparation of SEDDS was done on the basis of their aptitude to solubilize maximum amount of drug. This might be attributed to the fact that in SEDDS drug should be in its dissolved state, as this form have been reported to possess greater concentration of drug. The high concentration gradient provides driving force for the permeation of drug through GI tract. The results of solubility’s of Atorvastatin in different oils are summarized in Fig. 5. The data suggested that the solubility of drug in novel synthetic oil was maximum as compared to other natural/edible oil. Among the oils tested, Labrafac PG, which showed the highest solubility of Atorvastatin (2.83 ± 0.083 mg/mL), was selected as the oil phase for further study.

![Fig. 5: Solubility of Atorvastatin in various oils.](image)

Pseudo ternary Phase Diagram

On the basis of preliminary trials Labrafac PG (oil), Tween 80 (surfactant) and Transcutol (co surfactant) was selected for the preparation of SEDDS and pseudo ternary phase diagrams of the
three systems (with different surfactant and co-surfactant ratio) were constructed with the objective to study the relationship between the phase behavior and the to determine the concentration range of components for the formation of an emulsion. All the components were converted to weight/weight percent (%w/w) before constructing the phase diagrams. The pseudo ternary diagrams for three batches are shown in Fig. 6. The shaded area illustrated emulsification regions along with the highest probability to form nanoemulsion of less than 100 nm globule size whereas the part surrounding these areas illustrate formulations with poor emulsion forming ability with higher globule size. Within this shaded area the SEDDS form fine oil in water emulsion with only gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence then improves the thermodynamic stability of the emulsion formulation. Furthermore, co-surfactants increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules.

![Fig. 6: Ternary phase diagrams of different selected systems. The shaded area represents self-emulsifying region. a) Smix at a 1:1 ratio; b) Smix at a 1:2 ratio; c) Smix at a 2:1 ratio.](image-url)
The ability to form nanoemulsion with only gentle agitation was investigated by measuring percentage transmittance of all 27 formulations which was tabulated in table 3-5 and Fig. 7.

![Visual appearance of different emulsions](image)

**Fig. 7:** Visual appearance of different emulsions formed on gentle agitation at different Smix ratio. (a) 1:1; (b) 1:2; (c) 2:1.

**Table 3:** Percentage transmittance and visual appearance of various prepared formulation with Smix at 1:1 ratio

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Oil: Smix</th>
<th>% Transmittance</th>
<th>Visual appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:9</td>
<td>98.2</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>2:8</td>
<td>86.9</td>
<td>Transparent</td>
</tr>
<tr>
<td>3</td>
<td>3:7</td>
<td>42.3</td>
<td>Milky white</td>
</tr>
<tr>
<td>4</td>
<td>4:6</td>
<td>37.1</td>
<td>Milky white</td>
</tr>
<tr>
<td>5</td>
<td>5:5</td>
<td>35.8</td>
<td>Milky white</td>
</tr>
<tr>
<td>6</td>
<td>6:4</td>
<td>32.2</td>
<td>Milky white</td>
</tr>
<tr>
<td>7</td>
<td>7:3</td>
<td>24.5</td>
<td>Milky white</td>
</tr>
<tr>
<td>8</td>
<td>8:2</td>
<td>17.6</td>
<td>white</td>
</tr>
<tr>
<td>9</td>
<td>9:1</td>
<td>3.5</td>
<td>white</td>
</tr>
</tbody>
</table>
Table 4: Percentage transmittance and visual appearance of various prepared formulation with Smix at 1:2 ratio

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Oil : Smix</th>
<th>% Transmittance</th>
<th>Visual appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 9</td>
<td>97</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>2 : 8</td>
<td>83.5</td>
<td>Clear</td>
</tr>
<tr>
<td>3</td>
<td>3 : 7</td>
<td>51.7</td>
<td>Milky white</td>
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<tr>
<td>4</td>
<td>4 : 6</td>
<td>30.5</td>
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<tr>
<td>5</td>
<td>5 : 5</td>
<td>27.3</td>
<td>Milky white</td>
</tr>
<tr>
<td>6</td>
<td>6 : 4</td>
<td>26.5</td>
<td>Milky white</td>
</tr>
<tr>
<td>7</td>
<td>7 : 3</td>
<td>24.1</td>
<td>Milky white</td>
</tr>
<tr>
<td>8</td>
<td>8 : 2</td>
<td>15.3</td>
<td>White</td>
</tr>
<tr>
<td>9</td>
<td>9 : 1</td>
<td>9.5</td>
<td>White</td>
</tr>
</tbody>
</table>

Table 5: Percentage transmittance and visual appearance of various prepared formulation with Smix at 2:1 ratio

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Oil : Smix</th>
<th>% Transmittance</th>
<th>Visual appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 : 9</td>
<td>97.8</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>2 : 8</td>
<td>96.3</td>
<td>Clear</td>
</tr>
<tr>
<td>3</td>
<td>3 : 7</td>
<td>80.7</td>
<td>Transparent</td>
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<tr>
<td>4</td>
<td>4 : 6</td>
<td>79.2</td>
<td>Transparent</td>
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<td>5</td>
<td>5 : 5</td>
<td>52.7</td>
<td>Milky white</td>
</tr>
<tr>
<td>6</td>
<td>6 : 4</td>
<td>43.7</td>
<td>Milky white</td>
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<tr>
<td>7</td>
<td>7 : 3</td>
<td>31.7</td>
<td>Milky white</td>
</tr>
<tr>
<td>8</td>
<td>8 : 2</td>
<td>23.8</td>
<td>White</td>
</tr>
<tr>
<td>9</td>
<td>9 : 1</td>
<td>10.8</td>
<td>White</td>
</tr>
</tbody>
</table>

Globule size determination

The rate and extend of drug release as well as absorption mainly depends upon the globule size of the emulsion. Hence, globule size determination is a crucial factor for self emulsifying drug delivery system. In most of the cases increasing the surfactant concentration leads to smaller mean droplet size, this could be explained by the stabilization of the oil droplets as a result of localization of the surfactant molecules at the oil-water interface. However, higher oil proportion forms larger globules. The smaller the droplet size, the larger is the interfacial surface area provided for drug absorption. The mean globule size of the selected formulation was in the found to be 24.40±2.07 nm. The polydispersity index (PDI) reflects the uniformity of particle diameter and can be used to depict the size distribution of the nanoemulsion population. PDI varies from 0.0 to 1.0. The closer to zero the polydispersity value, the more homogeneous the particles are. The PDI of selected formulation was found to be 0.299, indicating good uniformity of droplets within the formulation (Fig. 8).
Zeta potential
Zeta potential governs the degree of repulsion between adjacent, similarly charged, and dispersed droplets. ZP measurement is used to identify the charge of the droplets. It has been suggested that ZP may serve as a partial indicator for the physical stability of the emulsion being formed. If the ZP is reduced below a certain value, (which depends on a particular system being used) the attractive forces exceed the repulsive forces, and the globules come together leading to flocculation. The zeta potential of the formulations was found to be -24.5 mV. The negative value of ZP was possibly due to the presence of negatively charged fatty acid esters in Labrafac PG in the formulation.

Self emulsification time
The rate of emulsification is an important parameter for the assessment of the efficiency or spontaneous emulsification of formulation without aid of any external thermal or mechanical energy source. Formulation should disperse completely and quickly when subjected to aqueous dilution under mild agitation of GIT due to peristaltic activity. It has been reported that self emulsification mechanism involves the erosion of a fine cloud of small droplets from the monolayer around emulsion droplets, rather than progressive reduction in droplet size. The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet. The results of self emulsification time were shown in Table 6. The results revealed that self emulsification time depends upon the individual composition and its proportion of oil, surfactant and co-surfactant. However, higher the percentage of surfactant system greater the spontaneity of emulsification, due to excess diffusion.
of aqueous phase into oil phase causing significant interfacial disruption and discharge of droplet into the bulk aqueous phase.

**Drug Content:**

Drug content from all the formulations (F1-F5) was determined in methanol. Good uniformity of drug content among the batches was observed with all formulations. Despite of difference in composition, the drug content of formulations F1 to F5 was found in range of 97.77–99.12% (Table 6). The results indicate that the process employed to prepare formulations in this study was capable of producing SEDDS with uniform drug content and minimal variability.

**Table 6: Drug content, emulsification time and percentage transmission values of selected formulations.**

<table>
<thead>
<tr>
<th>Formulation Code (Smix=2:1)</th>
<th>Drug Content (%)</th>
<th>Emulsification time (sec)</th>
<th>Percentage Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>98.63±4.26</td>
<td>32±2.33</td>
<td>102%</td>
</tr>
<tr>
<td>F2</td>
<td>99.12±3.89</td>
<td>48±2.14</td>
<td>100%</td>
</tr>
<tr>
<td>F3</td>
<td>98.11±3.24</td>
<td>55±2.77</td>
<td>95%</td>
</tr>
<tr>
<td>F4</td>
<td>97.77±4.15</td>
<td>64±2.08</td>
<td>91%</td>
</tr>
<tr>
<td>F5</td>
<td>98.26±3.74</td>
<td>75±2.85</td>
<td>84%</td>
</tr>
</tbody>
</table>

*All data are mean± S.D.(n=3)*

*In vitro drug release:*

Drug release from the Atorvastatin loaded SEDDS formulations F1 to F5 was shown in Fig. 9 and Table 7. The release data was compared with commercially available 20 mg Atorvastatin tablet (Atorva 20). The release from selected and optimized formulations was found to be significantly higher as compared with that of marketed formulation (Fig. 9.). Thus, this greater availability of dissolved Atorvastatin from the SEDDS formulation could lead to higher absorption and thereby higher oral bioavailability.

Regarding the effect of oil concentration, it is obvious that on increasing oil concentration significant retardation in the drug dissolution was seen, which might be due to the fact that increasing the oil concentration led to an increase in the system viscosity that slows down system emulsification. This is in agreement with who reported that increasing the viscosity was the reason of retardation of drug dissolution.

The amount of surfactant was mainly responsible for the increase in cumulative percentage of drug released from the formulation, since as the concentration of surfactant increase the cumulative drug release also increases. The increase in cumulative drug release was mainly
attributed to rapid self-emulsification in the medium after dissolution of the capsule shell. As the amount of free energy required in the formation of an emulsion is very low, this results in the spontaneous formation of an oil-water interface. This increases the water penetration of oil droplets, resulting in disruption of the interface and thereby decreasing the droplet size and eventually increasing the release rate. It was also seen that the cumulative percentage of drug released was further improved by the addition of the co-surfactant. This phenomenon might be due to the penetration of the co-surfactant into the surfactant monolayer interface, which further enhances the self-emulsification performance of SEDDS.

**Table 7: Percent cumulative drug release from selected formulations**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>Marketed formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>41.5±2.32</td>
<td>39.5±2.86</td>
<td>37.8±2.04</td>
<td>26.4±1.99</td>
<td>22.2±2.11</td>
<td>7.25±1.85</td>
</tr>
<tr>
<td>60</td>
<td>59.63±2.14</td>
<td>44.7±2.92</td>
<td>41.3±2.16</td>
<td>39.6±2.58</td>
<td>37.8±2.63</td>
<td>15.98±2.50</td>
</tr>
<tr>
<td>90</td>
<td>68.12±2.08</td>
<td>47.9±2.56</td>
<td>43.43±2.74</td>
<td>41.57±2.64</td>
<td>40.88±2.54</td>
<td>29.05±2.44</td>
</tr>
<tr>
<td>120</td>
<td>80.26±3.54</td>
<td>51.7±2.48</td>
<td>45.1±2.66</td>
<td>43.28±2.53</td>
<td>46.23±2.25</td>
<td>35.67±1.98</td>
</tr>
<tr>
<td>150</td>
<td>89.58±2.11</td>
<td>55.2±2.36</td>
<td>51.95±2.93</td>
<td>50.64±2.95</td>
<td>49.59±2.64</td>
<td>39.5±2.09</td>
</tr>
<tr>
<td>180</td>
<td>91.88±2.68</td>
<td>82.9±3.54</td>
<td>64.66±2.87</td>
<td>63.77±3.05</td>
<td>59.45±2.82</td>
<td>42.26±2.18</td>
</tr>
<tr>
<td>210</td>
<td>96.72±3.06</td>
<td>89.5±3.26</td>
<td>82.9±3.24</td>
<td>81.56±2.84</td>
<td>79.88±2.34</td>
<td>48.11±2.63</td>
</tr>
<tr>
<td>240</td>
<td>98.26±2.98</td>
<td>96.6±2.88</td>
<td>92.87±2.87</td>
<td>91.67±2.75</td>
<td>88.35±2.95</td>
<td>51.01±2.58</td>
</tr>
</tbody>
</table>

*All data are mean ± S.D. (n=3)*

**Fig: 9:** Percent Cumulative Drug Release profile of different formulations.
Drug Release Kinetics:

Further, in order to study the mechanism of drug release from SEDDS and conventional tablets, data obtained from the In vitro release study was fitted to various kinetic equations. The kinetic models used were a zero-order equation, first-order equation, Higuchi’s square root of time equation (Table 8).

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>R² Values</th>
<th>KorsemeyerPeppa’s (n value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero Order Kinetics</td>
<td>First Order Kinetics</td>
</tr>
<tr>
<td>F1</td>
<td>0.834</td>
<td>0.978</td>
</tr>
<tr>
<td>F2</td>
<td>0.901</td>
<td>0.823</td>
</tr>
<tr>
<td>F3</td>
<td>0.889</td>
<td>0.789</td>
</tr>
<tr>
<td>F4</td>
<td>0.936</td>
<td>0.821</td>
</tr>
<tr>
<td>F5</td>
<td>0.945</td>
<td>0.854</td>
</tr>
<tr>
<td>Marketed Formulation</td>
<td>0.952</td>
<td>0.979</td>
</tr>
</tbody>
</table>

It has been reported the advantages of using empirical equations, they can be fitted very simply to experimental data. However, as main disadvantage it has been proposed that the constants in the equations lack physical meaning and are therefore dependent upon how the data are measured. Then it would be possible to extract the diffusion coefficients from the empirical constants, as long as the latter are calibrated.

The in vitro release pattern of various formulations was analyzed by fitting the dissolution data into various kinetic models (Table 8). It was observed that for formulations F4 and F5, the R² values were higher when fitted to a zero-order equation, which indicated a zero-order release from these formulations. Formulations F1, F2 and F3 followed the Higuchi release pattern, whereas marketed formulation followed first-order release.

Regarding the n values calculated for the studied formulations (except marketed formulation), in most cases a Fickian mechanism was found to be predominant, which indicated that the drug release follow diffusion pattern.

CONCLUSION

SEDDS formulations consist of oils, surfactants and co surfactants, which are emulsified by aqueous medium under gentle digestive motility in the gastrointestinal tract. It is considered that the excipient in SEDDS could increase the dissolution and permeability of the drug significantly by decreasing the droplet size. Atorvastatin calcium belongs to the BCS Class-II drug with low aqueous solubility and high permeability. The low bioavailability of Atorvastatin is due to its
poor solubility. In this present work, solubility of drug in various excipient was analyzed first, and then we prepared series of SEDDS formulations using different composition of oil, surfactants and co-surfactants. Based on preliminary evaluation, the best 27 self emulsifying region of different compositions were identified. Ternary phase diagram was constructed using CHEMIX ternary plot software. Best five combinations of oil and Smix were selected based on the physical appearance and % transmittance and drug was loaded in them and evaluated for their physicochemical parameters and dissolution profile (In-vitro) in comparison to the marketed tablet. Optimal formulations containing 2:1 mixture of Tween-80/ Transcutol HP showed maximum transmittance, a minimum droplet size and higher In-vitro drug release. This study indicates that the potential use of SEDDS for the oral delivery of atorvastatin calcium can be an alternative to improve its systemic availability.

REFERENCES


