

Bioavailability enhancement of poorly water soluble drugs using Self Emulsifying Drug Delivery System (SEDDS) and Solid Dispersion (SD) technology

A thesis submitted to the University of Dhaka in partial fulfillment of the requirements for the degree of Doctor of Philosophy

By

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Declaration

This is to certify that Mr. Sardar Mohammad Ashraful Islam has conducted his thesis work entitled "Bioavailability enhancement of poorly water soluble drugs using Self Emulsifying Drug Delivery System (SEDDS) and Solid Dispersion (SD) technology" under our joint supervision for the degree of Doctor of Philosophy in the Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka. This work on any part thereof has not been submitted anywhere for any other degree.

 25/5/2015

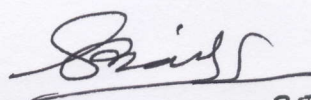
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Dedicated to my honorable teacher
Late Professor Dr. Md. Habibur Rahman

Acknowledgement

First I remember Late Professor Dr. Md. Habibur Rahman, my first supervisor who died on 18 February, 2011, at the middle of my research work. I am praying for the peace of the departed soul. May Allah forgive him and rest his soul in peace. I would like to express my profound sense of gratitude to my first co-supervisor Prof. Dr. Reza-ul Jalil, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, for his valuable advice, encouragement at the beginning of my research and especially for his help to enroll in the PhD program and to start the research work successfully.

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Abstract

The objective of the present work was to enhance bioavailability of poorly water soluble atorvastatin, simvastatin and rosuvastatin by Self Emulsifying Drug Delivery System (SEDDS) and Solid Dispersion (SD) technology. SEDDS and SD were prepared successfully and evaluated by *in-vitro*, *ex-vivo* and *in-vivo* techniques. Oil, surfactants and co-surfactants of SEDDS formulations were screened according to their solubilizing capacity. Solubility was determined by a validated RP-HPLC method. Oleic acid, Capryol™ 90 and peceol™ showed the highest solubilizing capacity for atorvastatin, simvastatin and rosuvastatin, respectively. Among the surfactants tween 80 showed higher solubilizing capacity for atorvastatin and simvastatin. Transcutol® showed good solubilizing capacity for atorvastatin, simvastatin and rosuvastatin as a co-surfactant. Composition of SEDDS was optimized by pseudo-ternary phase diagrams study. Microemulsion region in each diagram was plotted and compared. Formulations were initially checked for color, clarity and sedimentation. SEDDS formulations were found to be transparent and clear. Droplet size of SEDDS formulation was determined by Laser Diffraction Technology. Formulation containing 2:1 ratio of surfactants: co-surfactants produced smallest droplet size (121-150 nm) with higher *in-vitro* drug release (100% within 20 min) than other formulations. Primary solid dispersions of atorvastatin, simvastatin and rosuvastatin were prepared by solvent evaporation technique using poloxamer 407, croscarmellose sodium, sodium starch glycolate, hydroxypropylmethylellulose, povidone K-30 as carrier. Drug-carrier weight ratio was 1:1, 1:3 and 1:5. Physical mixtures and secondary and tertiary solid dispersions were also prepared and compared with the primary solid dispersions. The solid dispersions were investigated for dissolution behavior and SDs containing poloxamer 407 were found to be effective in enhancing drug release significantly. SDs containing higher amount of carriers showed higher drug release but the release was not found to be proportional to the amount of carrier. SDs were also evaluated by using Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Differential Scanning Calorimetry (DSC). The FTIR spectroscopic studies showed the stability of drug and absence of interaction between drug and carrier. The XRD, SEM and DSC studies indicated the amorphous state of drugs in SDs. SEDDS was formulated as capsules and SDs were formulated as tablets and they were evaluated and compared with liquid SEDDS and solid dispersions powder (SDs). Dissolution data of SDs and SEDDS

were compared by using both model dependant and model independent techniques. %DE (Dissolution Efficiency) was analyzed by ANOVA to test similarity among the SEDDS and SDs formulations. Response surface methodology (RSM) and 2^2 factorial designs were used to evaluate and characterize SEDDS and SDs. Both SEDDS and SDs were then tested for diffusion through dialysis cellulose tubing and permeability through chicken and rabbit intestinal sacs. *In-vivo* performance of rosuvastatin SEDDSs and SDs was evaluated by using its pharmacodynamic effects (hypolipidemic activity). The hypolipidemic activity of the SEDDS and SDs were found to be significantly more than the marketed products and also the placebo & control groups. . After oral administration of 6 mg kg^{-1} rosuvastatin to 6 rabbits, the oral bioavailability of SEDDS and SD was found to increase 1.68 and 1.43 fold, respectively as compared to the conventional tablets which infers that SEDDS and SDs have the potential to advance the oral bioavailability of poorly water soluble drugs. SEDDS was found to be more effective than SDs in enhancing the oral bioavailability. This study indicates that the potential use of SEDDS and SDs for the oral delivery of poorly water soluble drugs can be an alternative to improve their systemic availability.

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List of Abbreviations and Symbols

Abbreviations	
μg	microgram
ANOVA	analysis of variance
API	active pharmaceutical ingredient
ATV	Atorvastatin
ATV MP	atorvastatin commercially available local brand
ATV REF	atorvastatin innovator brand
AUC	Area under the serum concentration-time curve
BP	The British Pharmacopoeia
CCS	croscarmellose sodium
cm	centimeter
C_{max}	maximal concentration
DE	Dissolution efficiency
DF	Degree of freedom
DP	Droplet
DSC	Differential Scanning Calorimetry
DT	Desintegration Time
EHGCS	Empty Hard Gelatin Capsule Shell
f_1	difference factor
f_2	similarity factor
FTIR	Fourier Transform Infrared Spectroscopy
g	gram
HLB	hydrophilic-lipophilic balance value
HPLC	High Performance Liquid Chromatography
HPMC	hydroxypropylmethylellulose
hr	Hour
IS	Internal Standard
kg	kilogram
Km	ration of surfactant and cosurfactant mixture
l	litre
LC	Liquid Chromatography
LLOD	lower limit of detection
LLOQ	lower limit of quantification
mg	milligram
min	Minute
ml	milliliter
NAP	naproxen sodium
ng	nanogram
PM	Physical mixtures
POL	Poloxamer 407
POV	povidone K-30

Abbreviations

PSD	primary solid dispersion
PXRD	Powder X-ray Diffraction
ROS	rosuvastatin
ROS MP	rosuvastatin commercially available local brand
ROS REF	rosuvastatin innovator brand
rpm	rotations per minute
RSD	relative standard deviation
S.D.	standard deviation
SD	solid dispersion
SDs	solid dispersions
SDS	secondary solid dispersion
SEDDS	Self emulsifying drug delivery system
SEM	Scanning Electron Microscopy
SEM	Scanning Electron Microscope
SIM	simvastatin
SIM MP	simvastatin commercially available local brand
SIM REF	simvastatin innovator brand
Smix	surfactant and co-surfactant mixture
SSG	sodium starch glycolate
t	time
TDS	tertiary solid dispersion
T _g	Glass transition temperature of polymer
TGA	Thermogravimetric Analysis
T _m	Melting temperature of polymer
T _{max}	time to reach maximum serum concentration
USP	The United States Pharmacopoeia
UV	ultraviolet light
v/v	volume by volume
w/v	weight by volume
w/w	weight by weight

Symbols

°C	degree Celsius
μ	micron
®	Registered trademark
%	percent
λ _{max}	wave length of maximum radiation absorption
>	greater than
<	smaller than
=	equals to
α	proportional to
γ	gamma
&	and

Software used for statistical analysis of data

Design Expert 9.0 (Stat-Ease Inc., USA)

SPSS software (version 16.0; SPSS Inc., USA).

kinetica (version 5.0; ALFASOFT, UK).

SigmaPlot 10.0 software (USA)

Minitam® 17

Chapter One: Introduction

1.1 General Introduction

A large number of potential drug candidates suffer from low aqueous solubility and low dissolution rate. This results in low drug concentrations at the absorption sites and therefore low oral bioavailability (Leuner and Dressman, 2000). Amidon *et al.* (1995) classified such drugs in Biopharmaceutical Classification System as class II compounds. Recently, pharmaceutical technology provides many approaches to enhance the dissolution rate of such poorly soluble drugs. These include use of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins, nanoparticles and solid dispersions (Kumar *et al.*, 2010). Majority of these approaches have their limitations because of the need of specialized equipments, complicated manufacturing process, longer processing time, and regulatory complexity. For many compounds, however, decrease in the particle size may not lead to a significant or adequate increase in bioavailability. Salt formation may also be problematic, particularly with neutral compounds and weak acids (Paul *et al.*, 2012).

Lipid-based formulation approaches, particularly the self emulsifying drug delivery system (SEDDS), are well known for their potential as alternative approach for delivery of hydrophobic drugs (Pouton, 2000), which are associated with poor water solubility and low oral bioavailability (Kim *et al.*, 2000). SEDDSs are isotropic and thermodynamically stable solutions consisting of oil, surfactant, co-surfactant and drug mixtures that spontaneously form oil-in-water (o/w) emulsion when mixed with water under gentle stirring. The motility of stomach and intestine provides the agitation required for self-emulsification *in-vivo* (Shah *et al.*, 1994). This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption (Kommuru *et al.*, 2001). Apart from solubilization, the presence of lipid in the formulation further helps to improve bioavailability by enhancing the drug absorption (Constantinides, 1995). Selection of a suitable self-emulsifying formulation depends upon the assessment of the solubility of the drug in various components and the droplet size distribution of the resultant emulsion following self-emulsification (Kommuru *et al.*, 2001). SEDDS are mostly prepared in liquid dosage form in soft and hard gelatin capsules. Solid SEDDS are new approach to make solid dosage form such as tablets, capsules etc.

Solid dispersion technology is the science of dispersing one or more active ingredients in an inert matrix in the solid stage in order to achieve increased dissolution rate. Solid dispersions are prepared by various methods like fusion method, solvent evaporation method, fusion solvent method and supercritical fluid method (Sekiguchi and Obi, 1961). Solid dispersion method has been widely employed to improve the dissolution rate, solubility and oral absorption of poorly water soluble drugs. Numerous solid dispersion systems have been reported in the pharmaceutical literature along with various hydrophilic carriers, such as polyethylene glycols, polyvinylpyrrolidone, hydroxypropyl methylcellulose, gums, sugar, mannitol and urea (Tanaka *et al.*, 2006).

Most of the new chemical entities (NCE) under development are intended to be used as a solid dosage form through oral route (Serajuddin, 1999; Craig, 2002) as oral drug delivery is the simplest and easiest way of administering drugs. Currently Drugs are most often administered by this route. But oral delivery of approximately 40% of new drug candidates is difficult because of their low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality. Now it is one of the major challenges for the researchers and pharmaceutical companies to synthesize pharmacologically active new molecule, with high solubility and permeability. Drug release is a crucial and limiting step for oral drug bioavailability, particularly for drugs with low gastrointestinal solubility and high permeability. Self emulsifying drug delivery system (SEDDS) and solid dispersions (SDs) in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and therefore possibly bioavailability, for poorly water soluble hydrophobic drugs.

Atorvastatin, simvastatin and rosuvastatin are poorly water soluble drugs and they show dissolution rate limited bioavailability. Oral bioavailability of these drugs is less than 20%. They show high intra and inter-subject variability, and a lack of dose proportionality. In this study, attempts are taken to increase bioavailability of these drugs by SEDDS and SD technique. SEDDS and SD technique have been used previously to improve dissolution rate and oral bioavailability for other poorly water soluble drugs such as gliclazide (Nipun and Islam, 2014), spironolactone (Paul *et al.*, 2012), ibuprofen (Masum *et al.*, 2013), nifedipine (Alam *et al.*, 2013), furosemide (Chaulang *et al.*, 2008), halofantrine (Fattah and Bhargava., 2002) etc.

1.2 Poorly Water Soluble Drugs

The term “solubility” is defined as maximum amount of solute that can be dissolved in a given amount of solvent. Quantitatively it is defined as the concentration of the solute in a saturated solution at a certain temperature. In qualitative terms, solubility may be defined as an interaction of two or more substances to form a homogenous molecular dispersion (Tiwari *et al.*, 2009). Solubility of a drug substance plays a prime role in controlling its dissolution from dosage form. Aqueous solubility of a drug is a major factor which determines its dissolution rate.

The term low soluble or poorly soluble drug is not defined in British Pharmacopoeia (BP, 2014) or United States Pharmacopoeia (USP, 2012). According to BP (2014), very slightly soluble drugs are defined as solubility value of 0.1 mg/ml to 1 mg/ml; insoluble or practically insoluble drugs are those having a solubility value of < 0.1 mg/ml (100 µg/ml). The term low soluble or poorly soluble is defined in different ways in the literature. But all have the almost similar meaning, drugs that show dissolution limited oral absorption are known as poorly water soluble drugs. According to biopharmaceutical classification system (BCS), low solubility means drug will not dissolve in 250 ml of buffer solution throughout the pH range of 1 to 8 (Amidon *et al.*, 1995). According to Lindenberg *et al.* (2004), low solubility means dissolution time of dose will be greater than normal transit time through normal absorption regions of GI tract. A poorly water soluble drug, more recently, has been defined in general terms to require more time to dissolve in the gastrointestinal fluid than it takes to be absorbed in the gastrointestinal tract.

According to the FDA guidance for the industry, for the dissolution testing of immediate release solid oral dosage forms, 85% dissolution in 0.1 N HCl in 15 min ensures that the bioavailability of the drug is not limited by dissolution (FDA, 1997).

A solubility of >10 mg/ml in pH range 1 to 7 has been proposed as an acceptable limit to avoid absorption problems, while another suggestion is that drugs with water solubility less than 0.1 mg/ml often lead to dissolution limitations to absorption (Kaplan, 1972; Hörter and Dressman, 1997). Many new drug candidates are poorly water soluble and they show low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality.

1.3 Biopharmaceutical Classification System (BCS)

Biopharmaceutical classification system (BCS) is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. Biopharmaceutical classification system takes into account three major factors: solubility, intestinal permeability, and dissolution rate, all of which govern the rate and extent of oral drug absorption from immediate release (IR) solid oral-dosage forms (FDA, 2000; Amidon *et al.*, 1995).

Table 1.1: Biopharmaceutical classification system

Class	Solubility	Permeability
Class 1	High	High
Class 2	Low	High
Class 3	High	Low
Class 4	Low	Low

Class I Drugs

Class I drugs exhibit a high absorption and a high dissolution. The rate limiting step is drug dissolution. Gastric emptying rate becomes the rate determining step if dissolution is very rapid. Generally 85% drug is released within 15 min dissolution study. According to FDA (1997) guideline, bioavailability and bioequivalence studies are unnecessary for such products. IVIVC would not be expected for these drugs. Examples include amitriptyline hydrochloride, chloroquine phosphate, chlorpheniramine maleate, chlorpromazine hydrochloride, cloxacillin sodium, phenytoin sodium, prednisolone, promethazine, propranolol hydrochloride, quinine sulfate, verapamil hydrochloride and warfarin sodium etc (Kasim *et al.* 2004).

Class II Drugs

Class II drugs have a high absorption but a low dissolution rate. *In-vivo* drug dissolution is then a rate limiting step for absorption except at a very high dose number. These drug exhibited variable bioavailability and need the enhancement in dissolution for increasing the bioavailability. *In vitro- In vivo* correlation (IVIVC) is usually expected for class II drugs.

Examples include phenytoin, danazol, ketoconazole, mefenamic acid, nifedipine, felodipine, nicardipine, nisoldipine, atorvastatin calcium, simvastatin, rosuvastatin calcium etc (Kasim et al. 2004).

Class III Drugs

Permeability is rate limiting step for drug absorption of class III drugs. These drugs exhibit a high variation in the rate and extent of drug absorption. Since the dissolution is rapid, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage for factors. These drugs are problematic for controlled release development. These drugs showed the low bioavailability and need enhancement in permeability. Examples include cimetidine, ranitidine, acyclovir, alendronate, captopril, enalaprilat neomycin B, atenolol etc (Kasim et al. 2004).

Class IV Drugs

Class IV drugs exhibit poor and variable bioavailability. Several factors such as dissolution rate, permeability and gastric emptying are the rate limiting steps for the drug absorption. These drugs are not suitable for controlled release formulation. Examples include acetazolamide, allopurinol, dapsone, doxycycline, nalidixic acid, sulfadiazine, sulfamethoxazole, trimethoprim etc (Kasim et al. 2004).

Kasim *et al.* (2004) took attempts to classify WHO Essential Drugs (2002) which consists of a total of 325 medicines and 260 drugs, of which 123 are oral drugs in immediate-release (IR) products. Out of the 123 WHO oral drugs in immediate release dosage forms, 67% (82) were determined to be high-solubility drugs. The percentages of the drugs in immediate-release dosage forms that were classified as BCS Class I, Class II, Class III, and Class IV drugs using dose number and log P were as follows: 23.6% in Class I, 17.1% in Class II, 31.7% in Class III, and 10.6% in Class IV. The remaining 17.1% of the drugs could not be classified because of the inability to calculate log P values because of missing fragments.

Atorvastatin, simvastatin and rosuvastatin are considered as BCS II class drug as they are poorly soluble but highly permeable. Drugs having log P value more than 1.72 are considered as highly permeable drugs. Log p of atorvastatin, simvastatin and rosuvastatin are 6.36, 4.86 and 2.4 respectively (Patel *et al.*, 2013).

1.4 Bioavailability

Bioavailability is an important criteria of dosage form. According to Food and Drug Administration guideline, bioavailability is defined as the rate and extent to which an active ingredient is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the blood stream, bioavailability may be assessed by measuring the intended reflect and the rate and extent to which the active ingredient or active moiety becomes available at the site of action (FDA, 2003).

According to World Health Organization guidelines, Bioavailability is defined as: the rate and extent to which an active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action (WHO, 1986).

Comparative Bioavailability

Comparative or relative bioavailability refers to a comparison of two dosage forms in terms of their relative rate and extent of absorption. In some instances, two pharmaceutical alternatives exhibit markedly different bioavailability, for example, a rapidly absorbed elixir and more slowly absorbed capsule. In other cases, two different dosage forms may or may not exhibit very similar bioavailability.

Absolute Bioavailability

When an active pharmaceutical ingredient administered to reach the drug to the systemic circulation, the range of F (Fraction absorbed) may be zero (0) (No drug absorptions) to one (1) (if the drug is completely absorbed in the systemic circulation). Since the total amount of drug reaching the systemic circulation is directly proportional to the area under curve (AUC), F is determined by comparing the respective AUCs of the test product and the same dose of drug administered intravenously (Chereson, 1997; Allam, 2011).

Factors Influencing Bioavailability

The systemic absorption of an orally administered drug can alter the drugs bioavailability and thereby its therapeutic effects. There are many factors that can affect bioavailability. The factors can be broadly classified as dosage form related or patient related. Some of the important factors are as follows:

Disintegration of the drug product

Disintegration time measures the rate of breakup of the tablet or the capsule into the drug granules. Disintegration time of a tablet is a poor measure of the bioavailability of the contained drug. This is because, in addition to disintegration time and particle size, other factors such as crystalline form (polymorphism), saturation solubility of a drug may also affect bioavailability. The dissolution rate is perhaps a better parameter.

Dissolution of the drug in the fluid at the absorption site

The dissolution rate is the rate at which the drug goes into solution. After dissolution drug can pass the biological membrane. Poorly water soluble drug shows dissolution rate limited bioavailability.

Transfer of drug molecule across the membrane

Bioavailability depends on the capacity of drug molecule to cross the biological membrane. The availability of drug into the portal system or intestinal mucosa represents an upper limit to the amount of drug that can reach the systemic circulation. But It is difficult to make direct measurements the amount of drug the across the membrane of GI tract.

Study Design and Type of Studies

The pattern of bioavailability study is designed in such a way that the effect of formulation can be easily distinguished from other effect. For instance, if two formulations are to compare, through an open label, balanced, randomized model, two way cross over design is the design of choice. Generally in practice there are two types (fasting and fed) of study were under taken under bioavailability and bioequivalence studies.

Fasting Study

Subjects are fasted for 10 hours prior to product administration. Normally, the highest safe strength/dose of the test or reference product should be administered on the experimental day with about 250 ml water. Further fluid will be withheld for 2 hours standardized meals are to be permitted after four hours after drug administration.

Fed Study

This type of study is performed under fed conditions, the composition of the meal is recommended to be according to the protocol of the originator product. If no specific recommendation is given in the originator protocol, the meal should be a high-fat (approximately 50 percent of total caloric content of the meal). The composition of the meal should be described with regard to protein, carbohydrate and fat.

1.5 Self Emulsifying Drug Delivery System (SEDDS)

Self-emulsifying drug delivery system (SEDDS) are relatively newer lipid-based technological innovations with immense promise in oral bioavailability enhancement of lipophilic drugs. SEDDS are defined as isotropic mixtures of drug, natural or synthetic oils, solid or liquid surfactants or alternatively, one or more hydrophilic solvents and co-solvents/co-surfactants that have a unique ability of forming fine oil-in-water (o/w) microemulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids (Katteboina *et al.*, 2009). The resultant small droplet size from SEDDS provides a large interfacial surface area for drug release and absorption, and the specific components of SEDDS promote the intestinal lymphatic transport of drugs. SEDDS is a broad term typically producing emulsions with a droplet size ranging between a few nanometers to several microns. Depending upon the size of droplets, they may be microemulsions, or nanoemulsions (Joshi *et al.*, 2008). Self-microemulsified drug delivery system (SMEDDS) indicates the formulations forming transparent microemulsions with the oil droplet size range between 100 and 250 nm. Self-nanoemulsified drug delivery system (SNEDDS) is relatively a recent term indicating the globule size less than 100 nm (Pouton and Porter, 2008).

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

lipid vehicle there is less potential for precipitation on dilution in the GI tract, as partitioning kinetics will favor the drug remaining in the lipid droplets (Amidon *et al.*, 1995).

Ease of manufacturing and scale up is one of the most important advantages that make lipid based formulations unique when compared to other bioavailability techniques like solid dispersions, liposomes and nanoparticles. SEDDS require very simple and economical manufacturing facilities like simple mixer with agitator and volumetric liquid filling equipment for large-scale manufacturing (Vilas *et al.*, 2013). Lipid based formulations especially SEDDS also provide the advantage of increased drug loading capacity when compared with conventional lipid solution as the solubility of poorly water soluble drugs with intermediate partition coefficient ($2 < \log P < 4$) are typically low in natural lipids and much greater in amphiphilic surfactants, co-surfactants and co-solvents (Shukla *et al.*, 2010). SEDDS overcomes the drawback of layering of emulsions after stored for a long time. SEDDS can be stored easily because it is classified to a thermodynamics stable system (Sheth and Mistry, 2011). For the drug substances which are not soluble in both organic and aqueous solvents, complexation with cyclodextrin technique is not applicable. SEDDS has higher drug solubilization capacity.

Selective targeting of drug toward specific absorption window in GIT is also possible with SEDDS (Patel *et al.*, 2008). There are several drugs which show large inter-subject and intra-subject variation in absorption leading to decrease performance of drug and patient non-compliance. SEDDS offers reproducibility of plasma profile (Shukla *et al.*, 2010). Lack of good predicative *in-vitro* models for assessment of the formulations is a disadvantage of SEDDS as traditional dissolution methods do not work due to dependence on digestion prior to release of the drug (Porter and Charman, 2001). To mimic this, an *in-vitro* model simulating the digestive processes of the duodenum has been developed. The large quantity of surfactant in self emulsifying formulations (30-60%) irritates GIT. Volatile co-solvents in the conventional self-emulsifying formulations migrate into the shells of soft or hard gelatin capsules, resulting in the precipitation of the lipophilic drugs. (Shukla *et al.*, 2010). The precipitation tendency of the drug on dilution may be higher due to the dilution effect of the hydrophilic solvent. Further development of formulation is based only on *in-vitro* and *in-vivo* correlations (Shukla *et al.*, 2010).

1.5.1 Excipients Used in SEDDS Formulation

SEDDS formulations generally contain the following components:

Oils

Oil represents one of the most important excipients in the SEDDS formulation because it can solubilize the required dose of the lipophilic drug, facilitate self emulsification, increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the oil (Kimura *et al.*, 1994). The melting point of oil increases with increase in degree of un-saturation that also increases the relative susceptibility to oxidation. Triglycerides are synthetically hydrogenated to decrease the degree of un-saturation. Some examples of the oils used in the marketed preparations are cited in the Table 1.2.

Table 1.2: Oils used in marketed SEDDS

Type of oil	Marketed Product	Drug
Corn oil	Depakene capsule	Valproic acid
Olive oil	Sandimmune oral solution	Cyclosporine
Sesame oil	Marinol soft gelatin capsule	Dronabinol
Soya bean oil	Accutane soft gelatin capsule	Isotretinoin
Peanut oil	Prometrium soft gelatin capsule	Progesterone
Bees wax	Vesanoid soft gelatin capsule	Tretinoin
Hydrogenated soya bean oil	Accutane soft gelatin capsule	Isotretinoin

Both long and medium chain triglyceride (LCT and MCT) oils with different degrees of saturation have been used for the design of self-emulsifying formulations. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification systems with a large number of surfactants approved for oral administration and exhibit better drug solubility properties (Shukla *et al.*, 2010). They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semi-synthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties,

are progressively and effectively replacing the regular medium chain triglyceride (MCT) oils in the SEDDS (Lawrence and Rees, 2000).

Surfactants

Several surfactants are used in self-emulsifying systems. The most widely recommended ones being the non-ionic surfactants with a relatively high hydrophilic-lipophilic balance (HLB). The commonly used emulsifiers are various solid or liquid ethoxylated polyglycolized glycerides and polyoxyethylene 20 oleate. Safety is a major determining factor in choosing a surfactant. Emulsifiers of natural origin are preferred since they are considered to be safer than the synthetic surfactants (Kimura *et al.*, 1994). However, these surfactants have a limited self-emulsification capacity. Non-ionic surfactants are less toxic than ionic surfactants but they may lead to reversible changes in the permeability of the intestinal lumen (Lawrence and Rees, 2000). The usual surfactant strength ranges between 30–60% w/w of the formulation in order to form a stable SEDDS. The lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS (Hauss *et al.*, 1998). The surfactants being amphiphilic in nature can solubilize higher quantities of hydrophobic drug. This can prevent precipitation of the drug within the GI lumen and for prolonged existence of drug molecules (Crison and Amidon, 1999). Some of the surfactants used in the marketed preparations are given in the Table 1.3.

Table 1.3: Surfactants used in marketed SEDDS

Surfactant	Marketed Product	Drug
Span 80, Tween 80	Gengraf soft gelatin capsule	Cyclosporine
Tween 20	Targretin Hard gelatin Capsule	Bexarotene
Cremophor RH 40	BCNU self emulsifying implant	Carmustine
Labrafil M 1944 CS	Sandimmune oral solution	Cyclosporine

There is a relationship between the droplet size and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to droplets with smaller mean droplet size, this could be explained by the stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil-water interface (Karim *et al.*, 1994). On the other hand, in some cases the mean droplet size may increase

with increasing surfactant concentrations. The surfactants used in these formulations are known to improve the bioavailability by various mechanisms including: improved drug dissolution, increased intestinal epithelial permeability, increased tight junction permeability and decreased/inhibited p- glycoprotein drug efflux. However, the large quantity of surfactant may cause moderate reversible changes in intestinal wall permeability or may irritate the GI tract (Shukla *et al.*, 2010).

Surfactant molecules may be classified based on the nature of the hydrophilic group within the molecule (Shukla *et al.*, 2010). The four main groups of surfactants are defined as follows:

Anionic surfactants: Where the hydrophilic group carries a negative charge such as carboxyl (RCOO⁻), sulphonate (RSO₃⁻) or sulphate (ROSO₃⁻). Examples: Potassium laurate, sodium lauryl sulphate.

Cationic surfactants: Where the hydrophilic group carries a positive charge. Example: quaternary ammonium halide.

Ampholytic surfactants: (also called zwitterionic surfactants) contain both a negative and a positive charge. Example: sulfobetaines.

Nonionic surfactants: where the hydrophilic group carries no charge but derives its water solubility from highly polar groups such as hydroxyl or polyoxyethylene (OCH₂CH₂O). Examples: Sorbitan esters (Spans), polysorbates (Tweens).

Co-surfactant

The production of an optimum SEDDS requires relatively high concentrations (generally more than 30% w/w) of surfactants, thus the concentration of surfactant can be reduced by incorporation of co-surfactant. Generally co-surfactant of HLB value 10-14 is used with surfactant together to lower the interfacial tension to a very small even transient negative value. The selection of surfactant and co-surfactant is crucial not only to the formation of SEDDS, but also to solubilization of the drug in the SEDDS. Hydrophilic co-surfactants are preferably alcohols of intermediate chain length such as hexanol, pentanol, and octanol which are known to reduce the oil water interface and allow the spontaneous formation of microemulsion (Revathi and Raju, 2012).

Co-solvent

Co-solvents like diethylene glycol monoethyle ether, PEG 400, propylene glycol, polyethylene glycol, polyoxyethylene, propylene carbonate, tetrahydrofurfuryl alcohol polyethylene glycol ether 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-surfactant in the micro emulsion systems. These excipients are widely used in soft gelatin capsule formulations but find limited use in conjunction with hard gelatin capsules due their hygroscopic and resultant effects on gelatin moisture content, which can compromise capsule physical integrity. Propylene glycol, a pharmaceutically acceptable, monomer solvent possessing humectants and plasticizing properties, finds application for soft gelatin capsule formulations of poorly water-soluble drugs (Sheth and Mistry, 2011).

Consistency Builder

Additional material can be added to alter the consistency of the emulsion; such materials include tragacanth, acetyl alcohol, stearic acid and/or beeswax etc (Revathi and Raju, 2012).

Polymers

Inert polymer matrix representing from 5 to 40% of composition relative to the weight, which is not ionizable at physiological pH and being capable of forming matrix are used. Examples are hydroxy propyl methyl cellulose, ethyl cellulose, etc (Vilas *et al.*, 2013).

1.5.2 Formulation of SEDDS

Apparent solubility of drug is determined in different oils, surfactants and co-surfactants at ambient temperature. Based on solubility data, excipients are selected and formulated in SEDDS with varying ratios of surfactant and co-surfactant by mixing the components.

The addition of a drug to a SEDDS is critical because the drug interferes with the self-emulsification process to a certain extent, which leads to a change in the optimal oil-surfactant ratio. So, the design of an optimal SEDDS requires preformulation-solubility and phase-diagram studies. In the case of prolonged SEDDS, formulation is made by adding the polymer or gelling agent (Nazzal and Khan, 2006).

Construction of Pseudo Ternary Phase Diagram

The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. Compositional variables can also be studied as a function of temperature and, pressure, although with the exception of microemulsion prepared using supercritical or near critical solvents, or with liquefied chlorofluorocarbon and HFA propellants. The phase behavior of simple microemulsion systems comprising oil, water and surfactant can be studied with the aid of ternary phase diagram in which each corner of the diagram represents 100% of that particular component (Vilas *et al.*, 2013).

In the case where four or more components are investigated, pseudo-ternary phase diagrams are used where a corner will typically represent a binary mixture of two components such as surfactant / Co-surfactant, water /drug or oil / drug. The number of different phases present for a particular mixture can be visually assessed. Phase diagrams are used to determine the number and types of phases, the wt % of each phase and the composition of each phase.

A Titration method is generally employed to construct phase diagram. Mixture of oil with surfactant is prepared at different ratios (e.g. 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8,1:9, 0:10) into different vials. A small amount of water in 5 % (w /w) increments is added into the vials. The resulting mixture is evaluated by visual and microscopy observation. For phase diagram the micro emulsion is the region of clear and isotropic solution.

1.5.3 Self-Emulsification Characterization

A number of tests are carried out for characterization and evaluation of SEDDS.

Rheological Properties Determination

The SEDDS system is generally administered in soft gelatin capsules, where, it should have appreciable flow properties for processing. The rheological properties (viscosity, flow, thixotropy, static yield, creep value) of formulation (diluted to 5 % v/v water) are determined by rotational viscometers, digital instruments coupled with either cup and bob or coaxial measuring device (Atef and Belmonte, 2008). A type of rotational viscometer has also been used for determination of viscosity of fresh as well as other SEDDS formulations which has been stored for longer duration of time (Cirri *et al.*, 2007).

Viscosity determination of liquid SEDDS also indicates whether the system is o/w or w/o, as low viscosity systems are o/w and high viscosity systems are usually w/o in nature. Viscosity of formulation is inversely proportional to dilution.

Thermodynamic Stability Studies

The physical stability of a formulation is very important for its performance as it can be adversely affected by precipitation of the drug in excipients matrix. Poor physical stability of formulation can lead to phase separation of excipients which affects bioavailability as well as therapeutic efficacy. Also the incompatibilities between formulation and gelatin shell of capsule (if formulation filled in capsule) may cause brittleness, softness and delayed disintegration or incomplete release of drug. The following cycles are carried out for these studies-

- (i) Heating cooling cycle: - Six cycles of cooling and heating between refrigerator temperature (4°C) and elevated temperature (45°C) with exposure at each temperature for not less than 48 hours are carried out. Those formulations, which are stable, are then subjected to centrifugation test (Shafiq *et al.*, 2007).
- (ii) Centrifugation: - Formulations which pass the heating cooling cycle are centrifuged at 3500 r/min for 30 min. Those formulations that doesn't show any phase separation are taken for the freeze thaw stress test.
- (iii) Freeze thaw stress cycle:- Three freeze thaw cycles b/w -21° C and 25° C with storage at each temperature for not less than 48 hours. Those formulations which pass this test show good stability with no phase separation, cracking or creaming. The formulations that pass this test are then further taken for dispersibility test for assessment of self emulsification efficiency (Bachhav and Patravale, 2009).

Robustness to Dilution

Nanoemulsions, resulting from dilution with various dissolution media, must be robust to all dilutions, and should not show any phase separation or drug precipitation even after 12h of storage (Patel and Sawant, 2007; Date and Nagarsenker, 2007).

Dispersibility Test

The dispersibility test of SEDDS is carried out to assess its capability to disperse into emulsion and the size of resulting droplets to categorize them as SNEDDS. It is carried by

using a standard USP dissolution apparatus II (Paddle Type) (Shafiq *et al.*, 2007; Pouton, 1985). 1 ml of each formulation is added to 500 ml of water at $37 \pm 0.5^\circ\text{C}$ and A standard stainless steel dissolution paddle is rotated at 50 rpm. The *in-vitro* performance of the formulations is visually assessed from such dispersion, using a suitable grading system (Shafiq *et al.*, 2007). A grading system has been reported to be based upon the formation of a microemulsion (o/w or w/o), microemulsion gel, emulsion or emulgel.

Turbidimetric Evaluation

Turbidity is a parameter for determination of droplet size and self emulsification time. Fixed quantity of SEDDS is added to fixed quantity of suitable medium (0.1 N HCl or phosphate buffer) under continuous stirring at 50 rpm on magnetic stirrer at optimum temperature and the turbidity is measured using a turbidimeter. Since the time required for complete emulsification is too short, it is not possible to monitor the rate of change of turbidity i.e. rate of emulsification (Atef and Belmonte, 2008). Turbidimetric evaluation is carried out to monitor the growth of droplet after emulsification.

Droplet Size Analysis

This is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as the stability of the emulsion. Photon Correlation Spectroscopy (PCS) or Dynamic Light Scattering (DLS) or Laser Diffraction Techniques are used to determine droplet size of emulsion. A number of equipments are available for measurement of particle size viz. Particle Size Analyzer, Mastersizer, Zetasizer etc which are able to measure sizes between 10 and 5000 nm. In many instances nanometric size range of particle is retained even after 100 times dilution with water which indicates the system's compatibility with excess water (Atef and Belmonte, 2008).

Self Emulsification Time

The self emulsification time is determined by using USP dissolution apparatus II at 50 rpm, where 0.5 g of SEDDS formulations is introduced into 250 ml of 0.1N HCl or 0.5% SLS solution. The time for emulsification at room temperature is indicated as self emulsification time for the formulation. Less emulsification time indicates good dispersibility. The self emulsification time depends on the amount of surfactants and co-surfactants.

Zeta Potential Determination

The stability of emulsion is directly related to the charge present on mobile surface, which is termed as zeta potential. Zetasizer, Mastersizer etc are often used to determine zeta potential. The Zetasizer uses light scattering techniques to determine globule size, zeta potential and molecular weight of nanoparticulate systems. The instrument determines size and zeta potential for optimization of stability and shelf life and speeding up the formulation development. The SEDDS formulation is generally diluted in a ratio of 1: 2500 (v/v) with distilled water with constant stirring for determination of zeta potential.

Liquefaction Time

This test is done to determine the time required by solid SEDDS formulation to melt *in-vivo* in the absence of agitation in simulated gastric fluid. The formulation is packed in a transparent polyethylene film and tied to the bulb of thermometer. The thermometer is then placed in round bottom flask in which simulated gastric fluid without pepsin is filled. The temperature is maintained at $37 \pm 0.5^{\circ}\text{C}$ by using heating mantle.

Refractive Index (RI) and Percent Transmittance

Refractive Index and percent transmittance are determined to check the transparency of formulation. Refractive Index of the formulation is measured by refractometer by placing drop of solution on slide and then compare it with water (RI=1.333). The percent transmittance of the formulation is measured at a particular wavelength using UV spectrophotometer by using distilled water as blank (Patel *et al.*, 2008). If RI of formulation is similar to that of water and formulation having percent transmittance is greater than 99%, then the formulation are transparent in nature.

Differential Scanning Calorimetric Analysis

Differential scanning calorimetric analysis for SMEDDS can be determined using DSC. Liquid sample and solid sample should be placed in the aluminum pan and result can be recorded. Any type of chemical interaction can be determined using DSC.

***In-Vitro* Diffusion Study**

This study is done to determine release behavior of formulation using dialysis technique where phosphate buffer (pH 6.8) is generally used as dialysing medium. One end of the

dialysis membrane is tied with a thread and 1 ml of the SEDDS formulation along with 0.5 ml of dialysing medium are filled in the membrane. The other end of membrane is also tied with thread and then allowed to rotate in dialyzing medium at 100 rpm using magnetic stirrer or dissolution apparatus. Samples are withdrawn at different time intervals and then after suitable dilution are analyzed. Volume of samples withdrawn is replaced with fresh dialysing medium (Patil *et al.*, 2004).

***In-Vitro* Dissolution Study**

The quantitative *in-vitro* dissolution studies are carried out to assess drug release from oil phase into aqueous phase by USP type II dissolution apparatus using 500 ml of simulated gastric fluid containing 0.5% w/v of SLS (Sodium Lauryl Sulphate) at 50 rpm and maintaining the temperature at $37 \pm 0.5^\circ\text{C}$. Samples taken are then analyzed by using UV spectrophotometer or any other suitable technique (Vilas *et al.*, 2013; Sapra *et al.*, 2012).

Permeation Studies

For information about oral bioavailability enhancement of a formulation, one must have to perform *in-vitro* or *ex-vivo* studies. For these studies, isolated and perfused organ systems have been developed (Nipun and Islam., 2014). These organ systems have the advantage that research scientist works with an intact organ, where physiological cells remain in contacts intracellular matrices are preserved (Levet-Trafit *et al.*, 1996). A number of techniques are available for such *in-vitro* studies. First is *In-Situ* Single Pass Perfusion Technique (SPIP) in which perfusion solution is passed through the jejunum (a part of intestine) and the experimental conditions provided are closer to the *in-vivo* conditions. This technique is also able to determine exact absorption mechanism that is passive or active or carrier mediated absorption. Permeability parameters are determined by calculating the amount of drug which is not absorbed from intestine (Sapra *et al.*, 2012).

Second technique is everted sac technique in which a small part of intestine (2-4 cm) is tied at one end and everted using a glass rod or thread. The technique is used to determine kinetic parameters. In the presence of sensitive detection methods (such as radiolabelled compounds), drug transport across the intestine and through the epithelial cells can be studied (Leppert and Fix, 1994). The method is suitable for calculating absorption at

different sites in small intestine and estimating the first pass metabolism of xenobiotics in intestinal epithelial cells. The limitation of this technique is that muscularis mucosa is present which is usually not removed from everted sac preparations. That is why this method is not preferred for accurate determinations.

Third technique is Diffusion cell technique in which diffusion across a small part of intestine or any other tissue (such as buccal, rectal, skin, lung, gastric) is studied using the media with specific pH and temperature conditions. On both sides of diffusion membrane, buffer solution is continuously gassed with carbogen.

1.5.4 Factors Affecting SEDDS

Polarity of Lipophilic Phase

The polarity of the lipid phase is one of the main factors that govern the drug release from the micro-emulsions. The polarity of the droplet is governed by the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular weight of the hydrophilic portion and the concentration of the emulsifier. In fact, the polarity reflects the affinity of the drug for oil and/or water, and the type of forces formed. The high polarity will promote a rapid rate of release of the drug into the aqueous phase. This is confirmed by the observations of Sang-Cheol Chi, who observed that the rate of release of idebenone from SEDDS is dependent upon the polarity of the oil phase used (Kim *et al.*, 2000). The highest release was obtained with the formulation that had oil phase with highest polarity (Kyatanwar *et al.*, 2010).

Nature and Dose of the Drug

Drugs which are administered at very high dose are not suitable for SEDDS unless they have extremely good solubility in at least one of the components of SEDDS, preferably lipophilic phase. The drugs which have limited or less solubility in water and lipids are most difficult to deliver by SEDDS. The ability of SEDDS to maintain the drug in solubilised form is greatly influenced by the solubility of the drug in oil phase. As mentioned above if surfactant or co-surfactant is contributing to the greater extent in drug solubilisation then there could be a risk of precipitation, as dilution of SEDDS will lead to lowering of solvent capacity of the surfactant or co-surfactant. Equilibrium solubility measurements can be carried out to anticipate potential cases of precipitation in the gut.

However, crystallisation could be slow in the solubilising and colloidal stabilizing environment of the gut. Pouton's study reveal that such formulations can take up to five days to reach equilibrium and that the drug can remain in a super-saturated state for up to 24 hours after the initial emulsification event. It could thus be argued that such products are not likely to cause precipitation of the drug in the gut before the drug is absorbed, and indeed that super-saturation could actually enhance absorption by increasing the thermodynamic activity of the drug. There is a clear need for practical methods to predict the fate of drugs after the dispersion of lipid systems in the gastro-intestinal tract (Kyatanwar *et al.*, 2010).

1.5.5 Mechanism of Enhancing Bioavailability

Enhancement of Permeability

Permeability of drugs is influenced by interplay of complex set of barriers that are specific to each drug molecule. Further studies are needed to better understand these mechanisms in relation to different types of lipids. However, there is strong evidence confirming the role of lipids in fluidization of intestinal cell membranes by caprylocaproyl polyoxyglycerides (Labrasol[®]) (Koga *et al.*, 2006), ethoxylated castor oil (Cremophor[®] EL) or polysorbates 80 (Tween[®] 80) (Rege *et al.*, 2002); opening of tight junctions by Labrasol[®] and inhibition of efflux mechanisms with most of the lipid-based surfactants i.e. d-alpha-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) (Collnot *et al.*, 2007; Rege *et al.*, 2002.), polyoxyglycerides and PEG esters like Labrasol[®], Gelucire[®] 44/14, Mirj[®] 52 or Solutol[®] HS 15, esters of sorbitan (Ethoxylated or not) (Collnot *et al.*, 2007; Rege *et al.*, 2002; Cornaire *et al.*, 2004) or even partial glycerides. Properties needed by lipid-based excipients to inhibit P-gp are: water solubility, ester bonds, medium chain fatty acids and polyoxyethylene groups. Labrasol[®], an excipient possessing all the after mentioned properties was identified as the most effective excipient among the 10 products tested by Cornaire *et al.*(2004).

Lymphatic Absorption

The lymphatic route of absorption offers a window of opportunity to enhance the bioavailability of highly lipophilic drugs (Log P>5) with high solubility in triglycerides (Cs>50 mg/ml). Lipid systems consisting of alcohol esters of unsaturated long chain fatty

acids (LCFA) have been shown to enhance the bioavailability of certain drugs by facilitating lymphatic route of uptake. Examples of successes include saquinavir with polyglyceryl oleate (Plurol® Oleique CC497) or ethoxylated castor oil (Cremophor® EL) (Griffin and O'Driscoll, 2006), ontazolast with glyceryl monooleate (Peceol™) (Hauss *et al.*, 1998), and halofantrin with various triglycerides and derivatives (Charman and Stella, 1991; O'Driscoll, 2002).

Enhancing *in-vivo* Solubility

The primary role of lipids in oral delivery is solubilisation and emulsification of drug in the gastro-intestinal tract. In screening for a suitable lipid, the conventional tendency is to include or alternatively exclude excipients merely based on solubility data which often do not correlate with the physiological behaviour of the dosage form. A drug solubilised in hydrophilic cosolvents (PEG, propylene glycol, ethanol, etc) once in the gastro-intestinal tract, is subjected to dilution by the surrounding physiological fluids which can lead to rapid loss in solubility and precipitation of the drug. An emulsified drug, on the other hand, is less prone to precipitation or binding with other elements. Hence, the emulsification or micellar dispersion of the drug in the gastro-intestinal milieu and not the solubility in the dosage form alone should be of primary concern. Once a list of suitable excipients is identified, a binary drug-excipient screening for solubility, compatibility, and stability should follow in order to identify the lipid system(s) most appropriate for the drug in question. For design of SMEDDS or SNEDDS which require multiple excipients, although it is necessary to assess the relative solubility and affinity of the drug for each component, the focus should be the overall solubilising/dispersing power of the system and not so much as the solubility of the drug in the individual components. Whether the aim is to achieve drug solubility that is sustainable *in-vivo*; protection of the drug against binding to elements in the gastro-intestinal tract; inhibition or saturation of efflux and metabolic barriers; and or promotion of lymphatic transport, defining the main objectives will help narrow the focus on the appropriate type of excipient(s) to address the set of barriers specific to the drug molecule. Successful LBDD hence requires a holistic approach to formulation. A systemic elucidation of the rationale may be achieved by i) pre-selecting excipients for their fatty acid make up, melt characteristics, HLB or emulsification properties, potential effect on enterocytes-based drug transport and

disposition and overall digestibility; ii) conducting binary screening with pre-selected excipients for drug solubility, compatibility, stability and dissolution/ dispersion properties (in biorelevant media) to identify one or more suitable systems for further studies; iii) identifying the formulation technique(s) suitable for the dosage form intended; iv) confirming the *in-vivo* performance of the chosen formulation system(s) in appropriate animal models; v) optimizing the formulation for drug loading or dissolution profile and if necessary, gain control of the oxidative and polymorphic changes (Cade and Madit, 1996; Cole, 1989).

1.6 Solid Dispersion (SD)

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, in amorphous particles or crystalline particles (Chiou and Riegelman, 1971). The concept of solid dispersions was originally proposed by Sekiguchi and Obi (1961), who investigated the generation and dissolution performance of eutectic melts of a sulfonamide drug and a water-soluble carrier in the early 1960s. The solid dispersion is based on the concept that the drug is dispersed in an inert water soluble carrier at solid state. Several water soluble carriers, such as methyl cellulose, urea, lactose, citric acid, polyvinyl pyrrolidone and polyethylene glycols 4000 and 6000 are used as carriers for solid dispersion. The most commonly used hydrophilic carriers for solid dispersions include polyvinylpyrrolidone (povidone, PVP), polyethylene glycols (PEGs), surfactants like tween-80, pluronic-F68, and sodium lauryl sulphate (SLS) etc (Gupta *et al.*, 2004; Fattah and Bhargava, 2002; Sinha *et al.*, 2010).

Solid dispersion is one of the techniques for the improvement of solubility of poorly water soluble drugs. Solubility behavior is the most challenging aspect for various new chemical entities. Approximate 40% of new chemical entities (NCE) being synthesized by combinatorial screening programs possessing superior pharmacological activities are poorly soluble, which is a great obstacle in formulation development. This is the biggest reason for new drug molecules not reaching to the market or not reaches to full potential. Solid dispersion is an important approach for improvement of bioavailability of poorly water-soluble drugs. Solid dispersion has been widely used to improve the dissolution

rate, solubility, and oral absorption of poorly water-soluble drugs. Solid dispersion was first introduced to overcome the low bioavailability of lipophilic drugs by forming eutectic mixture of drugs with water soluble carriers.

Poor aqueous solubility and bioavailability of drugs into the body after administration are two prime issues which are faced by the pharmaceutical industry at the present time. This problem has been the major problem hampering the release of new chemical entities into the market. Every year more than 50% of the potentially active pharmaceutical ingredients get rejected due to the above stated problems. During the last decade, more than 40% of the new chemical entities launched in the U.S. pharmaceutical market faced the problem of adequate aqueous solubility. Therefore, pharmaceutical companies are focusing on finding a method or technology by which they can enhance the aqueous solubility and bioavailability of the drug. Solid dispersion is one of these methods, which was most widely and successfully applied to improve the solubility, dissolution rates and consequently the bioavailability of poorly soluble drugs. Solid dispersion technique was successfully used for the improvement of dissolution of paracetamol with polyvinyl pyrrolidone as carrier (Simonelli *et al.*, 1969).

The formulation of solid dispersion of a poorly water soluble drug has several advantages which include the enhancement of aqueous solubility which may lead to increased bioavailability. Along with this advantage, solid dispersion techniques also have a few disadvantages which hamper their extensive use in the improvement of the pharmaceutical formulation. These disadvantages include poor flowability, poor compressibility, and most importantly is conversion from amorphous state to crystalline state in solid dispersion during storage of SD. All the disadvantages limit the use of solid dispersions in the pharmaceutical industry. This limitation can be overcome by adsorbing the melt, which is made up of the drug and dispersion carrier, to an inert core material which is compressible and has good flowability. Various adsorbents such as talc, fumed silicon dioxide and many more can be used.

Solid dispersions are generally formulated as tablet or capsules. Properties of tablet prepared from solid dispersion depend on the physio-chemical nature of the solid dispersions. Solvent residue can also affect the properties of tablets. Tablets of desired properties can be prepared by adding appropriate excipients in the formulations.

1.6.1 Classification of Solid Dispersion

Physicochemical Classifications of Solid Dispersion

Simple eutectic mixtures: These are prepared by rapid solidification of the fused melt of two components that show complete liquid miscibility and negligible solid solubility. Thermodynamically, such a system is an intimately blended physical mixture of two crystalline components. Thus the X-ray diffraction pattern of a eutectic constitutes will be an additive composite of two components (Hörter and Dressman., 1997). chloramphenicol-urea; paracetamol-urea; griseofulvin and tolbutamide-PEG 2000 are examples of eutectic mixtures.

Solid solutions: In a solid solution the two components crystallize together in a homogeneous one phase system. The particle size of the drug in the solid solution is reduced to its molecular size. Thus, a solid solution can achieve a faster dissolution rate than the corresponding eutectic mixture. Solid solutions can be classified by two methods. According to the extent of miscibility of the two components, they may be classified as continuous or discontinuous. In continuous solid solutions, the two components are miscible in the solid state in all proportions (Kaur *et al.*, 2012).

Continuous solid solutions

In a continuous solid solution the components are totally miscible with one another in all proportions in both the liquid and solid state. The lattice energy of the continuous solid solution at all compositions is higher than that of the respective pure components in the solid state, because the heteromolecular bonding strength is higher than the homomolecular bonding strength in order to form a continuous solid solution (Giri *et al.*, 2010).

Discontinuous solid solution

In discontinuous solid solutions, the miscibility or solubility of one component in the other is limited. Below a certain temperature, the mutual solubilities of the two components start to decrease. Goldberg *et al.* (1965) showed that the term solid solution should only be applied when the mutual solubility of the two components exceeds 5%. Formulation of dosage form with solid solution will depend on both the mutual solubilities of the two components and dose of the drug component. The maximum limit

of a tablet or capsule is about 1 gm. Assuming that the solubility of the drug in the carrier is 10%, doses of above 100 mg would not be feasible with this strategy. If the drug solubility in the carrier is significantly higher than 10%, larger doses can be entertained.

Current Trends in Solid Dispersion Techniques

New manufacturing processes to obtain solid dispersions have also been developed to reduce the draw backs of the initial process. It is intended to discuss the recent advances related on the area of solid dispersions. According to implementation and recent advancement SD may be classified as first, second and third generation solid dispersion.

First generation solid dispersions

The first description of solid dispersions was from Sekiguchi and Obi in 1961. They noted that the formulation of eutectic mixtures improves the rate of drug release and consequently, the bioavailability of poorly water soluble drugs. In the same decade, several solid dispersions were described using poorly water soluble drugs, such as sulfathiazole (Sekiguchi and Obi, 1961) and chloramphenicol (Sekiguch and Obi, 1964) using urea as high water soluble carrier. These solid dispersions produced faster release and higher bioavailability than conventional formulations of the same drugs. The small particle size and the better wet ability of the drug were the main reasons for the observed improvements in bioavailability. The observed improvements were attributed to faster carrier dissolution, releasing microcrystals or particles of drug (Simonelli *et al.*, 1969). These solid dispersions, which could be designed as first generation solid dispersions, were prepared using crystalline carriers. Crystalline carriers include urea (Sekiguchi and Obi, 1961; Sekiguchi and Obi, 1964) and sugars, which were the first carriers to be employed in solid dispersions. They have the disadvantage of forming crystalline solid dispersions, which were more thermodynamically stable and did not release the drug as quickly as amorphous ones.

Second generation solid dispersions

In the late sixties it was observed that solid dispersions, where the drug was maintained in the crystalline state, might not be as effective as the amorphous, because the former were more thermodynamically stable (Urbanetz, 2006). Therefore, a second generation of solid dispersions appeared, containing amorphous carriers instead of crystalline. Indeed, the

most common solid dispersions do not use crystalline carriers but amorphous. In the latter, the drugs are molecularly dispersed in an irregular form within an amorphous carrier, which are usually polymers (Vilhelmsen, 2005). Polymeric carriers have been the most successful for solid dispersions, because they are able to originate amorphous solid dispersions. They are divided into fully synthetic polymers and natural product-based polymers. Fully synthetic polymers include povidone (Karavas, 2006; Drooge, 2006; Pokharkar, 2006; Hasegawa, 2005; Lloyd, 1999; Yoshihashi, 2006), polyethyleneglycols (Urbanetz, 2006) and polymethacrylates. Natural product based polymers are mainly composed by cellulose derivatives, such as hydroxypropyl methyl cellulose (HPMC), ethylcellulose or hydroxypropyl cellulose (Tanaka, 2005) or starch derivatives, like cyclodextrins (Won, 2005; Tanaka, 2005; Garcia-Zubiri, 2006). Amorphous solid dispersions can be classified according to the molecular interaction of drug and carriers in solid solutions, solid suspensions or a mixture of both (Drooge, 2006). In amorphous solid solutions, drug and carrier are totally miscible and soluble, originating a homogeneous molecular interaction between them (Van, 2006). In these systems, the drug and carrier interaction energy is extremely high, resulting in a really true solution. The use of polymers in the preparation of a true solid solution creates an amorphous product in which the crystalline drug is dissolved (Vanden, 2006). This type of amorphous solid dispersion is homogeneous on a molecular level. Therefore, only one phase is present (Van, 2006). Amorphous solid suspensions occur when the drug has limited carrier solubility or an extremely high melting point. Molecularly, the obtained dispersion does not have a homogeneous structure, but is composed of two phases. Small drug particles, when dispersed in polymeric carriers, are able to provide an amorphous final product. When a drug is both dissolved and suspended in the carrier, a heterogeneous structure is obtained with mixed properties of amorphous solid solutions and amorphous solid suspensions (Van, 2006; Goldberg *et al.*, 1966). In second generation solid dispersions, the drug is in its supersaturated state because of forced solubilization in the carrier. These systems are able to reduce the drug particle size to nearly a molecular level, to solubilize or co-dissolve the drug by the water soluble carrier, to provide better wettability and dispersibility of the drug by the carrier material, and to produce amorphous forms of the drug and carriers. In these solid dispersions, the carrier dissolution (or mixtures of carriers) dictates the drug release profile.

Third generation solid dispersions

Recently, it has been shown that the dissolution profile can be improved if the carrier has surface activity or self-emulsifying properties, therefore third generation solid dispersions appeared. These contain a surfactant carrier, or a mixture of amorphous polymers and surfactants as carriers. These third generation solid dispersions are intended to achieve the highest degree of bioavailability for poorly soluble drugs and to stabilize the solid dispersion, avoiding drug re-crystallization. The use of surfactants such as inulin (Van, 2006), inutec SP1 (Vanden, 2006), compritol 888 ATO, gelucire 44/14 and poloxamer-407 (Chauhan, 2005) as carriers was shown to be effective in originating high polymorphic purity and enhanced *in-vivo* bioavailability. The association of amorphous polymers and surfactants has also been reported. For instance, the dissolution rate and bioavailability of LAB68, a poor water soluble drug, were improved after being dispersed in a mixture of PEG and polysorbate 80. The bioavailability of this solid dispersion was 10-fold higher compared to the dry blend of micronized drug. In addition, the solid dispersion system was physically and chemically stable for at least 16 months. HPMC was also associated with poloxamer and polyoxyethylene hydrogenated castor oil to prepare an amorphous felodipine solid dispersion (Won, 2005). The inclusion of surfactants in the formulation containing a polymeric carrier may help to prevent precipitation and/or protect a fine crystalline precipitate from agglomeration into much larger hydrophobic particles (Hörter and Dressman, 1997)

1.6.2 Carriers for Solid Dispersion

Suitable properties of carrier

Dissolution enhancement largely depends on the properties of carriers. Following criteria are generally considered during selection of carriers (Tiwari *et al.*, 2009):

- a) High water solubility. It improves wettability and enhances dissolution
- b) High glass transition point that improves stability
- c) Minimal water uptake (reduces T_g)
- d) Soluble in common solvent with drug –solvent evaporation
- e) Relatively low melting point –melting process
- f) Capable of forming a solid solution with the drug-similar solubility parameters

Classification of Carrier

First generation carrier

First generation solid dispersions are prepared using crystalline carriers such as urea and sugar, which were the first carriers to be employed in solid dispersion.

Second generation carrier

Second generation solid dispersions include amorphous carriers instead of crystalline carriers which are usually polymers. These polymers include synthetic polymers such as povidone (PVP), polyethylene glycols (PEG) and polymethacrylates as well as natural product based polymers such as hydroxy propyl methyl cellulose (HPMC), ethyl cellulose and hydroxy propyl cellulose or starch derivatives like cyclodextrins.

Third generation

Recently, it has been shown that the dissolution profile can be improved if the carrier has surface activity or self emulsifying properties. Therefore, third generation solid dispersions appeared. The uses of surfactant such as inulin, inutec SP1, compritol 888 ATO and poloxamer 407 as carriers are shown to be effective in originating high polymorphic purity and enhanced *in-vivo* bioavailability (Kapoor *et al.*, 2012).

1.6.3 Solvent for Solid Dispersion Systems

In solvent evaporation method a solvent is used to dissolve or suspend carrier and drug. It is better to use a non toxic solvent as it is difficult to remove the solvent completely. In order to prepare solid dispersion, solvents should be selected on the basis of following criteria:

- a) Solvent should dissolve both drug and carrier
- b) Toxic solvents to be avoided due to the risk of residual levels after preparation e.g. chloroform and dichloromethane
- c) Ethanol is a less toxic alternative
- d) Water based systems preferable
- e) Use of surfactants to create carrier drug solutions but care should be taken as they can reduce the glass transition point (Kaur *et al.*, 2012).

Classification of Solvents

Class I solvents (solvents to be avoided)

Solvents in class I should not be employed in the manufacture of drug substances, excipients and drug products because of their deleterious environmental effect. Benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, 1,1,1-trichloroethane are included in this group.

Class II solvents (solvents to be limited)

Solvents such as chlorobenzene, chloroform, cyclohexane, ethylene glycol, methanol, pyridine, toluene should be limited use in pharmaceutical products because of their inherent toxicity.

Class III solvents (solvents with low toxic potential)

Solvents in class III (acetic acid, acetone, 1-butanol, 2-butanol, ethanol, ethylacetate, ethyl ether, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 1-pentanol, 1-propanol, 2-propanol) may be regarded as less toxic and of lower risk to human health. These solvents are not human health hazard at level normally accepted in pharmaceuticals.

Class IV solvents (solvents for which no adequate toxicological data was found)

Some solvents may also be of interest to manufacturers of excipients, drug substances, or drug products for example petroleum ether, isopropyl ether. However, no adequate toxicological data was found (Tiwari *et al.*, 2009).

1.6.4 Methods of Preparation of Solid Dispersions

Solvent Evaporation Method

Drug and carrier were dissolved in a common solvent and solvent was evaporated to form the solid mass. Basically, this solvent evaporation method involves two steps and these are: (i) preparation of a solution containing both matrix material or carrier and drug and (ii) the removal of the solvent resulting in the formation of the solid mass (Jagadeesan and Radhakrishnan, 2013). Thermal decomposition of drugs or carriers can be prevented because of the low temperature required for the evaporation of organic solvents (Karanth

et al., 2006). However completely removing the organic solvent is a major disadvantage of this method (Karanth *et al.*, 2006).

Modified Solvent Evaporation Method

Drug is dissolved in organic solvent at its saturation solubility with continuous stirring for some time. Polymer is suspended in sufficient amount of water. The drug solution is poured at once into polymer suspension. The entire solvent is evaporated. The mass obtained is dried (Rane *et al.*, 2007).

Melting/Fusion Method

The fusion method is sometimes referred to as the melt method, which is correct only when the starting materials are crystalline. Therefore, the more general term fusion method is preferred. The first solid dispersions created for pharmaceutical applications were prepared by the fusion method (Sekiguchi and Obi, 1961; Sayyad and Sawant, 2010). The main advantage of direct melting method is its simplicity and economy. In addition melting under vacuum or blanket of an inert gas such as nitrogen may be employed to prevent oxidation of drug or carrier (Sekiguchi and Obi, 1961; Sayyad and Sawant, 2010). However degradation of the drug or matrix can occur during heating to temperatures necessary to fuse matrix and drug (Sekiguchi and Obi, 1961)

Solvent Melting Method

Accurately weighed drug is dissolved in organic solvent and the solution is incorporated into the melt of mannitol by pouring into it. It is then suddenly cooled. The mass is kept in desiccators for complete drying. The solidified mass is crushed, pulverized and passed through sieve (Kalia and Podder, 2011).

Kneading Method

A mixture of accurately weighed drug and carrier is wetted with solvent and kneaded thoroughly for some time in a glass mortar. The paste is dried under vacuum for 2 hours. Dried powder is passed through sieves and stored in a desiccator. Solid dispersion of valdecoxib with povidone was prepared by kneading technique.. Furosemide and crospovidone solid dispersions were prepared by this method (Chaulang *et al.*, 2008). However this method cannot be applied to all poorly water soluble drugs.

Co-Grinding Method

Accurately weighed drug powder and the carrier are mixed for some time using a blender at a specified speed. The mixture is then charged into the chamber of a vibration ball mill. A certain number of steel balls are added. The powder mixture is ground. Then the sample is collected and kept at room temperature in a screw capped glass vial until use. Chlordiazepoxide and mannitol solid dispersion were prepared by this method (Nokhodchi *et al.*, 2007).

Co-Precipitation Method (Co-Evaporates)

Accurately weighed carrier is dissolved in water and drug is dissolved in organic solvent. After complete dissolution, the aqueous solution of carrier is then poured into the organic solution of the drug. The solvents are then evaporated. The dispersion is pulverized with pestle and mortar, sieved and dried (Shin and Cho, 1997).

Spray Drying

Spray drying method consists of dissolving or suspending the drug and polymer in a common solvent or solvent mixture and then drying it into a stream of heated air flow to remove the solvent. Due to the large surface area of the droplets, the solvent rapidly evaporates and solid dispersion is formed within seconds, which may be fast enough to phase separation. Spray drying usually yields drugs in the amorphous state, but sometimes the drug may be partially crystallized during processing (Jagadeesan and Radhakrishnan, 2013).

Gel Entrapment Technique

Carrier is dissolved in organic solvent to form a clear and transparent gel. Then drug is dissolved in gel by sonication for few minutes. Organic solvent is evaporated under vacuum. Solid dispersions are reduced in size by glass mortar and passing through sieves (Kalyanwat and Patel, 2010).

Direct Capsule Filling

Direct filling of hard gelatin capsules with the liquid melt of solid dispersions avoids grinding-induced changes in the crystallinity of the drug (Karanth *et al.*, 2006). This molten dispersion forms a solid plug inside the capsule on cooling to room temperature,

reducing cross contamination and operator exposure in a dust-free environment, better fill weight and content uniformity was obtained than with the powder-fill technique. However, PEG was not a suitable carrier for the direct capsule-filling method as the water-soluble carrier dissolved more rapidly than the drug, resulting in drug-rich layers formed over the surface of dissolving plugs, which prevented further dissolution of the drug (Serajuddin and Sheen, 1988).

Lyophilization Technique

In order to get a porous, amorphous powder with high degree of interaction between drug and carrier, lyophilization technique is considered suitable. In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug and carrier at reduced pressure. Thermolabile substances can be successfully made into complex form by this method. Lyophilization technique is considered as an alternative to solvent evaporation and involves molecular mixing of drug and carrier in a common solvent. The limitations of this technique are the use of specialized equipment, time consuming process, and yield poor flowing powdered product (Cao *et al.*, 2005).

Electrospinning

Electrospinning is a process in which solid fibers are produced from a polymeric fluid stream solution or melt delivered through millimeter scale nozzles. This process involves the application of a strong electrostatic field over a conductive capillary attaching to a reservoir containing a polymer solution or melt and a conductive collection screen. Upon increasing the electrostatic field strength up to but not exceeding a critical value, charge species accumulated on the surface of a pendant drop destabilize the hemispherical shape into a conical shape. Beyond the critical value, a charged polymer jet is ejected from the apex of the cone. The ejected charged jet is then carried to the collection screen via the electrostatic force. The coulombic repulsion force is responsible for the thinning of the charged jet during its trajectory to the collection screen. The thinning down of the charged jet is limited by the viscosity increase, as the charged jet is dried (Chiou and Rigelman, 1971; Sharma and Joshi, 2007). This technique can be utilized for the preparation of solid dispersions in future (Chiou and Rigelman, 1971; Sharma and Joshi, 2007)

Supercritical Fluid Method

Supercritical fluid methods are mostly applied with carbon dioxide, which is used as either a solvent for drug and matrix or as an anti-solvent. When supercritical CO₂ is used as solvent, matrix and drug are dissolved and sprayed through a nozzle, into an expansion vessel with lower pressure and particles are immediately formed. The adiabatic expansion of the mixture results in rapid cooling. This technique does not require the use of organic solvents and since CO₂ is considered environmentally friendly, this technique is referred to as 'solvent free'. The technique is known as Rapid Expansion of Supercritical Solution (Goldberg *et al.*, 1966; Sekiguchi and Obi, 1961).

Dropping Solution Method

The dropping method facilitates the crystallization of different chemicals and produces round particles from melted solid dispersions. In laboratory-scale preparation, a solid dispersion of a melted drug-carrier mixture is pipetted and then dropped onto a plate, where it solidifies into round particles. The size and shape of the particles can be influenced by factors such as the viscosity of the melt and the size of the pipette. Because viscosity is highly temperature-dependent, it is very important to adjust the temperature so that when the melt is dropped onto the plate it solidifies to a spherical shape (Kalia and Poddar, 2011).

1.6.5 Characterization of Solid Dispersion

Detection of Crystallinity in Solid Dispersions

Many attempts have been made to investigate the molecular arrangement in solid dispersions. However most effort has been put into differentiate between amorphous and crystalline materials. Many techniques are available which detect the amount of crystalline material in solid dispersion. The amount of amorphous material is never measured directly but is mostly derived from the amount of crystalline material in the sample (Kaushal *et al.*, 2004). It should be noted that through the assessment of crystallinity as method to determine the amount amorphous drug it will not be revealed whether the drug is present as amorphous drug particles or as molecularly dispersed molecules. The following techniques are available to detect crystallinity (Dhirendra *et al.*, 2009).

Powder X-ray Diffraction Study (XRD)

X-ray diffraction can be used to detect qualitatively material with long range order. Sharper diffraction peaks indicate more crystalline material. Recently developed X-ray equipment is semi-quantitative.

Fourier Transformed Infrared Spectroscopy (FTIR)

It can be used to detect the variation in the energy distribution of interactions between drug and matrix (Forster *et al.*, 2001). Sharp vibrational bands indicate crystallinity (Bugay, 2001). Fourier Transformed Infrared Spectroscopy (FTIR) was used to accurately detect crystallinities ranging from 1 to 99% in pure material. However in solid dispersions only qualitative detection is possible (Broman *et al.*, 2001).

Water Vapor Sorption

Water Vapor Sorption can be used to discriminate between amorphous and crystalline material (Backton and Darcy, 1995). This method requires accurate data on the hygroscopicity of both crystalline and amorphous samples.

Isothermal Microcalorimetry

It measures the crystallization energy of amorphous material that is heated above its glass transition temperature, T_g (Sebhatu *et al.*, 1994). However, this technique has some limitations. Firstly, this technique can only be applied if the physical stability is such that only during the measurement crystallization takes place. Secondly, it has to be assumed that all amorphous material crystallizes. Thirdly, in a binary mixture of two amorphous compounds a distinction between crystalline energies of drug and matrix difficult.

Dissolution Calorimetry

Dissolution Calorimetry measures the energy of dissolution, which is dependent on the crystallinity of the sample (Pikal *et al.*, 1978). Usually, dissolution of crystalline material is endothermic, whereas dissolution of amorphous material is exothermic.

Microscopic Technique

Microscopic techniques that measure mechanical properties that are different for amorphous and crystalline material can be indicative for the degree of crystallinity. Density measurements and Dynamic Mechanical Analysis (DMA) determine the modulus

of elasticity and viscosity and thus affected by the degree of crystallinity. However, also these techniques require knowledge about the additives of these properties in intimately mixed binary solids.

Differential Scanning Calorimetry

A frequently used technique to detect the amount crystalline material is Differential Scanning Calorimetry (DSC) (Kerc and Srcic, 1995). In DSC, samples are heated with a constant heating rate and the amount of energy necessary for that is detected. With DSC the temperatures at which thermal events occur can be detected. Thermal events can be a glass to rubber transition, crystallization, melting or degradation. Furthermore, the melting energy can be used to detect the amount of crystalline material. Possibly, the recrystallization energy can be used to calculate the amount of amorphous material provided, that all amorphous material is transformed to the crystalline state (Kerc and Srcic, 1995).

Detection of Molecular Structure in Solid Dispersions

The properties of a solid dispersion are highly affected by the uniformity of the distribution of the drug in the matrix. The stability and dissolution behavior could be different for solid dispersions that do not contain any crystalline drug particles, i.e. solid dispersions of type V and VI or for the type II and III. However not only the knowledge for the on the physical state is important; the distribution of the drug as amorphous or crystalline particles or as separate drug molecules is relevant to the properties of the solid dispersion too. Nevertheless, only very few studies focus on the discrimination between amorphous incorporated particles versus molecular distribution or homogenous mixtures.

Confocal Raman Spectroscopy

It was used to measure the homogeneity of the solid mixture of ibuprofen in PVP (Breitenbach *et al.*, 1999). It was described that a standard deviation in drug content smaller than 10% was inactive of homogenous distribution. Because of the pixel size of 2 μm , uncertainty remains about the presence of nano-sized amorphous drug particles.

Infrared Spectroscopy

Using FTIR, the extent of interactions between drug and matrix can be measured. The interactions are indicative for the mode of incorporation of the drug, because separately

dispersed drug molecules will have more drug-matrix interactions than when the drug is present in amorphous clusters or other multi molecule arrangements (Li *et al.*, 2002; Rogers *et al.*, 2002).

Temperature Modulated Differential Scanning Calorimetry (TMDSC)

Temperature Modulated Differential Scanning Calorimetry (TMDSC) can be used to assess the degree of mixing of an incorporated drug. Due to modulation, reversible and irreversible events can be separated. For example, glass transitions (reversible) are separated from crystallization or relaxation (irreversible) in amorphous materials. It has been shown that the sensitivity of TMDSC is higher than conventional DSC (De *et al.*, 1999). Therefore this technique can be used to assess the amount of molecularly dispersed drug (Cilurzo *et al.*, 2002) and from that the fraction of drug that is dispersed as separate molecules is calculated (Vasanthavada *et al.*, 2004).

1.6.6 Mechanism Responsible for Solubility Enhancement from Solid Dispersion

A number of mechanisms are responsible to improve solubility of poorly water soluble drug from SD and further to improve its bioavailability.

Reduced particle size: When solid dispersion is exposed to aqueous media, the carrier dissolve and the drug release as fine colloidal particles. The resulting enhanced surface area results in higher dissolution rate of poorly water soluble drugs.

Drug in amorphous state in SD: Crystalline drugs generally remain in amorphous state in SD. Amorphous state have higher solubility as no energy is required to break crystal lattice in amorphous state during dissolution.

Particles with high porosity: Particles in solid dispersion have been found to have high porosity. The increased porosity of solid dispersion particles hastens the drug release profile. Increase in porosity depends on carrier properties, i.e., linear polymers results in larger and more porous particles than that of reticular particles.

Particles with improved wettability: A strong contribution to the enhancement of drug solubility is related to the drug wettability improvement. It has been verified in case of SD. Hydrophilic carrier increases wettability and helps to drug release rapidly.

Use of Surfactant: Some carriers have surface active properties. The utility of surfactant systems in solubilization is well known. Adsorption of surfactant on solid surface can modify their hydrophilicity, surface charge, and other key properties that govern interfacial processes such as dispersion, floatation and wetting. Surfactants have also been reported to cause solvation/plasticization, manifesting in reduction of melting the active pharmaceutical ingredients, glass transition temperature and the combined glass transition temperature of solid dispersions. Because of these unique properties, surfactants have attracted the attention of investigators for preparation of solid dispersions. Solid dispersions using pluronic f-68 (a type of poloxamer) as a carrier were studied for improving the dissolution and bioavailability of abt-963, a poorly water- soluble compound. Results showed that the solid dispersion substantially increased the *in-vitro*-dissolution rate of ABT-963. A significant increase of oral bioavailability compared with conventional capsule formulation was also reported. (Sharma and Joshi, 2007).

1.7 Drug Dissolution Study

The process of dissolution plays a vital role in liberating a drug from its dosage form and making it available for subsequent gastrointestinal absorption. Dissolution is dependent on many factors, which include not only the physiochemical properties of the drug, but also the formulation of the dosage form (including coating formulation) and the process of manufacturing. An immediate-release drug product is considered rapidly dissolving when not less than 85% of the label amount of drug substance dissolves within 30 minutes using USP Apparatus I at 100 rpm or Apparatus II at 50 rpm in a volume of 900 ml or less in each of the following media:

1. Acidic media such as 0.1 N HCl or Simulated Gastric Fluid USP without enzymes.
2. A pH 4.5 buffer.
3. A pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes.

1.7.1 Dissolution Process

For many drugs, particularly those, that are poorly soluble in the gastric fluid, the rate-limiting step in the absorption process is the dissolution rate and a dissolution rate determination can therefore be a useful guide to comparative bioavailability. Since drug absorption and physiological availability depend on the availability of the drug substance

in the dissolved state, suitable dissolution characteristics are important property for a satisfactory tablet. The dissolution test measures the amount of time required for certain percentage of the drug substance in a tablet to go into solution under a specified set of conditions. It describes a step towards physiological availability of the drug substance, but it is not designed to measure the safety or efficacy of the tablet being tested. It provides *in-vitro* control procedure to eliminate variation among production batches. The dissolution medium must be aqueous and the pH of the medium should be controlled and should simulate *in-vivo* conditions.

The most well established apparatuses are those described in the pharmacopoeias. Four methods, mainly intended for oral solid dosage forms, which are described in the U.S. Pharmacopoeia (USP, 2012) are as follows:

- The rotating basket method (USP I).
- The rotating paddle method (USP II).
- The reciprocating cylinder (USP III).
- The flow through method (USP IV).

All the methods, except for the reciprocating cylinder, are also described in the European Pharmacopoeia (EP).

1.7.2 Dissolution Profile Comparison

Under appropriate test conditions, a dissolution profile can characterize the product more precisely than a single point dissolution test. A dissolution profile comparison between a test and reference product helps assure similarity in product performance and signals bioequivalence. Among several methods for dissolution profile comparison, similarity factor (f_2) is the simplest. The mathematical approach to compare the dissolution profile using two factors, dissimilarity factor (f_1), similarity factor (f_2) and the dissolution efficiency (%DE are very helpful.

Similarity Factor (f_2)

As the name specifies, it stresses on the comparison of closeness of two comparative formulations. Therefore the f_2 factor measures the closeness between two profiles and it is expressed by the following equation:

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^n (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

Where R_t and T_t are the cumulative percentage dissolved at each of the selected n time point of the reference and the test product respectively. The f_2 factor is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time-points. Because of the nature of measurement f_2 is described as similarity factor by FDA (1997).

FDA has set a public standard of f_2 value of (50-100) to indicate similarity between two dissolution profiles. An f_2 value of 50 or greater ensures similarity or equivalence of the two curves and thus the performance of the two products. The dissolution measurements of the two products should be under same test conditions and the time points should be same for each dissolution profile as f_2 values are sensitive to the number of dissolution time points, only one measurement should be considered after 85% dissolution of the product.

For products which are rapidly dissolving, i.e., more than 85% in 15 minutes or less, a profile comparison is not necessary. In dissolution profile comparisons, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are, and to have a measure which is more sensitive to large differences at any particular time point. For this reason, the f_2 comparison has been the focus in most guidance.

Dissimilarity Factor (f_1)

Difference factor focuses on the difference in percent dissolved between reference and test products at various time intervals and it is expressed by the following equation:

$$f_1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100$$

Where R_t and T_t are the cumulative percentage dissolved at each of the selected n time point, points of the reference and the test product respectively. The f_1 factor is

proportional to the average difference between the two profiles. Difference factor of 0-15 ensures minor difference between two products.

Dissolution Efficiency (%DE)

Dissolution efficiency (%DE) was employed to compare drug release from various brands. %DE is the area under the dissolution curve within a time range (t_2-t_1) and is defined as:

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

where y is the drug percent dissolved at time t . The reference and test products are considered equivalent if the difference between their dissolution efficiencies is within appropriate limits ($\pm 10\%$ is often used) (Anderson *et al.*, 1998).

1.7.3 Drug Release Kinetics

The purpose of an immediate oral solid dosage form is to provide rapid dissolution (% drug release greater than $\geq 90\%$) if there is no GIT instability. Now since it is apparently impossible not to have any kind of gastric instability or other problems, the FDA has defined an appropriate immediate oral solid dosage form by having $\geq 85\%$ drug release in gastric media within 30 minutes of administration (FDA, 1997). However, various formulation factors like drug form, drug crystallinity, excipients, formulation process and also various pH range of GIT may affect the drug release from the immediate oral solid dosage form and cause it to be slow. Then the formulation will not be able to meet the USP requirement. Thus, drug release kinetic study is important for immediate oral solid dosage form. There are different drug release kinetics, mathematical models, such as Zero-order kinetic model, First-order kinetic model, Hixson-Crowell kinetic model and the Higuchi kinetic model. Immediate oral solid dosage form can follow any one of the mathematical models.

Zero order equation

The equation assumes that the cumulative amount of drug release is directly related to time. The equation may be as follows:

$$C = K_0 t \text{-----} (1)$$

Where, K_0 is the zero order rate constant expressed in unit concentration/time and t is the time. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes.

First order equation

The release behavior of first order equation is expressed as log cumulative percentage of drug remaining vs time. The equation may be as follows (Wagner, 1969):

$$\text{Log } C = \text{Log } C_0 - kt / 2.303 \text{-----} (2)$$

where, C = the amount of drug un-dissolved at t time,

C_0 = drug concentration at $t = 0$,

k = corresponding release rate constant.

Higuchi square root law

The Higuchi release model describes the cumulative percentage of drug release vs square root of time. The equation may be as follows (Higuchi, 1961):

$$Q = K\sqrt{t} \text{-----} (3)$$

Where, Q = the amount of drug dissolved at time t . K is the constant reflecting the design variables of the system. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Hixson-Crowell cube root law

It is the law that represents idea about the evaluation of drug release pattern changes with the surface area and the diameter of the particles/tablets (Hixon *et al.*, 1931). It is mentioned as the cube root of the percentage of drug remaining in the matrix vs time. The equation may be as follows

$$Q_0^{1/3} - Q_t^{1/3} = k_{HC} \times t \text{-----} (4)$$

where, Q_0 = initial amount of the drug in the tablets

Q_t = the amount of drug release in time t

k_{HC} = the rate constant for the Hixson-Crowell cube root law

Korsmeyer–Peppas equation

Korsmeyer *et al.* (1983) developed a simple, semi-empirical model relating exponentially the drug release to the elapsed time. The equation may be as follows:

$$Q/Q_0 = Kt^n \text{ ----- (5)}$$

where, Q/Q_0 = the fraction of drug released at time t

k = constant comprising the structural geometric characteristics

n = the diffusion exponent that depends on the release mechanism.

If $n \leq 0.5$, the release mechanism follows a Fickian diffusion, and if $0.5 < n < 1$, the release follows a non-Fickian diffusion or anomalous transport (Peppas, 1985). The drug release follows zero order drug release and case II transport if $n = 1$. But when $n > 1$, then the release mechanism is super case II transport. This model is used in the polymeric dosage form when the release mechanism is unknown or more than one release phenomena is present in the preparation.

To characterize the drug release rate in different experimental conditions MDT (mean dissolution time), $T_{50\%}$, $T_{80\%}$ and dissolution efficiency (DE) are also calculated from dissolution data according to the following equations (Giri *et al.*, 2010).

$$T_{50\%} = (0.5/k)^{1/n}$$

$$T_{80\%} = (0.8/k)^{1/n}$$

$$MDT = (n/n+1) \cdot K^{-1/n}$$

where k is the antilog of intercept and n is a release exponent of Korsmeyer's plot. Mean dissolution time (MDT) value is used to characterize the drug release rate from the matrix. A higher value of MDT indicates a lower drug releasing ability of the solid dispersion and vice-versa. Besides, %DE is the area under the dissolution curve up to a certain time t , expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. Larger the value of DE, higher is the dissolution rate. These parameters along with f_1 , f_2 are important for release profile comparison. Additionally analysis of variance (ANOVA) may be calculated for comparison.

1.8. Drug Profile: Statins

Statins are among the most widely prescribed classes of medicines in the world (Baigent *et al.*, 2005). Lovastatin is the first statins that was developed in 1978 at the Merck Research Laboratories in a fermentation broth of *Aspergillus terreus*. Lovastatin was approved for marketing in 1987 followed by simvastatin (1991), pravastatin (1991), fluvastatin (1994), atorvastatin (1996), cerivastatin (1998) and rosuvastatin (2003) (Schachter, 2004). Clinical trials over more than 2 decades have shown that statins are safe and prevent cardiovascular (CV) deaths, major CV events (stroke, myocardial infarction) and total mortality (Mancini *et al.*, 2011). Cardiovascular diseases (CVD) are the most prevalent cause of death and disability in both developed as well as developing countries (Chaturvedi and Bhargava, 2007). South Asians around the globe have the highest rates of Coronary Artery Disease (CAD) (Enas *et al.*, 2007).

Mevastatin was the first HMG-CoA reductase inhibitor and was isolated from *Penicillium citrinum*. Other statins as simvastatin, lovastatin and pravastatin are also fungal derivatives while atorvastatin, cerivastatin, fluvastatin, pitavastatin and rosuvastatin are fully synthetic compounds (Wierzbicki and Anthony, 2003). The use of statins (simvastatin, pravastatin, lovastatin, fluvastatin, rosuvastatin and atorvastatin) has become the preferred method for treating elevated LDL-C levels in children and adolescents who meet the criteria for drug therapy. In fact, their use is generally safe and well tolerated. However, it must be remembered that cholesterol is an essential structural component of cells, a precursor for steroid hormones, vitamin D metabolites and bile acids, and an important factor in neural myelinization and brain growth (Arambepola *et al.*, 2007).

Rosuvastatin is an important statins which is selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts -3-hydroxy-3- methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. *In-vivo* studies in animals and *in-vitro* studies in cultured animal and human cells have shown rosuvastatin to have a high uptake into, and selectivity for, action in the liver, the target organ for cholesterol lowering. In *in-vivo* and *in-vitro* studies, rosuvastatin produces its lipid-modifying effects in two ways. First, it increases the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL. Second, rosuvastatin inhibits hepatic synthesis of VLDL, which reduces the total number of VLDL and LDL particles. Cholesterol

biosynthesis pathway demonstrates the step inhibited competitively by statins (Schachter, 2004).

Rosuvastatin reduces total cholesterol (total-C), LDL-C, ApoB, and non HDL-C (total cholesterol minus HDL-C) in patients with homozygous and heterozygous familial hypercholesterolemia (FH), nonfamilial forms of hypercholesterolemia, and mixed dyslipidemia (Schachter, 2004). Rosuvastatin also reduces TG and produces increases in HDL-C. Rosuvastatin reduces total-C, LDL-C, VLDL-cholesterol (VLDL-C), ApoB, nonHDL-C and TG, and increases HDL-C in patients with isolated hypertriglyceridemia. The effect of rosuvastatin on cardiovascular morbidity and mortality has not been determined. Atorvastatin, simvastatin and rosuvastatin are poorly water soluble drugs. Kasim *et al.* (2004) enlisted atorvastatin and simvastatin as BCS II class drug. So these three drugs (atorvastatin, simvastatin and rosuvastatin) are suitable candidate to increase dissolution rate to enhance bioavailability. Comparative properties of three important statins are shown in Table 1.4.

Table 1.4: Comparison of properties of three statins

Properties	Atorvastatin Ca	Simvastatin	Rosuvastatin Ca
MF	$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$	$C_{25}H_{38}O_5$	$(C_{22}H_{27}FN_3O_6S)_2Ca$
MW	1209	418.6	1001.14
Water	3.5 - 5.5%		<6%
Dissociation Constant. pKa	4.46		
Partition Coefficient. Log P (octanol/water)	6.36	4.68	2.4
Anti log P	2290867	47863	246
Melting Point	152.85°	135° to 138°.	124°-127°
Specific optical rotation		+ 285 to + 300	
Optimal time of dosing	Any time of day	Evening	Any time of day
Bioavailability (%)	12	5	20
Solubility	Lipophilic	Lipophilic	Less Lipophilic
Effect of food Bioavailability	decreased	No effect	No effect
Protein binding (%)	98	95–98	90
Active metabolites	✓	×	Minor
Elimination half-life (h)	14	2	19
Renal excretion (%)	<5	13	10
T _{max} (h)	2.17	2.81	4.08
C _{max} (ng/ml)	7.5	18.19	4.16□

1.9 Excipients profile

Labrasol[®] is a liquid used in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations. It can be used in self emulsifying lipid formulations. It is a solubilizer/penetration enhancer for topical formulations. It is a surfactant for microemulsions in topical formulations.

Chemistry	: Caprylocaproyl macroglycerides (Polyoxylglycerides)
Physical appearance	: Liquid
Functional Category	: Liquid bioavailability enhancer
Use in SEDDS	: Self type SMEDDS
HLB Value	: 14

Labrafil[®] M 1944 CS is also use in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations. It can be used in self emulsifying drug delivery system. It is a co-emulsifier/penetration enhancer for topical formulations. It is an oily phase for emulsions/microemulsions in topical formulations.

Chemistry	: Oleoyl macroglycerides (polyoxylglycerides)
Physical appearance	: Liquid
Functional category	: Liquid bioavailability enhancer
Use in SEDDS	: SEDDS/SMEDDS
HLB value	: 4

Labrafil[®] M 2125 CS is liquid in at room temperature. It is generally used in oral and topical formulations. It is a solubilizer/bioavailability enhancer. It can be used in self emulsifying lipid formulations. It is a co-emulsifier/penetration enhancer for topical formulations.

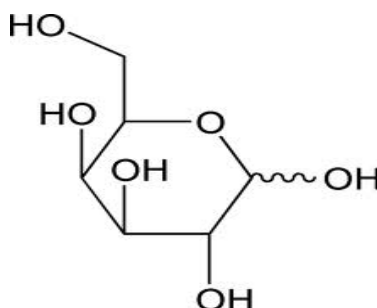
Chemistry	: Linoleoyl macroglycerides (polyoxylglycerides)
Physical appearance	: Oily liquid
Functional category	: Liquid bioavailability enhancer
Use in SEDDS	: SEDDS/SMEDDS/SNEDDS
HLB value	: 4

Capryol[™] 90 is an oily liquid used in both oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations. It can be used in self emulsifying lipid formulations such as SEDDS and SMEDDS. It is a

Bioavailability enhancement of poorly water soluble drugs using Self Emulsifying Drug Delivery System (SEDDS) and Solid Dispersion (SD) technology

solubilizer/penetration enhancer for topical formulations. It is a co-surfactant for microemulsions in topical formulations.

Chemical Structure:



Chemistry	: Propylene glycol monocaprylate
Physical appearance	: Oily liquid
Functional category	: Liquid solubility enhancer
Use in SEDDS	: Self type SMEDDS
HLB value	: 6

Capryol™ PGMC is suitable for oral and topical formulations. It can be used in self emulsifying lipid Formulations. It is a solubilizer/penetration enhancer for topical formulations..

Chemistry	: Propylene glycol caprylate
Physical appearance	: Oily liquid
Functional category	: Liquid solubility enhancer
Use in SEDDS	: SELF type SMEDDS
HLB value	: 5

Lauroglycol™ 90 is a liquid utilized in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations. It can be used as a surfactant in SEDDS. It is a solubilizer/penetration enhancer for topical formulations. It is a co-surfactant for microemulsions in topical formulations.

Chemistry	: Propylene glycol monolaurate
Physical appearance	: Liquid
Functional category	: Liquid solubility enhancer
Use in SEDDS	: surfactant
HLB value	: 5

Lauroglycol™ FCC is a liquid that can be used in oral and topical formulations. It can be used as a surfactant in SMEDDS. It is a solubilizer/penetration enhancer in semisolid preparations. It is a co-surfactant for microemulsions.

Chemistry	: Propylene Glycol Laurate
Physical appearance	: Liquid
Functional category	: Liquid solubility enhancer
Use in SEDDS	: SELF type SMEDDS/SNEDDS
HLB value	: 4

Plurol® Oleique CC 497 is an oily liquid that can be used in oral and topical formulations. It is a solubilizer/bioavailability enhancer for oral formulations. It can be used in SENDDS. It is a solubilizer/penetration enhancer for topical preparations. It is a co-surfactant for microemulsions.

Chemistry	: Polyglyceryl oleate
Physical appearance	: Visquous liquid
Functional category	: Liquid solubility enhancer
Use in SEDDS	: SELF type SMEDDS
HLB value	: 6

Transcutol® HP is a highly purified product, offering greater than 99.9% purity. This grade is suitable for oral dosage forms. It is a high performance solubilizer/solvent for many poorly soluble compounds. It is soluble in both water and oil.

Chemistry	: Diethylene glycol monoethyl ether
Physical appearance	: Liquid
Functional category	: soluble in both water and oil

Labrafac™ Lipophile WL 1349 is a liquid oily vehicle for use in oral and topical formulations. For oral formulations, it has solubilizing properties for lipophilic drugs. It is a bioavailability enhancer. It can be used in Self Emulsifying Lipid Formulations. It is an oily phase for microemulsions in oral and topical formulations.

Chemistry	: Medium chain triglycerides
Physical appearance	: Liquid
Functional category	: Liquid oily vehicle
Use in SEDDS	: SEDDS/SMEDDS
HLB value	: 2

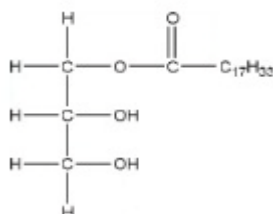
Labrafac™ PG is a liquid oily vehicle for use both in oral and topical formulations. It has solubilizing properties for lipophilic drugs. It can be used in Self Emulsifying Lipid Formulations (SEDDS). It is an oily phase for microemulsions in topical formulations.

Chemistry	: Propylene glycol dicaprylocaprate
Physical appearance	: Oily liquid
Functional category	: Liquid oily vehicle
Use in SEDDS	: SEDDS/SMEDDS/SNEDDS
HLB value	: 2

Maisine™ 35-1 is a liquid oily vehicle for use in oral dosage forms. It has solubilizing properties for lipophilic drugs. It can be used in self emulsifying lipid formulations.

Chemistry	: Glycerol mono-linoleate
Physical appearance	: Liquid
Functional category	: Liquid oily vehicle
Use in SEDDS	: SEDDS/SMEDDS
HLB value	: 4

Pecol™ is a liquid oily vehicle for use in oral dosage forms. It has solubilizing properties for lipophilic drugs. It can be used in self emulsifying lipid preparations.



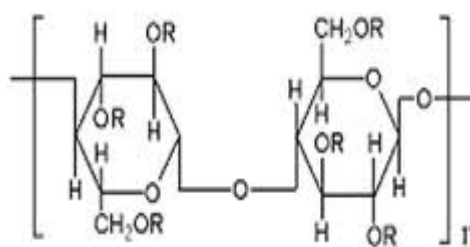
Chemistry	: Glycerol mono-oleate
Physical appearance	: Liquid
Functional category	: Liquid oily vehicle
Use in SEDDS	: SEDDS/SMEDDS/SNEDDS
HLB value	: 3

Tween 80 is polyoxyethylene sorbitan monooleate. Its chemical name is 2-[2-[3,4-bis(2-hydroxyethoxy)oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl (E)-octadec-9-enoate.

Chemical Formula	: C ₃₂ H ₆₀ O ₁₀
Physical State	: Amber colored viscous liquid

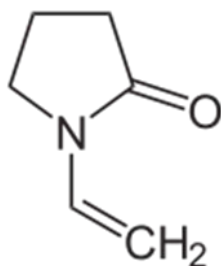
HLB value	: 15
Boiling Point	: > 100°C.
Solubility	: Soluble in water, oil, ethyl acetate, methanol, toluene and ethanol (96%).
Functional Category	: Emulsifying agent; emulsion stabilizer; nonionic surfactant; solubilizing agent, foaming agent.
Applications	: It is used to stabilize aqueous formulations of medications for parenteral administration. It is used as an emulsifier in the manufacture of the popular anti-arrhythmic amiodarone.

HPMC 6 cps is an odorless and tasteless, white or creamy-white fibrous or granular powder.



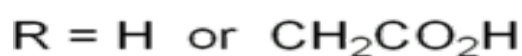
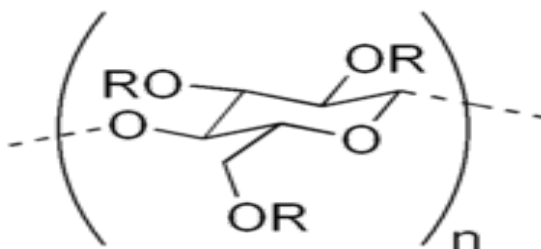
Molecular Weight	: Approx 86,000
Appearance	: White to creamy white fiber or granular powder
Melting point	: 1900C - 2000C
Density	: 1.326 g/cm ³
Acidity	: pH 5.5 – 8.0 for a 1% w/w aqueous
Solubility	: Soluble in cold water. Practically insoluble in chloroform, ethanol (95%), ether; but soluble in mixture of ethanol and dichloromethane, mixture of methanol and dichloromethane and mixture of alcohol and water.

Povidone K-30 is white or yellowish-white powder or flakes, hygroscopic in nature. Its chemical name is 1-Ethenyl-2-pyrrolidone homopolymer and chemical structure is as follows:



Empirical Formula	: (C ₆ H ₉ NO) _n
Molecular Weight	: 50000
Appearance	: White to creamy white
Melting point	: 1500C
pH	: 4.0-7.0
Solubility	: Freely soluble in methanol, ethanol, acids, chloroform and water.

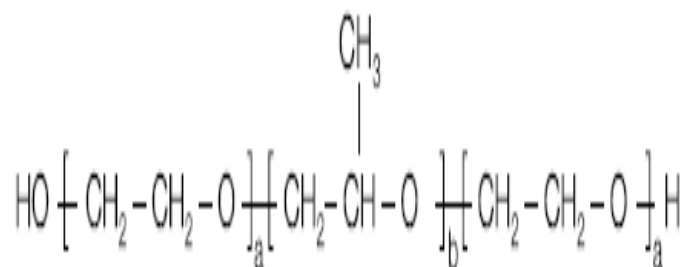
Croscarmellose sodium is an internally cross-linked sodium carboxymethylcellulose for use as a disintegrant in pharmaceutical formulations. Croscarmellose sodium occurs as an odorless, white or grayish white powder. Croscarmellose sodium is a crosslinked polymer of carboxymethylcellulose sodium.



Chemical Name	: Cellulose, carboxymethyl ether, sodium salt
Molecular Weight	: 90000-700000
Appearance	: White to almost white granular powder.
Melting point	: 227-252 ⁰ C
pH	: 6.5 – 8.5
Solubility	: Sparingly soluble in water and methanol. Slightly soluble in ethanol.

Poloxamer 407

Poloxamer 407 is a hydrophilic non-ionic surfactant of the more general class of copolymers known as poloxamers. Poloxamer 407 is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol. The approximate lengths of the two PEG blocks is 101 repeat units while the approximate length of the propylene glycol block is 56 repeat units. This particular compound is also known by the BASF trade name Pluronic F127. Poloxamers generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless. Chemical structure and properties of poloxamer 407 are as follows:



Molecular Weight	: 9800-14600
Appearance	: White, waxy, free flowing prilled granules
Melting point	: 52-57 ⁰ C
pH	: 5.0-7.5
Solubility	: Sparingly soluble in water and ethanol.

Sodium starch glycolate:

Sodium starch glycolate is a white or almost white free-flowing very hygroscopic powder. The granules show considerable swelling in contact with water.

Functional Category	: Tablet and capsule disintegrant.
Melting Point	: Does not melt, but chars at approximately 200°C.
Particle Size	: 100% of particles less than 106 µm in size. Average
Distribution	particle size (d50) is 38 µm and 42 µm for Primojel by microscopy and sieving respectively.
Solubility	: Practically insoluble in methylene chloride. It gives a translucent suspension in water.

Purified Talc is a very fine, white to grayish-white, odorless, impalpable, unctuous, crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness. It is used as dusting powder (con.90.0–99.0%). glidant and tablet lubricant (1.0–10.0%) and tablet and capsule diluent (5.0–30.0 %).

Molecular Weight	: 480.90.
Functional Category	: Anticaking agent, glidant, tablet and capsule diluent, tablet and capsule lubricant.
Solubility	: Practically insoluble in dilute acids and alkalis, : organic solvents, and water.
Moisture content	: Talc absorbs insignificant amounts of water at 25°C and relative humidities up to about 90%.
Incompatibilities	: Incompatible with quaternary ammonium compounds.
Stability and storage condition	: Talc is a stable material and may be sterilized by heating at 160°C for not less than 1 hour. It may also be sterilized by exposure to ethylene oxide or gamma irradiation. Talc should be stored in a well-closed container in a cool, dry place.

Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. Magnesium stearate is widely used in cosmetics, foods, and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet. Magnesium stearate is stable and should be stored in a well-closed container in a cool, dry place.

Functional Category	: Tablet and capsule lubricant.
Density (bulk)	: 0.159 g/cm ³ .
Density (tapped)	: 0.286 g/cm ³ .
Density (true)	: 1.092 g/cm ³ .
Melting point	: 117–150°C (commercial samples); 126–130°C (high purity magnesium stearate).
Solubility	: Practically insoluble in ethanol, ethanol (95%), ether : and water; slightly soluble in warm benzene and warm ethanol (95%).
Loss on drying	: ≤0.6 %.

1.10.1 Reports of Research Work Carried out to Enhance Bioavailability of Atorvastatin, Simvastatin and Rosuvastatin (1991- 2007)

Atorvastatin, simvastatin and rosuvastatin are three important lipid lowering drugs. Simvastatin was approved for marketing in December, 1991 followed by atorvastatin (17 December, 1996). On the other hand rosuvastatin was approved on 12 August, 2003. A very little works was carried out and reported on their dissolution enhancement before 2007.

Ambike *et al.* (2005) described spray drying technique to prepare free flowing, stable, amorphous solid dispersions (SDs). Simvastatin (SIM) was used as a model drug with relatively lower glass transition temperature (T_g). Povidone K-30 used as a carrier. The study demonstrated high potential of spray drying technique for obtaining stable amorphous SDs of low T_g drug. *In-vivo* study in rats, also justified the improvement in rate and extent of *in-vitro* drug release.

Kang *et al.* (2005) prepared self-microemulsifying drug delivery system (SMEDDS) for oral bioavailability enhancement of a poorly water soluble drug, simvastatin. Optimized formulations used for *in-vitro* dissolution and bioavailability assessment contained carpryol 90 (37%), cremophor EL (28%), and carbitol (28%). The prepared SMEDDS was compared with the conventional tablet (Zocor[®]) by administering the prefilled hard capsules to fasted beagle dogs. The absorption of simvastatin acid from SMEDDS form resulted in about 1.5-fold increase in bioavailability compared with the conventional tablet

Another study conducted by patil *et al.* (2007) describes new SEDDS formulations of simvastatin containing captex 355, lauroglycol 90, cremophor EL and capmul MCM. The developed SEDDS were evaluated for turbidimetry, droplet size analysis, drug content and *in-vitro* diffusion profiles. *In-vivo* performance of the optimized formulation was evaluated in rats using pharmacodynamic marker parameters like plasma total cholesterol (CH), Test formulation showed enhanced pharmacodynamic performance compared to reference formulation in rats.

Shen and Zhong, (2006) carried out a study to prepare SEDDS of atorvastatin by using labrafil, estol and labrafac as oil, cremophor RH40 as surfactant, propylene glycol (g) as

co-surfactant. The release of atorvastatin from SMEDDS capsules was studied using the dialysis bag method in 0.1 M HCl and phosphate buffer (pH 7.4), compared with the release of atorvastatin from a conventional tablet. A pharmacokinetic study was performed in 6 beagle dogs after oral administration of 6mg kg⁻¹ atorvastatin. The bioavailability of atorvastatin SMEDDS capsules was significantly increased compared with that of the conventional tablet.

1.10.2 Reports of Research Work Carried out to Enhance Bioavailability of Atorvastatin, Simvastatin and Rosuvastatin (2008- 2015)

Rao *et al.* (2010) investigated to increase dissolution rate of simvastatin by surface solid dispersion (SSDs). SSDs of simvastatin with two different superdisintegrants in three different drug-carrier ratios were prepared by a co-evaporation method. Surface solid dispersions were characterized by differential scanning calorimetry (DSC), powder x-ray diffractometry (PXRD), scanning electron microscopy (SEM), and infrared spectroscopy (IR) and evaluated for drug content, saturation solubility, pH-dependent solubility, solubility in biorelevant media and performed *in-vivo* studies by a triton-induced hypercholestermia model in rats.

Boddupalli *et al.* (2010) also reported same type of findings of increasing dissolution rate of simvastatin by surface solid dispersion (SSDs). Three different superdisintegrants were used to prepare SSDs using solvent evaporation method. Tablets containing SSDs were also prepared and compared with SSDs.

Jatwani *et al.* (2010) prepared solid dispersion of simvastatin using hydrophilic carriers polyethylene glycol 6000 (PEG 6000), sorbitol, gelucire 44/14 by fusion and solvent evaporation method to increase its aqueous solubility. It was concluded that solid dispersions prepared using multiple carriers showed enhanced dissolution as compared to the ones prepared using single carriers. Finally solid dispersion of simvastatin: PEG 6000: Sorbitol: Gelucire 44/14 prepared in the ratio of 1:1:1:1 (SIM 17) showed excellent physico-chemical characteristics and better release profile than the other solid dispersions.

Sukanya and Kishore (2012) & Sunita *et al.* (2012) used polyethylene glycol (PEG 4000 and PEG 6000, respectively) to prepare SDs of simvastatin. Both the study showed that

dissolution rate of simvastatin can be enhanced to considerable extent by solid dispersion technique with polyethylene glycol.

Parmar *et al.* (2012) investigated a new method (microwave induced fusion method) to prepare simvastatin SDs. They concluded that higher solubility was found with simvastatin with gelucire 44/14 after 10 mins time interval as compare to poloxamer 407 and β -cyclodextrin. Solubility of simvastatin increased higher with gelucire 44/14 by using microwave induced fusion method as compare to other technique. Using gelucire 44/14 with simvastatin showed 94% increase in solubility of simvastatin as compare to pure drug in water.

During 2008-2015 different researchers used different carriers to prepare SDs of ATV such as polyethylene glycol (PEG) 6000 (Narasaiah *et al.*, 2010a; Sharma *et al.*, 2012) PEG 4000 (Narasaiah *et al.*, 2010b; Sharma *et al.*, 2012; Sharma *et al.*, 2013; Bobe *et al.*, 2011), mannitol (Bobe *et al.*, 2011) and skimmed milk (Choudharya *et al.*, 2012). Drug release from these SDs was found to be higher than pure drug powder and conventional tablets.

Kadu *et al.* (2011) carried out a study to prepare SEDDS of atorvastatin by using various vehicles such as captex 355, captex 355 EP/NF, ethyl oleate, capmul MCM, capmul PG-8. gelucire 44/14 were used as oil and tween 80, tween 20 were used as surfactants where as PEG 400 was used as co-surfactant. Prepared formulations were tested for microemulsifying properties and evaluated for clarity, precipitation, viscosity determination, drug content and in vitro dissolution. The optimized formulation further evaluated for particle size distribution, zeta potential and product stability.

Nainwal *et al.* (2011) prepared solid dispersion of rosuvastatin by solvent evaporation method. PEG (Polyethylene glycol) 4000, mannitol and urea were used as carriers. It was observed that the solubility increased with the increase in the concentration of hydrotropic agents. Swathi *et al.* (2013) also used PEG 4000 as carrier to prepare SDs of rosuvastatin.

Kumar *et al.* (2014) carried out a study to prepare, characterize and evaluate starch phosphate solid dispersions for enhancing the dissolution rate of rosuvastatin calcium. Solid dispersions (SD) of rosuvastatin calcium in starch phosphate were prepared by solvent evaporation method, (drug: starch phosphate 1:1, 1:2, 1:3, and 1:4). *In-vitro*

Dissolution studies were performed and it followed first order kinetics. SD which released the drug faster was compressed into tablets for comparison with SDs powder.

Kumar, (2013) prepared SEDDS containing two drugs (finofibrate and rosuvastatin) by using capmul MCM, labrafac (LF), isopropyl myristate (IPM). SNEDDS were prepared using the concentration oil (2:1) and SCoS (1:1) which resulted in enhanced extent of absorption and relative bioavailability of 1.69 (fibrate) and 1.64 (statin). An approximately 40% of drug was available for systemic circulation via lymphatic route of absorption. The results showed a significant difference between the marketed products and the SNEDDS.

Patel *et al.* (2013) used capmul-PG8, acconon-MC8, tween-80 and propylene glycol for development of rosuvastatin calcium with highest efficiency. *Ex-vivo* study as a surrogate of *in-vivo* study was performed by non-everted chick ileum sac absorption model. Self-emulsifying formulation shows 30% drug diffusion/cm² area of ileum as compare to 6% of powdered drug.

Amrutkar *et al.* (2014) carried out an investigation to improve solubility and hence bioavailability of rosuvastatin calcium using self nanoemulsifying drug delivery system (SNEDDS) by using capmul MCM, tween 20 and PEG 200. The prepared formulation were evaluated for self emulsification time, dispersibility, average globule size, polydispersibility index (PDI). *In vitro* drug release studies showed remarkable increase in dissolution of SNEDDS compared to marketed formulation.

Rokad *et al.* (2014) preparer solid SEDDS of rosuvastatin calcium (ROS) with the least amount of surfactant which could enhance its solubility and oral bioavailability by using capmul MCM (oil), cremophor ELP (surfactant) and propylene glycol (co-surfactant). The solid-SEDDS (S-SEDDS) was prepared using an adsorbent, consequently the prepared S-SEDDS were filled up in hard gelatin capsule which were evaluated for various physicochemical parameters. The S-SEDDS formulations were prepared from the optimized liquid SEDDS, which revealed maximum release rate (97.7%) among all the prepared S-SEDDS formulation and marketed formulation.

Kulkarni *et al.* (2015) carried out a study to develop rosuvastatin calcium-loaded self-nanoemulsifying powder for improved oral delivery of the drug by using maisine 35-1 as

oil phase and tween 20 with lutrol E400 as surfactant mixture (Smix). The liquid formulations were adsorbed onto aerosil 200 in a ratio of 1: 0.25 % w/w to convert them into a solid form. The formulations were evaluated for globule size, zeta potential, and emulsion properties. Transmittance study, scanning electron microscopy, and *in-vitro* dissolution studies were also carried out.

1.11 Purpose of the Research

Atorvastatin, simvastatin and rosuvastatin are the most widely prescribed lipid lowering drugs (statins). They are poorly water soluble and show dissolution rate limited bioavailability. Oral bioavailability of these drugs varies from 5% to 20%. They show high intra- and inter-subject variability, and a lack of dose proportionality. The purpose of the present study was to increase bioavailability of these drugs by SEDDS and SD techniques.

Rosuvastatin is relatively a new drug having poor water solubility. It was approved on 12 August, 2003 as a lipid lowering agent. No studies regarding dissolution enhancement using solid dispersion or SEDDS formulations of rosuvastatin were reported before 2011. However a few number of research works has been carried out in this field from 2011 which are reported earlier in section 1.10.2. In most of the cases polyethylene glycol (PEG) was used as a carrier for SD preparation and *in-vitro* dissolution study was carried out for evaluation of SDs. But no studies have been reported so far on the secondary, tertiary solid dispersion of rosuvastatin and their evaluation by *in-vitro* diffusion, *ex-vivo* permeability, *in-vivo* performance and *in-vivo* bioavailability study. So, an initiative was taken to prepare primary, secondary and tertiary solid dispersions of rosuvastatin by using seven different carriers (poloxamer 407, croscarmellose sodium, sodium starch glycolate, hydroxypropylmethylellulose, povidone K-30, lactose and aerosil-200) and to evaluate them by *in-vitro*, *ex-vivo* and *in-vivo* techniques by applying model dependant and model independent approaches for data analysis along with ANOVA and Bonferroni test for multiple comparison. Response surface methodology (RSM) and 2² factorial designs were also used to evaluate and characterize SDs. *t*- test were also done in some cases for comparison of two variables (% drug release, % DE etc)

On the other hand capmul MCM was used as an oil phase for the preparation of SEDDS of rosuvastatin in most of the previous works. The purpose of the present study was to

develop innovative SEDDS formulation by using new excipients with the intention to improve dissolution rate and bioavailability and to evaluate the formulation by *in-vitro*, *ex-vivo* and *in-vivo* techniques as used for SDs. Comparison studies between SDs and SEDDS by different techniques such as diffusion through cellulose tubing, permeability through chicken and rabbit intestinal sacs, *in-vivo* performance by using hypolipidemic activity and finally by *In-vivo* bioavailability test in rabbits were another objective of the current study.

Simvastatin (approved on 23 December, 1991) and atorvastatin (approved on 7 December, 1996) are another two poorly water soluble drugs. An initiative was taken to prepare SDS and SSDs of these two drugs with the same carrier (poloxamer 407, croscarmellose sodium, sodium starch glycolate, hydroxypropylmethylcellulose, povidone K-30, lactose and aerosil-200) to compare the relative effectiveness of these carriers for three drugs. In the same time, preparation of SEDDS of these two drugs and to compare them with rosuvastatin SEDDS was another intention of this study.

Like 40 percent of new chemical entities atorvastatin, simvastatin and rosuvastatin are poorly soluble or lipophilic compounds. Characterization of these drugs is essential before formulation development. Characterization of atorvastatin, simvastatin and rosuvastatin by *in-vitro* dissolution study at three different biowaiver media (pH 1.2, pH 4.5 and pH 6.8.), FTIR, DSC, XRD and SEM was another important objective of current study.

The solubility issues are complicating the delivery of atorvastatin, simvastatin and rosuvastatin as these drugs show unpredictable absorption, high intra subject and inter subject variability. So, constant surveillance on the marketed poorly water insoluble drugs by the government, manufacturers and independent research groups is essential to ensure availability of quality medicines. To evaluate *in-vitro* equivalence of these tablets marketed in Bangladesh with REF products under biowaiver conditions was another purpose of the study. The *in-vitro* equivalence test was carried out at three different medium (pH 1.2, pH 4.5 and pH 6.8.). The test results were subjected to statistical analysis to compare the dissolution profiles by using model independent approaches of difference factor (f_1), similarity factor (f_2) and dissolution efficiency (%DE).

Chapter Two: Materials and Methods

2.1 Materials

Drug: Standard atorvastatin calcium (ATV POW), simvastatin (SIM POW), rosuvastatin calcium (ROS POW) and naproxen sodium (NAP) were kind gift from Incepta Pharmaceuticals Ltd., Bangladesh.

Excipients: PeceolTM (Glyceryl mono-oleate), MaisineTM 35-1 (Glyceryl mono-linoleate), LabrafacTM Lipophile WL 1349 (Medium chain triglycerides), LabrafacTM PG (Propylene glycol dicaprylocaprate), Transcutol[®] HP (Diethylene glycol monoethyl ether), Labrafil[®] M 1944 CS (Oleoyl macrogolglycerides), Labrafil[®] M 2125 CS (Linoleoyl macrogolglycerides) Labrasol[®] (Caprylocaproyl macrogolglycerides), CapryolTM 90 (Propylene glycol monocaprylate), CapryolTM PGMC (Propylene glycol caprylate), LauroglycolTM 90 (Propylene glycol monolaurate), LauroglycolTM FCC (Propylene glycol laurate), Plurol[®] Oleique CC 497 (Polyglyceryl oleate) were supplied by Gattefosse Co. (France). Oleic acid (Merck, Germany), Tween 20 and 80 (BDH Chemicals Ltd, England) were collected from the manufacturers. Poloxamer 407 (POL), croscarmellose sodium (CCS), sodium starch glycolate (SSG), hydroxypropylmethylellulose (HPMC 5 cps), povidone K-30 (POV), lactose, avicell PH 101, purified talc were collected from Incepta Pharmaceuticals Ltd., Bangladesh. Ludiflash was received from BASF (Germany) as a gift. Colloidal silicon dioxide, magnesium stearate, cross povidone and Pharmabust was received from Eskayef Bangladesh limited as a gift.

Empty Hard Gelatin Capsule Shell (EHGCS): Empty capsules (size 2 and 0) were supplied by Global Capsules Limited, Bangladesh.

Dosage form: Innovator brand of atorvastatin (Lipitor), simvastatin (Zocor) and rosuvastatin (Crestor) are coded as ATV REF, SIM REF and ROS REF, respectively. Four brands of 10 mg atorvastatin tablets (coded as ATV MP-1 to ATV MP-4), four brands of simvastatin (coded as SIM MP-1 to SIM MP-4) and four brands of rosuvastatin (coded as ROS MP-1 to ROS MP-4) were purchased from local drug store in Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, production and expiry dates. They were stored properly.

Solvents and Reagents: Acetonitrile and methanol were of HPLC grades. Methanol, diethyl ether, absolute ethanol, dichloromethane, chloroform, ethyl acetate, and n-hexane were purchased from Sigma–Aldrich (Oslo, Norway). Water was deionised and double distilled.

2.2 Identification of Drugs

FTIR Study

Drugs were identified by FTIR spectrum. FTIR studies were carried out for pure drug using FTIR spectrophotometer (IR Prestige 21, Shimadzu, Japan). The powdered sample was intimately mixed with dry powdered potassium bromide. The mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in IR spectrophotometer using sample holder and spectrum was recorded. Spectra were compared with standard value.

Melting Point Determination

Melting point of atorvastatin calcium, simvastatin and rosuvastatin calcium were determined by open capillary method.

Specific Optical Rotation Determination

0.125 gm simvastatin was dissolved in acetonitrile and diluted up to 250 ml BP (2014). Optical rotation was measured by a calibrated polarimeter (Model CDP-001, Contech Instruments Ltd., India)

Moisture Content Determination

Karl Fischer reagent VS was used to determine the amount of water in atorvastatin calcium and rosuvastatin calcium. 20 mg drug was dissolved in anhydrous methanol R and taken to the titration vessel. The solution was stirred for 1 min and titrated to the amperometric end-point with the Karl Fischer reagent VS (T50 Titrator, Metler-Toledo).

.Presence of Calcium

Samples were ignited and dissolved in 5 ml of acetic acid R and filtered. 0.5 ml of potassium ferrocyanide solution R was added in the solution. The solution remains clear.

Then 50 mg of ammonium chloride R was added. A white, crystalline precipitate was formed (BP, 2014)

2.3 Analytical Method Development

2.3.1 UV Spectrophotometric Analytical Method Development and Validation

UV analysis method for atorvastatin, simvastatin and rosuvastatin were developed and validated in different dissolution media (pH 1.2, pH 4.5 and pH 6.8). Solutions containing 20 µg/ml and 10 µg/ml of drug in different media were scanned separately in the range of 200-800 nm to find out the wavelength of maximum absorption. The proposed method was validated for the parameters like linearity, accuracy and precision as per ICH guidelines.

Instruments

A double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer, Model UV-1700 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of +0.5 nm (with automatic wavelength correction) was used. The drug analysis data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC. For scanning, the wavelength range selected was from 800 nm to 200 nm with medium scanning speed.

Preparation of Different Dissolution Media

Buffer solution of pH 1.2 was prepared by dissolving 22.365 gm potassium chloride and 43.35 ml 37% hydrochloric acid in 6000 ml distilled water. Acetate buffer solution of pH 4.5 was prepared by dissolving 22.17 g of sodium acetate trihydrate and 9.744 ml of glacial acetic in 6000 ml distilled water. Phosphate buffer solution of pH 6.8 was prepared by dissolving 40.8 g of potassium dihydrogen phosphate and 5.34 g of sodium hydroxide in 6000 ml distilled water.

Preparation of Standard Solution of Drug in Different Dissolution Media

Standard solution of atorvastatin and rosuvastatin were prepared in different dissolution media (pH 1.2, pH 4.5 and pH 6.8) by dissolving 10 mg equivalent drug in 500 ml volumetric flask. Atorvastatin calcium equivalent to 10 mg atorvastatin and rosuvastatin calcium equivalent to 10 mg rosuvastatin were accurately weighed and transferred to 500

ml separate volumetric flasks. Buffer solution was added and sonicated to dissolve drug. The volume was made up to the mark with the same buffer to get 20 µg/ml drug concentration. In the same way 20 µg/ml of simvastatin solution in phosphate buffer of pH 6.8 was prepared but due to poor solubility 10 µg/ml of simvastatin solution was prepared in hydrochloric acid buffer (pH 1.2) and in acetate buffer (pH 4.5).

Linearity

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. Linearity of the method was determined by constructing calibration curves. 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of the stock solution (20 µg/ml) were transferred to a series of nine 10 ml volumetric flasks. The volume in each flask was adjusted to the mark and mixed so as to obtain solutions of final concentrations in the range of 2 to 20 µg/ml (2 to 10 µg/ml for simvastatin in case of in hydrochloric acid buffer of pH 1.2 and in acetate buffer of pH 4.5). These solutions were analyzed using UV-Visible Spectrophotometer (UV-1700 PC, Shimadzu, Japan) at wave length of maximum absorbance using respective buffer as blank. Each measurement was carried out in six replicates and the absorbances were plotted against the concentrations to obtain calibration curves and correlation coefficients. Characteristic parameters for regression equation ($y = a + bx$) of the method were obtained by least squares treatment of the results and these parameters were used to confirm the good linearity of the method.

Precision

Precision of the method was investigated with respect to reproducibility (inter laboratory trial). Reproducibility was determined by performing three repeated analysis of standard solutions (2 µg/ml, 6 µg/ml and 10 µg/ml) in two different labs by using two equipments (Shimadzu spectrophotometer model UV-1601 and UV-1700). The relative standard deviation (% RSD) was calculated in order to assess the precision of the method.

Accuracy

Accuracy indicates the deviation between the test result and true value. Accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments. The accuracy studies were carried out by spiking a

known concentration of standard (4 µg/ml) drug to the pre-analyzed sample (6 µg/ml). From the absorbance drug concentration was calculated. Then accuracy was assessed from the test results as the percentage of drug recovered by the assay.

2.3.2 RP-HPLC Analytical Method Development and Validation

A new, affordable, cost-effective and convenient HPLC method for determination of atorvastatin, simvastatin and rosuvastatin was developed and validated. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness.

Instrumentation

A Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A auto-sampler and CTO-10ASVP column oven was used. Ultraviolet detection was achieved with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analysis data were acquired and processed using LC solution (Version 1.2, Shimadzu, Japan) software running under Windows XP on a Pentium PC.

System Suitability Study

In order to assess the system suitability of the method solution containing 100% target concentration of drug was injected in six replicates and various chromatographic parameters such as retention time, peak area, tailing factor and theoretical plates (Tangent) of the column were determined. The method was evaluated by analyzing the parameters.

Selectivity

Selectivity was determined by injecting the pressing drugs containing common excipients used in SD and SEDDS formulation. Sample containing 100% nominal concentration was injected first. Then the samples of drug along with different oils and surfactants were injected to find out the selectivity of the method.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of drug of different concentrations level (2-20 µg/ml) were used for this purpose. Each measurement was carried out in six replicates to verify the reproducibility

of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves. The data were then subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

Accuracy was determined by means of spike and recovery method. Drug at different level (50%, 100% and 150% of nominal concentration) were added to placebo formulations. The accuracy was calculated as the percentage of the drug recovered by the assay.

Precision

The precision of the method was determined by intra-day (repeatability) and inter-day (ruggedness) study. Intra-day precision (repeatability) was determined by performing four repeated analysis of the standard solutions (100% of nominal concentration) on the same day but at different times and inter-day precision (intermediate precision) of the method was assessed by carrying out the analysis of standard solutions on three different days in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

Robustness

Robustness of the method was determined by the analysis of the samples under a variety of conditions making small changes in the mobile phase component ($\pm 0.5\%$), flow rate (± 0.05 ml/ min) and column temperature (± 2 °C).

2.4 Dissolution Study of Pure Drug (API) in Different Dissolution Media

Before preparing SEDDS and solid dispersion (SD) dissolution study of atorvastatin, simvastatin and rosuvastatin were carried out in different dissolution media [USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution)]. The test results were subjected to statistical analysis to compare the dissolution profile. The dissolution test was undertaken using USP Apparatus II (TDT-08L, Electrolab, India) at 75 rpm. The medium was maintained at 37 ± 0.5 °C in all the experiments. 5 mL of dissolution sample was withdrawn at 0, 5, 10, 15, 20, 30, 45 and 60 min and replaced with an equal volume to maintain sink conditions.

Samples were filtered and assayed by the validated UV spectroscopic method. The concentration of each sample was determined from calibration curve obtained from pure drug.

2.5 Analysis of Marketed Tablets of Atorvastatin, Simvastatin and Rosuvastatin

Comparative Dissolution Study

To know the current quality status of marketed products, drug release from innovator brand (REF) and marketed tablet (MP) of atorvastatin, simvastatin and rosuvastatin were studied in different dissolution media [USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution)].

The dissolution test was undertaken using USP Apparatus II (TDT-08L, Electrolab, India) at 75 rpm. The medium was maintained at 37 ± 0.5 °C in all experiments. 5 ml of dissolution sample was withdrawn at 0, 5, 10, 15, 20, 30, 45 and 60 min and replaced with an equal volume to maintain sink conditions. Samples were filtered and assayed by a validated HPLC method.

Other quality parameters of selected tablets like weight variation, hardness, friability, disintegration time were also tested to assess the quality of marketed products. The test results were subjected to statistical analysis for comparison.

Determination of Uniformity of Weight

20 tablets from each brand were weighed individually with an analytical weighing balance (AY-200, Shimadzu, Japan). The average weights for each brand as well as the percentage deviation from the mean value were calculated.

Hardness

The crushing strength was determined with an Automatic Tablet Hardness Tester (8M, Dr Schleuniger, Switzerland). Six tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability Twenty tablets of each brand were weighed and subjected to abrasion by employing a Veego friabilator (VFT-2, India) at 25 rev/min for 4 min. The tablets were then weighed and compared with their initial weights and percentage friability was calculated.

Assay

Validated reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was used to determine the potency of tablets. Standard and sample solutions were prepared by dissolving 10 mg drug and powdered tablets equivalent to 5 mg drug in 10 ml methanol separately. Then the solutions were diluted by the mobile phase to get suitable analytical concentration. After filtration samples were injected in Liquid Chromatographic (RP-HPLC) system and potency were calculated for each brand by comparing the standard and sample peak area.

2.6 Formulation Development of SEDDS**Solubility Analysis**

The solubility of atorvastatin, simvastatin and rosuvastatin in various lipids, surfactants and co-surfactants was determined by using shake flask method. Briefly, an excess amount of drug was added to each vial containing 1 ml of the selected vehicle. After sealing, the mixture was vortexed using a cyclomixer for 10 min in order to facilitate proper mixing of drug with the vehicles. Mixtures were then shaken for 48 h in a water bath shaker (Remi, Mumbai, India) maintained at room temperature (Ashok and Pradeep, 2007). Mixtures were centrifuged at 3000 rpm for 5 min using a centrifuge (Sigma 3K15; Sigma Co., USA) followed by filtration through membrane filter 0.45 μm to remove undissolved drug. Filtrate was suitably diluted with mobile phase and concentration of drug was determined by HPLC analysis.

Screening of Surfactants for Emulsifying Ability

Emulsification ability of various surfactants was screened out by transmittance study. Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45–60⁰C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed and diluted with double distilled water to 50 ml to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 638.2 nm by UV-1700 double

beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank (Nipun and Islam, 2014).

Screening of Co-Surfactants

The turbidimetric method was used to assess relative efficacy of the co-surfactant to improve the emulsification ability of the surfactants and also to select best co-surfactant. 0.2 g surfactant was mixed with 0.1 g of co-surfactant. Oil (0.3 g) was added to this mixture and the mixture was homogenized with the aid of the gentle heat (45–60 °C). The isotropic mixture, 50 mg, was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The resulting emulsions The emulsions were allowed to stand for 2 h and their transmittance was measured at 638.2 nm by UV-1770 double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank. (Wankhade *et al.*, 2010).

Construction of Solubility Ternary Phase Diagrams

Depending on the solubility and screening of surfactants and co-surfactants we selected two non-ionic surfactants, namely tween 80 and labrasol^(R), and 1 solubilizer as co-surfactants (transcutol^(R)). Lipids employed were oleic acid in case of atorvastatin, Peceol for rosuvastatin and capryol 90 for simvastatin. Surfactant was blended with co-surfactant in the ratio of 1:2, 1:1, 2:1, 3:1 (i.e. Km, w/w). Volumes of each surfactant and cosurfactant mixture (S_{mix}) were blended with lipid in a ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w) in captubes. Then drug was gradually added in each of the captube up to the maximum solubility capacity of lipid/surfactant/co- surfactant mixture. After each increment, the captube containing lipid/surfactant/co- surfactant/drug mixture was heated in sealed condition in a water bath at $\leq 90^{\circ}\text{C}$ for ≤ 5 minutes to facilitate the solubilization of drug. Solubility points (percentage of lipid, S_{mix} and drug) were then plotted in a ternary phase diagram by SigmaPlot 10.0 software (USA).

Construction of pseudo-ternary Phase Diagrams

Ternary diagrams of surfactant, co-surfactant mixture (S_{mix}) and oil were developed using water titration method. Ternary mixtures with varying compositions of surfactant, co-surfactant and oil were prepared. Water was folded in a drop-wise manner to each lipid– S_{mix} mixture under gentle shake at 40°C. After equilibrium, the appearance and

dispersibility of the formulation was observed and droplet size/distribution was analyzed. So it was distinguishable between the microemulsion which was clear and slight blue and the crude emulsion which had a white appearance. The amount of water, lipid and S_{mix} (surfactant and cosurfactant) was noted down. The pseudo-ternary phase diagrams were mapped with SigmaPlot 10.0 according to the data. The microemulsion region in the diagrams was plotted and the wider region indicated the better self-microemulsification efficiency.

Preparation of SEDDS Formulations

After the pseudo-ternary phase diagrams were plotted and compared, optimal surfactant, co-surfactant and lipid combinations were selected for three drugs. SEDDS formulations were prepared by dissolving drug into S_{mix} in a glass tube, heating at 40°C in a water-bath and using a vortex mixer to facilitate solubilization, then adding the required weight of lipid into the glass tube and mixing. The mixture was filled in capsules (Global Capsules Ltd., Bangladesh). The capsules were tightly sealed and stored at ambient temperature (25°C) until used.

2.7 Characterization of Optimized SEDDS

Optimized SEDDS were evaluated for emulsification time, optical clarity, droplet size, drug content, robustness to dilution, stability and *in-vitro* dissolution profile.

Assessment of Emulsification Time

Emulsification time of SEDDS formulations was determined in a USP dissolution tester (Electrolab, India). The SEDDS formulation equivalent to 10 mg of drug was added drop wise to 500 ml of distilled water maintained at $37 \pm 0.5^\circ\text{C}$. Gentle agitation was provided by a paddle rotating at 50 rpm. The emulsification time was then recorded (Mei *et al.*, 2006).

Spectroscopic Characterization of Optical Clarity

Each SEDDS formulation equivalent to 10 mg drug was diluted with 500 ml distilled water. The transmittance values of each emulsion were measured by a UV spectrophotometer (UV mini-1240, Shimadzu, Japan) at 400 nm (Subramanian *et al.*, 2004) just after dilution and at 10, 20, and 30 min after dilution.

Emulsion Droplet Size Measurement

Emulsion droplet size was determined by the Malvern particle size analyzer (Mastersizer 2000, Malvern, UK). Briefly, SEDDS formulations (equivalent to 10 mg drug) were diluted with 50 ml distilled water and thereafter, the droplet size was immediately determined. Each determination was done in triplicate.

Effect of Dilution Medium on Droplet Size

SEDDS formulations (equivalent to 10 mg drug) was diluted to 50 ml with media like double distilled water, SGF pH 1.2 and phosphate buffer pH 6.8. Visual observations were made immediately after dilution for assessment for self-emulsification efficiency, appearance (transparency), phase separation, and precipitation of drug. Droplet size distribution of SEDDS diluted with water was determined using by the Malvern particle size analyzer (Mastersizer 2000, Malvern, UK) based on the laser light scattering phenomenon. Diluted samples were directly placed into the module and measurements were made in triplicate after 2-min stirring. Droplet size was calculated from the volume size distribution. The resultant emulsions were also allowed to stand for 6 h at room temperature to assess dilution stability.

Effect of Drug Loading on Droplet Size

The increase or decrease in the amount of drug would influence the droplet size of the resultant SEDDS if drug were participating at interface of emulsion. In order to investigate role of drug, various formulations were prepared containing varying amount of drug from 1 to 3% (w/w). SEDDS, SEDDS formulations (equivalent to 10 mg drug) was diluted to 50 ml with SGF pH 1.2 and phosphate buffer pH 6.8 and the mean droplet size of resulting emulsions were determined (Mastersizer 2000, Malvern, UK).

Drug Content Analysis

Drug from pre-weighed SEDDS was extracted by dissolving in 25 ml methanol. Drug content in the methanolic extract was analyzed by the validated HPLC method.

Robustness to Dilution

Robustness of SEDDS to dilution was studied by diluting 50, 100 and 1000 times with various dissolution media viz. water, SGF pH 1.2 and phosphate buffer pH 6.8. The

diluted emulsions were stored for 12 h and observed for any signs of phase separation or drug precipitation.

Stability Study

Stability was assessed by analysing droplet size and distribution at 0.2, 1, 10, and 24 h after SEDDS formulation was dispersed. The optimal SEDDS formulations filled in capsules were tightly sealed for storage at ambient temperature (25°C) for one year. Drug content and droplet size of SEDDS were determined at predetermined intervals.

***In-Vitro* Dissolution Study**

In-vitro release profile of SEDDS was performed using USP XXIII apparatus II (TDT-08L, Electrolab, India) at $37 \pm 0.5^\circ\text{C}$ with a rotating speed of 75 rpm in dissolution media (pH 1.2). At designated time intervals (5, 10, 15, and 20 min), 5ml of release medium was collected and concentration of drug was determined by HPLC. Release percentages were calculated as the ratio of drug released to total drug. *In-vitro* dissolution profile of optimized SEDDS was compared with ref tablets and pure drugs.

2.8 Solid Dispersion

Preparation of Solid Dispersion and Physical Mixture

Solid Dispersion of atorvastatin, simvastatin and rosuvastatin were prepared in different carrier by solvent evaporation method

Solvent evaporation method

SDs of atorvastatin containing different weight ratios (1:1, 1:3, 1:15) were denoted as SD Drug: Carrier ratio (example: SD ATV:POL 1:1) and prepared by the solvent method. Respective amount of carrier was dissolved in glass beaker containing ethanol and drug was added in parts with continuous stirring. Then the solvent was removed by evaporation at 40°C under vacuum. Then the resulting residue was dried under vacuum for 3 h and stored in a desiccator. The material was then grounded in a mortar and passed through a sieve (#100). SDs of simvastatin and rosuvastatin were also prepared in the same way. In case of secondary and tertiary solid dispersions mixture of carriers were dissolved or suspended in the solvent followed by addition of drug in the mixed system.

Physical mixtures

Physical mixtures (PMs) having the same weight ratios, as described in the previous method, were prepared by thoroughly mixing appropriate amounts of drug and carrier in a mortar until a homogeneous mixture was obtained. The resulting mixtures were sieved through a sieve (#120) and denoted as PM Drug: Carrier ratio (example: PM ATV:POL 1:1).

2.9 *In-vitro* Dissolution Studies of SD

Dissolution studies of SD were performed using USP Apparatus II with 900mL dissolution medium (pH 1.2) at 37 ± 0.5 °C and 75 rpm for 45 min. Samples of pure drug and PMs and SDs equivalent to 10 mg of the drug were added to the dissolution medium. At fixed time intervals, 5 ml aliquots were withdrawn, filtered through a 0.22- μ m membrane filter, suitably diluted and assayed by HPLC. Equal volume of fresh medium pre-warmed at the same temperature was replaced in the dissolution medium after each sampling to maintain constant volume throughout the test. Each test was performed in triplicate, and release curves were plotted using calculated mean values of cumulative drug release. Similarity factor (f_2) and mean dissolution time (MDT) values were calculated to compare the extent of improvement in the dissolution rate from different samples.

2.10 Characterization of Solid Dispersion**Physical Appearance and Drug Content Analysis**

First solid dispersions were evaluated physically by observing their color and physical state after preparation and during storage. Color change and physical changes were also observed during preparation of SD. Validated reversed phase High Performance Liquid Chromatographic (RP-HPLC) method as discussed in section 2.3.2 was used to determine the potency of SDs. Standard and sample solutions were prepared by dissolving 10 mg drug and SD powder containing 10 mg drug in 10 ml methanol separately. Then the solutions were diluted to by the mobile phase to get suitable analytical concentration. After filtration samples were injected in Liquid Chromatographic (RP-HPLC) system and potency were calculated for each SD by comparing the standard and sample peak area.

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

FTIR studies were carried out for pure drugs, excipients, physical mixtures and solid dispersions using FTIR spectrophotometer (IRPrestige 21, Shimadzu, Japan). FTIR studies were conducted in Center for Advanced Research in Science (CARS), University of Dhaka. The powdered sample was intimately mixed with dry powdered potassium bromide. The mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in FTIR spectrophotometer using sample holder and spectrum was recorded. FTIR has been used to assess the interaction between carrier and drug molecules in the solid state.

Differential Scanning Calorimetric (DSC) Analysis

DSC studies were carried out for pure drugs, excipients, physical mixtures and solid dispersions to check any kind of incompatibilities that may give rise to change in the stability, solubility, dissolution rate and bioavailability of drug. Differential scanning calorimetric (DSC-60, Shimadzu, Japan) analysis of selected samples was conducted in Center for Advanced Research in Science (CARS), University of Dhaka. Sample was weighed (3-5mg) and placed in sealed aluminium pans. The coolant was liquid nitrogen. The samples were scanned at 10⁰C/ min from 30⁰ C to 300⁰ C. Nitrogen flow rate was 20 ml/min.

SEM Studies

Sample of pure drugs and solid dispersion formulations were mounted onto the stubs using double sided adhesive tape and then coated with gold palladium alloy (150-200Å) using fine coat ion sputter (Joel fine coat ion sputter, JFC-1100). The sample was subsequently analysed under the Scanning Electron Microscope ((JSM-6490, JEOL USA, Inc. USA) in Center for Advanced Research in Science (CARS), University of Dhaka. for external morphology.

Powder X-ray Diffraction (PXRD) Analysis

The powder X-ray diffraction patterns were traced employing X-Ray diffractometer (XPRT-PRO PW3050/60) for the samples using Ni filtered Cu (K- α) radiation, a

voltage of 40 KV, a current of 30 mA. The samples were analysed over 2θ range of 5-75° with scan step size of 0.020° (2 θ) and scan step time 0.30 S in BCSIR, Bangladesh.

Tablet Formulation of SD

Formulation excipients were selected on the basis of preliminary tests, which demonstrated no interference of these excipients with the drugs. Tablets containing SDs equivalent to 10 mg drug were made by direct compression using different formulation excipients such as directly compressible lactose (79%), colloidal silicon dioxide (1%), purified talc (1%) and magnesium stearate (1%). Three disintegrating agents (5%) were used in three different formulations (Cross povidone in TAB-1, Ludiflash in TAB-2 and Pharmabust in TAB-3). The blend was compressed on an eight-station single rotary machine (Shakti Pharmatech, India) using round-shaped, flat punches to obtain tablets of 40-50 N hardness and 2.78-2.82 mm thickness. For the assay, three tablets were crushed and a blend equivalent to 10 mg drug was weighed and dissolved in methanol and samples were analysed by HPLC method. The release profile of drug from tablets was studied in triplicate using the same dissolution media, conditions and procedure as described for *in-vitro* dissolution studies for SD.

2.11 In-Vitro Diffusion Study of SEDDS and SD through Cellulose Dialysis Tubing

In-vitro diffusion studies were carried out using cellulose dialysis tubing for selected SEDDS, SD along with API (atorvastatin, simvastatin, rosuvastatin) and REF tablets (ATV REF, ROS REF, SIM REF). SEDDS formulations (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3) having lowest droplet size and highest % DE value and SD formulations (SD ATV: POL 1:1, SIM: POL 1:1, ROS: POL 1:1) having highest % DE value were included in the study.

Membrane was purchased from Medical Membranes Limited, UK. The supplied membrane was cut into small pieces (9cm in length) and taken in 500 ml beaker containing de-ionized water. The membranes were immersed beneath the de-ionized water and heated for more than 10 hrs to remove sulfur as sulfur may interfere in the overall diffusion process. The temperature was controlled between 65-70°C and hot water was replaced by fresh de-ionized water at every one hour for efficient removal of sulfur.

The prepared pieces of membrane were kept in a beaker containing fresh de-ionized water and preserved in a refrigerator until use.

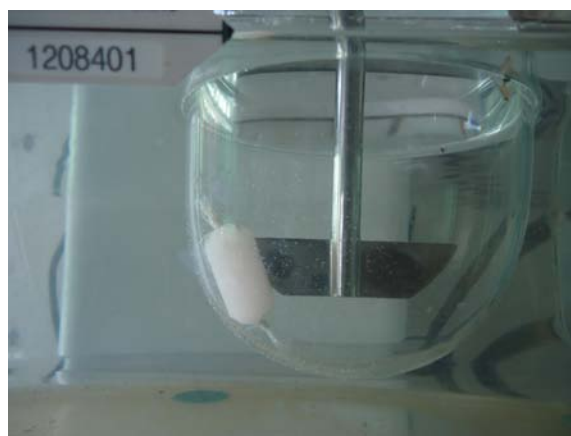


Figure 2.1: *In-Vitro* Diffusion Study of SEDDS and SD through Cellulose Dialysis Tubing

One end of pretreated cellulose dialysis tubing (9 cm in length) was tied with plastic thread. SEDDS (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3) SD (SD ATV: POL 1:1, SIM: POL 1:1, ROS: POL 1:1) drug powder (atorvastatin, simvastatin, rosuvastatin) and REF tablets (ATV REF, ROS REF, SIM REF) were placed in cellulose dialysis tubing along with 3 ml of dialyzing medium (pH 1.2). The other end of tubing was also secured with thread and was allowed to rotate freely in the dissolution vessel of a USP 24 type II dissolution test apparatus (Electro lab TDT-06P, India) that contained 900 ml dialyzing medium (buffer pH 1.2) maintained at 37 ± 0.5 °C and stirred at 75 rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through a 0.22- μ m membrane filter, suitably diluted, and were assayed by HPLC for drug content.

2.12 *Ex-Vivo* Permeability Study of SEDDS and SD through Chicken and Rabbit Intestinal Sac

Ex-vivo permeability study was carried out by using chicken and rabbit intestinal sac for selected SEDDS, SD along with API (atorvastatin, simvastatin, rosuvastatin) and ref tablets (ATV REF, ROS REF, SIM REF). SEDDS formulations (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3) having lowest droplet size and highest % DE value

and SD formulations (SD ATV: POL 1:1, SIM: POL 1:1, ROS: POL 1:1) having highest % DE value were included in the study.

Male white chicks weighing between 1.5 to 2 kg were bought from the local market. The Krebs–Ringer solution was prepared by dissolving 6.3 g NaCl, 0.35 g KCl, 0.14 g CaCl₂, 0.16 g KH₂PO₄, 0.15 g MgSO₄·7 H₂O, 2.1 g NaHCO₃ and 5 g glucose in one liter of distilled water. For isolation of everted intestine, the chicks were slaughtered, a median incision of the abdomen was performed, and the small intestine was freed. The lumen was carefully cleared from mucus by rinsing with a pH 7.4 buffer solution (Krebs–Ringer solution). An intestinal segment of the first 6- cm length was removed and transferred to oxygenated Krebs–Ringer solution. It was washed thoroughly with warm Krebs–Ringer solution. 6 cm long sacs were prepared by tying up the two end of the sac with plastic thread.

SEDDS formulations (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3) and SD formulations (SD ATV: POL 1:1, SIM: POL 1:1, ROS: POL 1:1) equivalent to 10 mg drug were taken inside the sac. Intestinal sac containing API (atorvastatin, simvastatin, rosuvastatin) and ref tablets (ATV REF, ROS REF, SIM REF) were also included in this study for comparison. The sacs were then taken into different dissolution baskets containing 900 ml dissolution medium (pH 1.2) maintained at 37 ± 0.5°C and stirred at 75 rpm. Samples were withdrawn at predetermined time intervals. Each time 5 ml of the sample was withdrawn with a calibrated plastic disposable syringe and media was replenished with fresh medium. The samples were analyzed by HPLC method. The permeability of drug was checked for 6 hours. The same procedure was followed for permeability testing through rabbit intestinal sacs but testing time was 45 minutes.

2.13 Effect of SEDDS and SD on Plasma Lipid Profiles of Albino Rats

The effect of different SD and SEDDS formulations on plasma lipid profiles was determined by compared in healthy albino rats (Wistar strain) of either sex and weighing between 100–120 g. Animals had free access to food and water. The animals were randomly divided into 5 treatment groups of 6 animals each, *viz.*, test treatment group for ROS SEDDS F-3 (SEDDS-TG), test treatment group for SD ROS: POL 1:1 (SD-TG) reference treatment group (R-TG), placebo treatment group (P-TG) and control treatment

group (C-TG). The treatment was given for 21 days. Each treatment group received daily 1.5 ml of coconut oil orally in the morning throughout 21 days. T-SEDDS, T-SD, R-TG and P-TG additionally received ROS SEDDS F-3, SD ROS: POL 1:1 aqueous suspensions of ROS REF tab and blank formulation respectively. The administered oral dose was equivalent to 10 mg kg⁻¹ per day. cholesterol level was measured by Blood Cholesterol Measuring KIT (**EasyMate[®] I**, Jhunan Township, Taiwan).

Statistical analysis for the determination of differences in lipid profiles of different treatments and control groups was done by the unpaired *t*-test and ANOVA (significance level $p < 0.05$). The results were confirmed by Bonferroni's multiple comparison as a post-hoc test.

2.14 *In-Vivo* Bioavailability Study of SEDDS and SD in Rabbits

Oral bioavailability study in rabbits was performed by determining the concentration of rosuvastatin in blood samples following oral administration. Six healthy rabbits, 1.5-1.7 kg, fasted for 24 h before the experiment, were allocated to three groups at random. Rabbits were administered rosuvastatin SEDDS (ROS SEDDS F-3), SD ROS:POL 1:1 and ref tablet (ROS REF) within three periods of experiment. Washout interval among the administrations was kept at 7 days.

Collection of Blood Samples

Blood samples (0.6 ml) were collected from marginal ear vein into glass tubes at the following times: immediately before administration, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after administration.

Separation and Storage of Blood Serum

The blood samples were immediately centrifuged at 3000 rev min⁻¹ for 20 min. Plasma samples were collected in capped tubes and stored at -20°C until assay.

Preparation of Standard Solutions

The stock solutions of rosuvastatin and naproxen sodium (IS) were prepared by dissolving appropriate amount corresponding to 1.0 mg/ml concentration of working standards in methanol. All stock solutions were stored at 2-8°C. The stock solutions of rosuvastatin were further diluted with the mobile phase methanol-water (68:32, v/v; pH

adjusted to 3.0 with trifluoroacetic acid) to give a series of standard mixtures having a final concentration in the range of 2.0–256 ng/ml. A working solution of the naproxen sodium (to give a final concentration of 400 ng/ml) was also prepared by diluting its stock solution and added to all standard mixtures and serum samples.

Precipitation of Protein and Sample Preparation for HPLC Analysis

HPLC method of Shah *et al.* (2011) was used for the analysis of plasma samples. Frozen plasma samples were thawed at room temperature just before assay. A simple two step liquid–liquid extraction (LLE) procedure was carried out for the extraction of rosuvastatin from serum samples. A volume (50µl) of the working solution of the naproxen sodium (to give a final concentration of 400 ng/ml) was added to 200µl of serum and mixed for approximately 10 s. Then absolute ethanol (600 µl) was added and vortex-mixed for 2min for deproteination. In step one, 1.0 ml of diethyl ether (extraction solvent 1) was added, vortex-mixed for 5min and centrifuged at 5000 rpm for 10 min. The supernatant organic layer was separated in a test tube. In step two, 0.5 ml of dichloromethane (extraction solvent 2) was added, vortexed for 5min followed by centrifugation at 5000rpm for 10 min. The organic layer was separated, collected in the same tube and evaporated to complete dryness under the gentle stream of nitrogen on a heating block maintained at 40⁰C. After drying, the residue was reconstituted in 500µl of mobile phase, vortex-mixed for 2min and 20µl sample was injected onto HPLC system.

HPLC Technique

Method development of rosuvastatin was done by a Reverse Phase High Performance Liquid Chromatography (Shimadzu LC-20 AT, Japan) and data was analyzed by LC Solution software (Version 1.2, Shimadzu, Japan). Separation of rosuvastatin calcium was successfully achieved on a phenomenex C₁₈ Column. The chromatographic system consisted of a column oven (model CTO-10AS VP, Shimadzu) equipped with prominence UV-VIS detector (model SPD-20A, Shimadzu), auto sampler (model SIL-20A, Shimadzu), degasser (model DGU-20A, Shimadzu) and liquid chromatography pump (model –LC20 AT).

Method developed and validated by Shah *et al.*, 2011 was used in this study for determination of rosuvastatin in serum using naproxen sodium as an internal standard.

The method showed adequate separation for rosuvastatin with phenomenex analytical C₁₈ column (150×4.6mm, 5µm) using methanol–water (68:32, v/v; pH adjusted to 3.0 with trifluoroacetic acid) as a mobile phase at a flow rate of 1.5 ml/min and wavelength of 241 nm. The calibration curves were linear over the concentration ranges of 2.0–256 ng/ml for rosuvastatin. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for rosuvastatin were 0.6 and 2.0 ng/ml.

Calibration Curve

Calibration curve was constructed by spiking a series of standard mixtures of rosuvastatin (2.0–256 ng/ml) and internal standard (400 ng/ml) into rabbit serum samples, extracting and analyzing in triplicate. Calibration curves for standard solutions and spiked serum samples were then acquired by plotting their response ratios (ratios of the peak area of the analytes to internal standard) against their respective concentrations. Linear regression was applied and slope (a), intercept (b), correlation coefficient (r) and standard error (Es) were determined.

Statistical Analysis of Pharmacokinetic Data

Statistical analysis of the pharmacokinetic data was performed based on a non-compartmental model with kinetica (version 5.0; ALFASOFT, UK). Data from the plasma concentration–time curve within 24 h after drug intake were used to obtain the peak plasma concentration (C_{max} , ngml⁻¹) and time of peak plasma concentration (T_{max} , h) for Reference tablet (ROS REF) and ROS SEDDS F-3 and SD ROS:POL 1:1. The area under the plasma concentration–time curve ($AUC_{0\rightarrow24}$ h) was calculated using the linear trapezoidal method. The relative bioavailability (Fr) of the SEDDS to the conventional tablet with the same dose was calculated as: $Fr = [AUC_{SEDDS\ 0\rightarrow24\ h} / AUC_{REF\ 0\rightarrow24\ h}] \times 100\%$. The relative bioavailability (Fr) of the SD to the conventional tablet with the same dose was calculated as: $Fr = [AUC_{SD\ (0\rightarrow24\ h)} / AUC_{REF\ (0\rightarrow24\ h)}] \times 100\%$. The pharmacokinetic parameters were analyzed statistically by ANOVA test using SPSS software (version 16.0; SPSS Inc., USA).

Chapter Three: Results and Discussion

3.1 Identification of Drugs

Identification of Atorvastatin Calcium

FTIR Spectrum: FTIR spectrum of atorvastatin calcium (Figure 3.1) was found similar to the standard spectrum of atorvastatin calcium. The spectrum of atorvastatin calcium shows the following functional groups at their frequencies:

3368.64 cm^{-1} (OH-stretching), 2953.02 cm^{-1} (CH-stretching), 1649.14 cm^{-1} (C=O-stretching), 1573.91 cm^{-1} (C=C-bending), 1315.45 cm^{-1} (C-N-stretching), 1226.73 cm^{-1} (C-F-stretching).

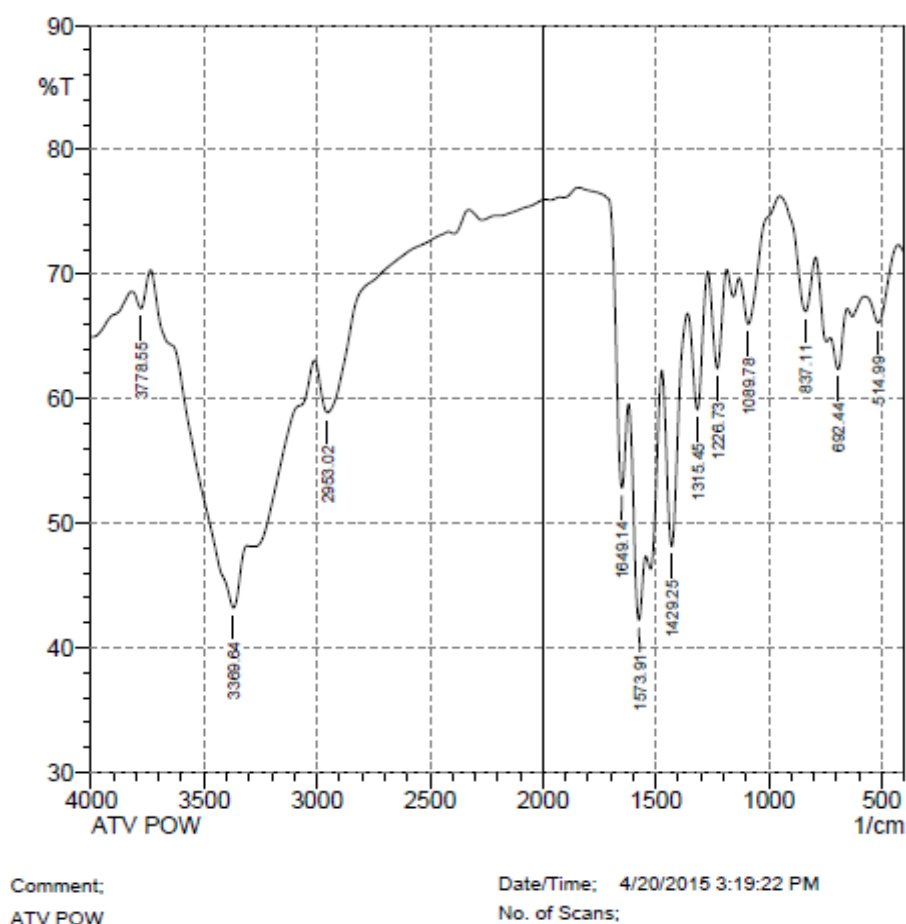


Figure 3.1: FTIR spectrum of atorvastatin calcium

Melting Point: Melting point of atorvastatin calcium was found 152.85°C which is within range as reported in the literature (Bobe *et al.*, 2011). This also indicates the purity of the substance.

Moisture Content: 3.78% moisture was found in atorvastatin calcium which complies with BP (2014) specification (3.5-5.5%).

Presence of Calcium: A white, crystalline precipitate was formed after addition of ammonium chloride R which proves the presence of calcium in the molecule (BP, 2014).

Identification of Simvastatin

FTIR Spectrum: FTIR spectrum of simvastatin (Figure 3.2) was found similar to the standard spectrum of simvastatin. The spectrum of simvastatin shows the following functional groups at their frequencies:

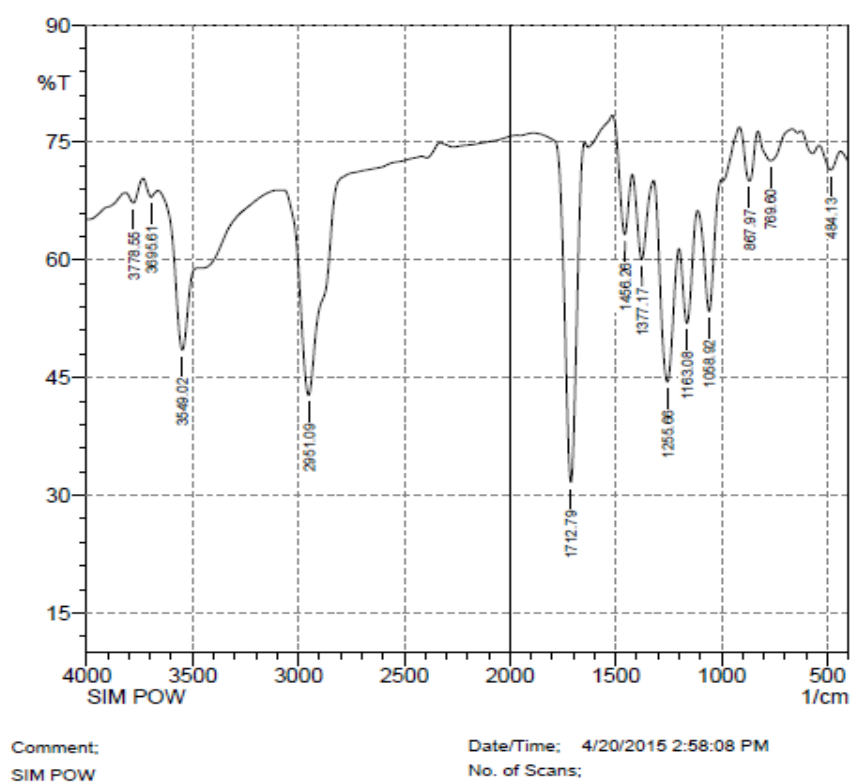


Figure 3.2: FTIR spectrum of simvastatin

3553 cm^{-1} (alcohol O-H stretching vibration), 2951.09 cm^{-1} (methyl and methylene C-H asymmetric and symmetric stretching vibration), 1712.79 cm^{-1} (Lactone C=O stretching vibration) and 1058.92 cm^{-1} (C-O stretching vibration).

Melting Point: Melting point of simvastatin was found 135.01°C which is within range as reported in the literature (Rao *et al.*, 2010). This also indicates the purity of the substance.

Specific Optical Rotation: Measured SOR was $+290^\circ$ which complies with BP (2014) specification ($+285^\circ$ to $+300^\circ$)

Identification of Rosuvastatin Calcium

FTIR Spectrum: FTIR spectrum of rosuvastatin calcium (Figure 3.3) was found similar to the standard spectrum of rosuvastatin calcium. The spectrum of rosuvastatin shows the following functional groups at their frequencies:

3390.86 cm^{-1} (OH-stretching), 2968.45 cm^{-1} (C-H-stretching), 1548.84 cm^{-1} (C=C-stretching), 1382.96 cm^{-1} (C-N-stretching), 1330.88 cm^{-1} (S=O-asymmetric), 1153.43 cm^{-1} (C-F-stretching).

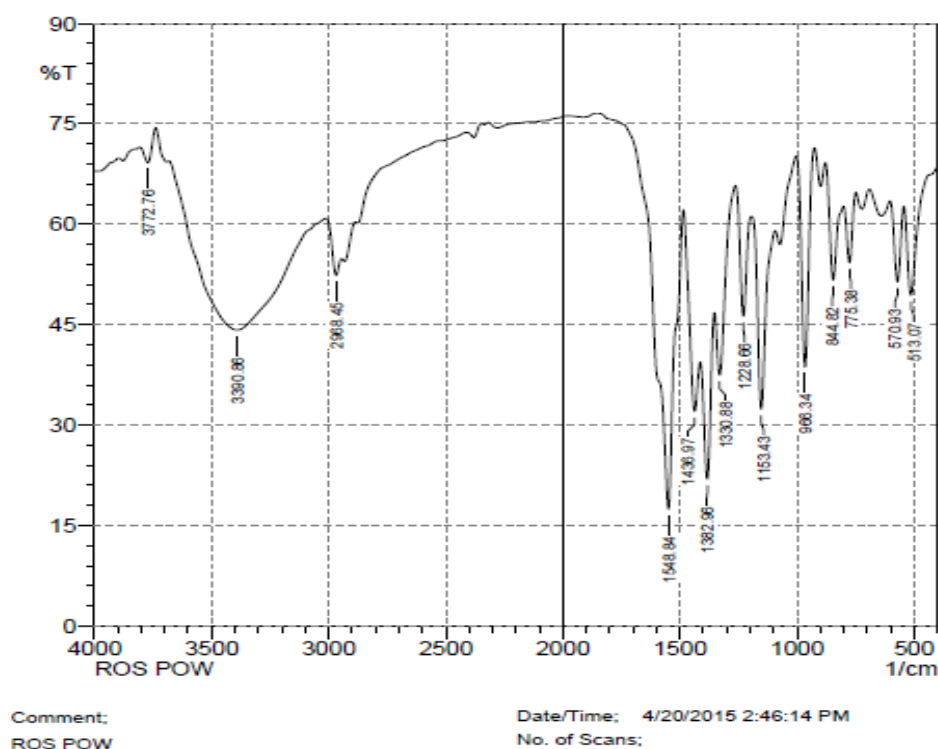


Figure 3.3: FTIR spectrum of rosuvastatin calcium

Melting Point: Melting point of rosuvastatin calcium was found 124.88°C which is within range as reported in the literature (Rokad *et al.*, 2014). This also indicates the purity of the substance.

Presence of Calcium: A white, crystalline precipitate was formed after addition of ammonium chloride R which proves the presence of calcium in the molecule (BP, 2014).

3.2 UV Spectrophotometric Analytical Method Development

UV analysis method for atorvastatin, simvastatin and rosuvastatin was developed and validated in different dissolution media (pH 1.2, pH 4.5 and pH 6.8). The methods were found linear, precise and accurate.

The plot of absorbance of each sample against respective concentration of drug was found linear (Figure 3.4). Regression equation and correlation coefficient of calibration curves for atorvastatin, simvastatin and rosuvastatin in different media (pH 1.2, pH 4.5 and pH 6.8) are shown in Table 3.1. Due to low solubility calibration curve of simvastatin in hydrochloric acid buffer (pH 1.2) and acetate buffer (pH 4.5) were constructed in the range of 2-10 $\mu\text{g/ml}$ (Figure 3.4). Higher value of correlation coefficients proves the linearity of the method.

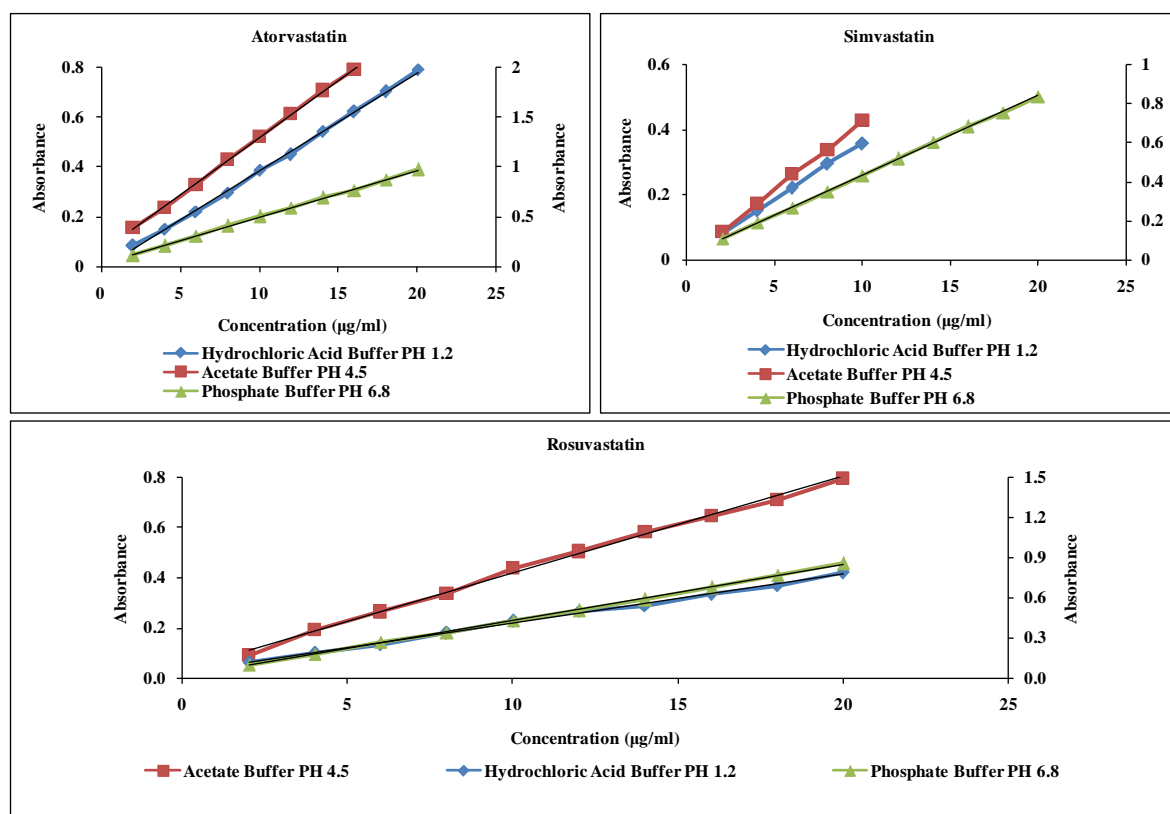


Figure 3.4: Standard calibration curves of atorvastatin, simvastatin and rosuvastatin in different media (pH 1.2, pH 4.5 and pH 6.8)

Table 3.1: Regression equation and correlation coefficients of atorvastatin, simvastatin and rosuvastatin

Media	Atorvastatin	Simvastatin	Rosuvastatin
	Regression equation and R ² value		
Hydrochloric Acid Buffer pH 1.2	y = 0.039x - 0.010, R ² = 0.998	y = 0.034x + 0.013 R ² = 0.999	y = 0.036x + 0.043 R ² = 0.997
Acetate Buffer pH 4.5	y = 0.046x + 0.059 R ² = 0.999	y = 0.042x + 0.001 R ² = 0.999	y = 0.038x + 0.032 R ² = 0.997
Phosphate Buffer pH 6.8	y = 0.047x + 0.023 R ² = 0.999	y = 0.040x + 0.028 R ² = 0.999	y = 0.041x + 0.013 R ² = 0.999

Table 3.2: Accuracy and reproducibility data of atorvastatin, simvastatin and rosuvastatin for UV analytical method validation

Drug	Accuracy	Reproducibility	
	% Recovery (mean ±SD)	% RSD (UV 1700)	% RSD (UV 1601)
Atorvastatin	99.04 ± 0.45	0.311	0.192
Simvastatin	99.94 ± 0.43	0.251	0.160
Rosuvastatin	99.97 ± 0.24	0.878	0.380

Results of reproducibility and accuracy are summarized in Table 3.2. Percent recovery was found 99.04% to 99.97% with % RSD value less than 1%. All the results indicate that the methods are highly accurate and reproducible.

3.3 RP-HPLC Analytical Method Development and Validation

Methods Development and Optimization

RP-HPLC methods with UV detection were developed for the determination of atorvastatin, simvastatin and rosuvastatin. The reversed-phase column, shim-pack CLC, ODS (C₁₈), 150 mm × 4.6 mm, 5 μm was used for separation. The mobile phase was chosen after several trials with different buffers and organic solvents. For simvastatin and rosuvastatin water and methanol were chosen as mobile phase. These two solvents were tested in various proportions like 70: 30, 60:40, 50:50, 40: 60, 30:70 and 35:65. Finally water: methanol at 40:60 was found as a best combination for short retention time of

simvastatin (3.723min) and rosuvastatin (2.143min). This solvent system (water: methanol at 40:60) was not suitable for atorvastatin. 0.025 M phosphoric acid and acetonitrile in various proportions like 70:30, 60:40, 50:50, 40: 60, 30:70 and 35:65 at different pH values were tested for atorvastatin. Finally a mobile phase constituting 0.025 M phosphoric acid solution: acetonitrile (60:40 v/v, pH 3.0 adjusted with 80% phosphoric acid) was selected to achieve maximum separation and sensitivity.

Different flow rates (0.50 to 2.0 ml /min) were studied. A flow rate of 1.5 ml /min was found suitable for optimal signal to noise ratio with a reasonable separation time in case of simvastatin and rosuvastatin. On the other hand 1.2 ml/min flow rate was found suitable for atorvastatin for short retention time (2.737 min).

Wave length for UV detection was determined by scanning individual standard of atorvastatin, simvastatin and rosuvastatin in the UV region. Then HPLC analysis of individual standard was measured at 245 nm (atorvastatin), 241 nm (simvastatin) and 242 nm (rosuvastatin). The developed methods are summarized in the Table 3.3.

For peak identification a blank sample was injected three times to observe the peak of the blank. No peak was observed. Then samples containing atorvastatin, simvastatin and rosuvastatin were then injected individually to identify peaks.

Table 3.3: Comparison of HPLC method for atorvastatin, simvastatin and rosuvastatin

Item	Atorvastatin	Simvastatin	Rosuvastatin
Stationary phase	Shim-pack C ₁₈ ; 5µm, 4.6×150mm.	Shim-pack C ₁₈ ; 5µm, 4.6×150mm.	Shim-pack C ₁₈ ; 5µm, 4.6×150mm.
Mobile phase	0.025M OPA (pH 3.0) : acetonitrile 60:40	water: methanol 40:60	water: methanol 40:60
Temperature	30°C	30°C	30°C
Flow rate	1.2 ml/minute.	1.5ml/minute.	1.5ml/minute.
Injection volume	10µl	10µl	10µl
Wave length	245 nm (λ_{max})	241 nm (λ_{max})	242 nm (λ_{max})
Run time	10 minutes.	10 minutes.	10 minutes.
R-Time (min)	2.737± 0.019	3.723 ± 0.017	2.143 ± 0.035

Peak area and retention time were found 325124 and 2.737 min for atorvastatin for 10µg/ml solution whereas peak area of 402215 with retention time of 3.723 min was observed for single injection of simvastatin at 10µg/ml nominal concentration. Solution

containing 10 μ g/ml rosuvastatin produced a peak at 2.143 min with an area of 212287.

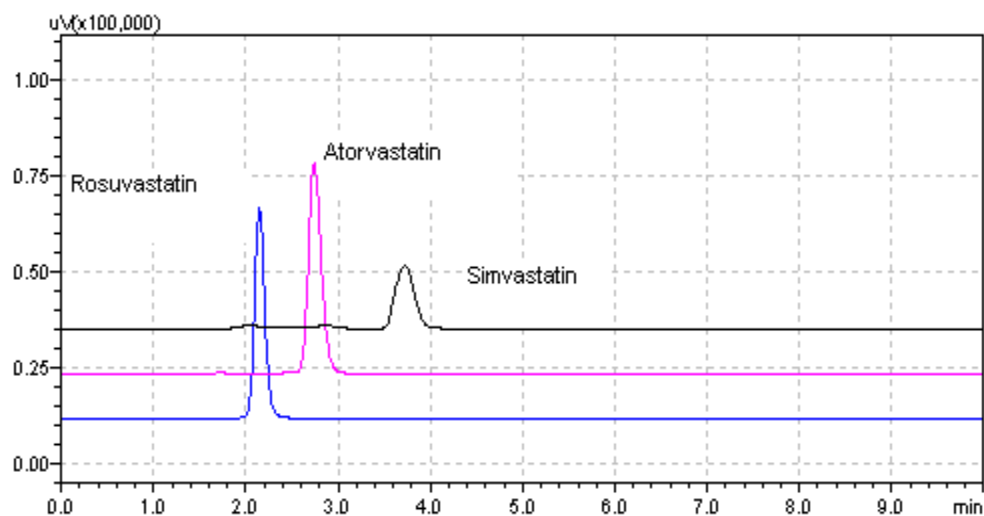


Figure 3.5: Typical HPLC chromatograms of atorvastatin, simvastatin and rosuvastatin

Validation of the Method: The methods were validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness.

System Suitability Test

System suitability tests were carried out on freshly prepared standard solution of drugs to evaluate the resolution and reproducibility of the system for the analysis and test result is summarized in Table 3.4. Average value, SD and % RSD of retention time, peak area, theoretical plates and tailing factor were calculated after six replicate injections. % RSD of retention time, peak area was within limit (< 2%) which indicates uniformity of test results. The tailing factor was found less than 1.5 which indicated symmetric nature of the column. High theoretical plate numbers suggested an efficient performance of the column.

Table 3.4: System suitability test results of HPLC analytical method validation

Parameters	Atorvastatin	Simvastatin	Rosuvastatin
	Average \pm SD, %RSD	Average \pm SD, %RSD	Average \pm SD, %RSD
Retention time	2.737 \pm 0.003, 0.11	3.723 \pm 0.022, 0.591	2.143 \pm 0.003, 0.139
Area	325124 \pm 500.18, 0.154	402215 \pm 3408, 0.847	212287 \pm 1315.12, 0.619
Theoretical plates	7255.5 \pm 25.34, 0.349	7255.5 \pm 25.33, 0.349	6559.33 \pm 38.375, 0.885
Tailing factor	1.255 \pm 0.0028, 0.223	1.23 \pm 0.002, 0.162	1.27 \pm 0.008, 0.629

Selectivity

The absence of additional peaks in the chromatogram indicated non-interference by the common excipients used in the SEDDS and SD that indicates the selectivity and specificity of the method.

Linearity

The linearity of the method was determined at ten concentration levels (2 to 20 µg/ml). The plot of peak area of each sample against respective concentration of drug was found linear in the range of 2-20 µg/ml (Figure 3.6). Regression equation, correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) were also calculated and summarized in Table 3.5.

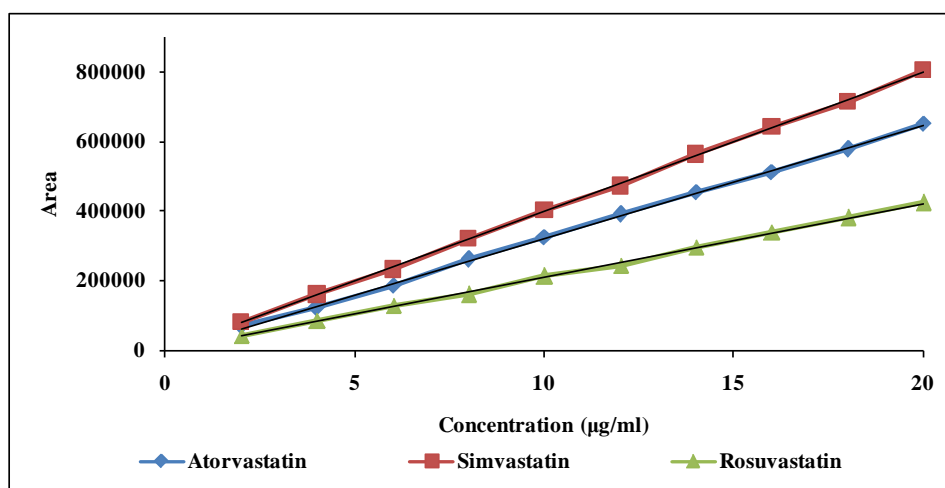


Figure 3.6: Linearity curves of atorvastatin, simvastatin and rosuvastatin for HPLC analytical method validation.

Table 3.5: Regression equation, correlation coefficients, LOD and LOQ of HPLC analysis method

Parameters	Atorvastatin	Rosuvastatin	Simvastatin
Regression equation	$y = 32480x - 2474.$	$y = 21271x - 2635.$	$y = 40130x - 2185.$
Correlation coefficient	$R^2 = 0.999$	$R^2 = 0.998$	$R^2 = 0.999$
LOD (µg/ml)	0.184	0.149	0.283
LOQ (µg/ml)	0.614	0.497	0.943

Accuracy

Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to placebo formulations and the proposed method was followed to determine % recovery. 50%, 100% and 150% of nominal concentration was included for this study. Six replicates of each concentration were measured. The percentage recovery value was 99.64 %-100.14% which indicates the accuracy of the method and absence of interference from the excipients present in the samples.

Precision

The precision is a measure of ability of a method to generate reproducible results. The precision of the methods was determined by repeatability (intra-day) and Intermediate precision (inter-day) and reported as %RSD. For repeatability, four determinations of 100% test concentration were measured from 9.00 AM to 6.00 PM and %RSD was calculated. For intermediate precision the same work was done in three consecutive days and average %RSD value was calculated. The intra-day and inter-day precision measurements showed, good reproducibility with percent relative standard deviation (%RSD) values < 2%. This indicates that method was highly precise (Table 3.6).

Table 3.6: Accuracy and precision results of HPLC method validation

Validation parameters		Atorvastatin	Simvastatin	Rosuvastatin
Accuracy	% Recovery	100.14 ± 0.57%	99.64 ± 0.74%	100.02 ± 0.15%
	%RSD	0.559	0.749	0.149
Precision (%RSD)	Repeatability	0.55	0.34	0.59
	(%RSD)			
	Ruggedness	0.58	0.72	0.27
	(%RSD)			

Robustness

Robustness was performed by small but deliberate variation in the chromatographic conditions. Robustness of the method was determined by the analysis of the samples under a variety of conditions making small changes in the mobile phase component ($\pm 0.5\%$),

flow rate (± 0.05 ml/ min) and column temperature (± 2 °C). It was observed that there were no marked changes in the chromatograms, which demonstrates that the method was robust.

3.4 Comparative Dissolution Study of Pure Drug in Different Dissolution Media

The results of dissolution studies of atorvastatin, simvastatin and rosuvastatin powder (API) are graphically represented in Figure 3.7. Dissolution study of API is not an official test but as dissolution of final product as well as bioavailability depends on the solubility and dissolution of API we studied drug release from pure drug powder and concentration of drug in the dissolution medium were calculated for comparison.

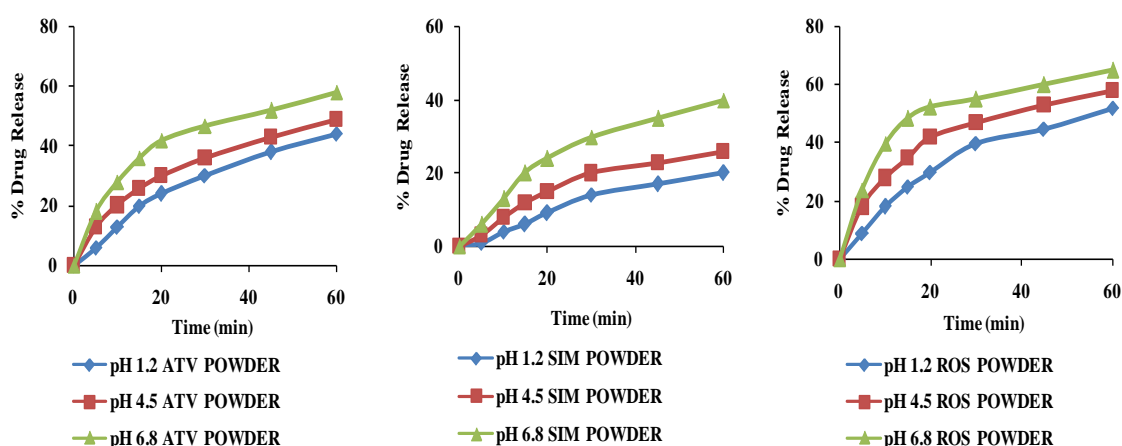


Figure 3.7: Dissolution profiles of atorvastatin, simvastatin and rosuvastatin in different media

Different bio-waiver dissolution media such as USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution) and pH 6.8 (phosphate buffer solution) were used in the study (FDA, 2000). Some differences were observed in percent drug release among the three drugs in different media, although none released more than 65% drug within one hour. The release of rosuvastatin was a little bit faster than that of atorvastatin and simvastatin in all media. Lowest drug release was found in case of simvastatin (20% drug was released in hydrochloric acid solution, pH 1.2 within one hour). On the other hand atorvastatin released 44% drug within one hour in acid medium.

Drug dissolved in different dissolution media was tested statistically to ascertain the effect of pH on drug release using one-way analysis of variance (ANOVA). When we compared the drug release in different media significant differences were found ($p < 0.001$). % drug

release for the time point of 30 min was analyzed by one-way analysis of variance (ANOVA) using SPSS software (version 16.0; SPSS Inc., USA). The results of ANOVA are shown in table 3.7 and results indicate that drug release was significantly different at 0.001 level. It implies that drug release is pH dependent.

Table 3.7: Results of analysis of variance for % drug release at 30 min time point for atorvastatin, simvastatin and rosuvastatin

Drug		Sum of Squares	DF	Mean Square	F	Sig.
SIM	Between Groups	786.17	2	393.09	4272.08	0.000
	Within Groups	1.38	15	0.09		
	Total	787.55	17			
ATV	Between Groups	895.53	2	447.77	1726.33	0.000
	Within Groups	3.89	15	0.26		
	Total	899.42	17			
ROS	Between Groups	679.71	2	339.85	858.18	0.000
	Within Groups	5.94	15	0.40		
	Total	685.65	17			

DF - Degree of freedom

According to the FDA guidance, for the dissolution testing of immediate release solid oral dosage forms, 85% dissolution in 0.1 N HCl in 15 min ensures that the bioavailability of the drug is not limited by dissolution (FDA, 1997). From Figure 3.7 we found that only 20% atorvastatin (dose 5-80 mg), 6% simvastatin (dose 5-80 mg) and 25% rosuvastatin (dose 5-40 mg) were dissolved within 15 min which implies that bioavailability of all the drugs is limited by dissolution rate.

According to Amidon *et al.* (1995) drug absorption is expected to be independent of drug dissolution if administered dose is completely dissolved in the 250 ml fluids within a range of pH 1 to 8. Drugs that do not fulfill this requirement are known as poorly water soluble drugs. From Figure 3.7 we found that only 1.2 mg atorvastatin (dose 5-80 mg), 0.5 mg simvastatin (dose 5-80 mg) and 1.4 mg rosuvastatin (dose 5-40 mg) were dissolved in 250

ml dissolution medium (pH 1.2). So all the drugs may be considered as poorly water soluble and their bioavailability will be dissolution rate limited.

A solubility of >10 mg/ml in pH range 1 to 7 has been proposed as an acceptable limit to avoid absorption problems, while another suggestion is that drugs with water solubility less than 0.1 mg/ml often lead to dissolution limitations to absorption (Kaplan, 1972; Hörter and Dressman, 1997). In this study, we found that final concentration of all the drugs were lower in hydrochloric acid (pH 1.2) medium (simvastatin 0.002 mg/ml, atorvastatin 0.004 mg/mL and rosuvastatin 0.005 mg/ml). Higher drug concentration was found in dissolution media with higher pH (pH 4.5 and 6.8). In phosphate buffer dissolution medium (pH 6.8) drug concentration (simvastatin 0.004 mg/ml, atorvastatin 0.006 mg/ml and rosuvastatin 0.007 mg/ml) was higher than acetate buffer (pH 4.5) dissolution medium (simvastatin 0.0022 mg/ml, atorvastatin 0.005 mg/ml and rosuvastatin 0.006 mg/ml). In all the cases concentration was < 0.1 mg/ml which implies that all the drugs will show dissolution rate limited bioavailability.

A little higher solubility was reported for all the drugs (atorvastatin 0.02 ± 0.52 mg/ml at pH 2.1, simvastatin 0.0145 mg/ml at pH 1.2 and rosuvastatin 0.125 mg/ml in water) (Popy *et al.*, 2012; Oishi *et al.*, 2011). British Pharmacopeia (BP, 2014) describes simvastatin as practically insoluble in water (< 0.1 mg/ml) and atorvastatin as very slightly soluble. Rosuvastatin is still an INN drug.

These three drugs are considered as BCS II class drug as they are poorly soluble but highly permeable. Drugs having log P value more than 1.72 are considered as highly permeable drugs (log p of atorvastatin, simvastatin and rosuvastatin are 6.36, 4.86 and 2.4, respectively (Patel *et al.*, 2013).

Depending on the solubility and permeability Kasim *et al* (2003) classified 123 drugs that were enlisted in WHO Essential Drug List. The WHO Essential Drug List consists of a total of 325 medicines and 260 drugs, of which 123 are oral drugs in immediate-release (IR) products (WHO, 2002). The percentages of the drugs in immediate-release dosage forms that were classified as BCS Class 1, Class 2, Class 3, and Class 4 drugs using dose number and log P were as follows: 23.6% in Class 1, 17.1% in Class 2, 31.7% in Class 3, and 10.6% in Class 4. The remaining 17.1% of the drugs could not be classified because of

the inability to calculate log P values because of missing fragments. The author also provided top 200 U.S. Drug Product List in the article. Atorvastatin and simvastatin are included in the list as BCS II class drug. So these three drugs (atorvastatin, simvastatin and rosuvastatin) are suitable candidate to increase dissolution rate to enhance bioavailability.

3.5 Comparative Dissolution Study of Commercially Available Tablets

Drug release from innovator brand and marketed tablet of atorvastatin, simvastatin and rosuvastatin were studied in different dissolution media [USP buffer solutions of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution), and pH 6.8 (phosphate buffer solution)]. Innovator brand of atorvastatin (Lipitor), simvastatin (Zocor) and rosuvastatin (Crestor) coded as ATV REF, SIM REF and ROS REF respectively and four brands of 10 mg atorvastatin tablets (coded as ATV MP-1 to ATV MP-4), four brands of 10 mg simvastatin (coded as SIM MP-1 to SIM MP-4) and 10 mg rosuvastatin tablet (coded as ROS MP-1 to ROS MP-4) were included in this study.

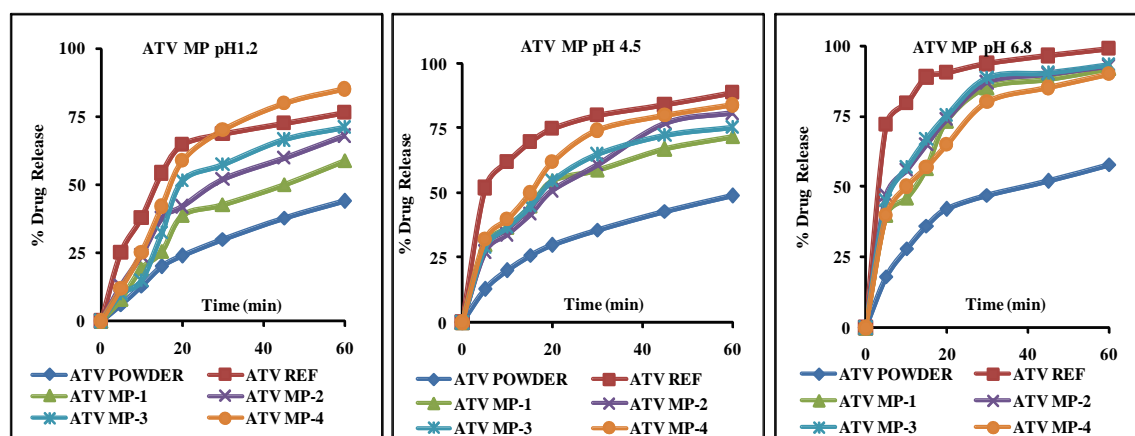


Figure 3.8: Dissolution profile of atorvastatin from marketed brands in different media

The results of dissolution studies are graphically represented in Figures 3.8-3.10. Both inter-brand (brand to brand) and intra-brand (within a brand) variations in dissolution profiles were observed. Drug release was found to be dependent on pH of the dissolution media. Higher drug release within 15 minutes was found 60 % (ROS REF), 79.62% (ATV MR-2) and 86.32% (ATV MR-2) in pH 1.2, 4.5 and 6.8 dissolution media respectively. On the other hand lowest drug release within 15 minutes was found 23.74% (SIM MP-4), 26.77% (SIM MP-1) and 43.16% (ROS MP-4) in pH 1.2, 4.5 and 6.8 dissolution media

respectively. From these data it is clear that the bands differ in case of drug release in different dissolution media.

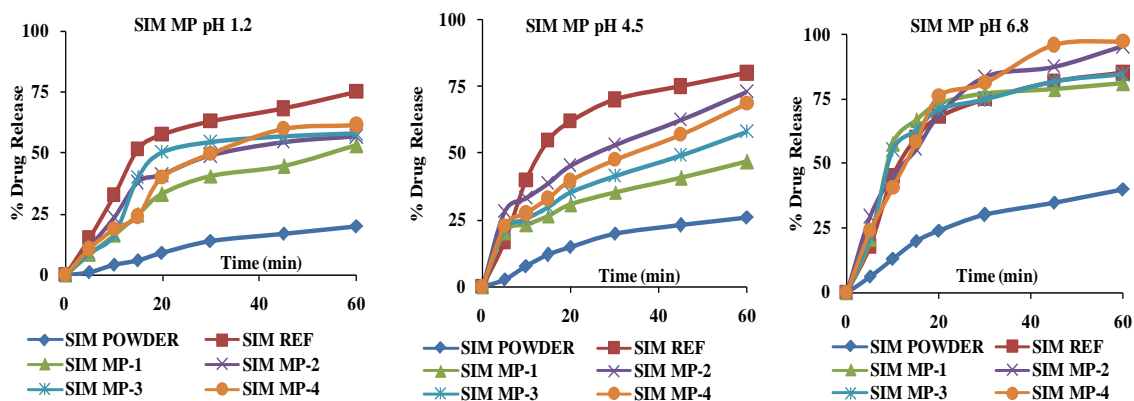


Figure 3.9: Dissolution profile of simvastatin from marketed brands in different media

The therapeutic efficacy of a drug depends on rate and extent of drug absorption by the gastrointestinal tract. The dissolution rate of poorly water-soluble drugs is often a rate-limiting step in their absorption from the GI tract (Chiba *et al.*, 1991). Such drugs suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability.

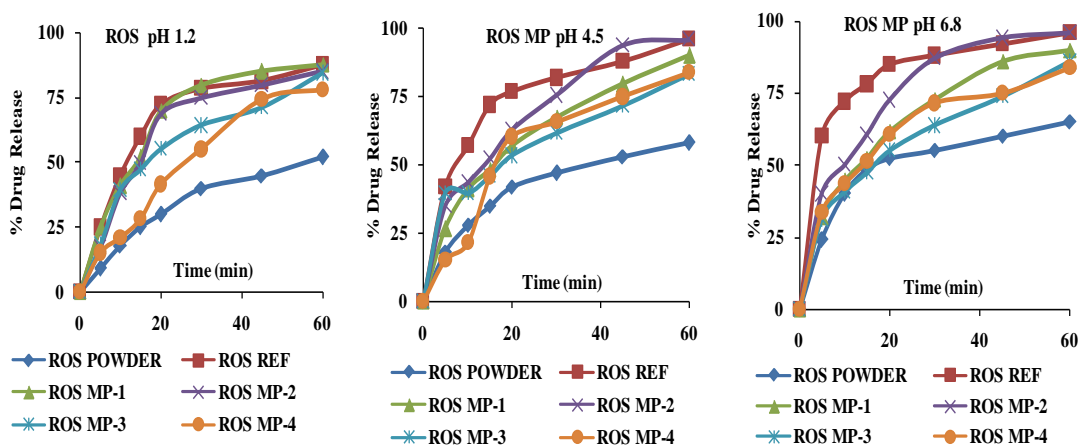


Figure 3.10: Dissolution profile of rosuvastatin from marketed brands in different media

All the drugs included in this study having limited oral bioavailability are often associated with high intra subject and inter subject variability due to high variations in dissolution profiles.

Comparison of Dissolution Data:

Difference factor (f_1), similarity factor (f_2) and dissolution efficiency (% DE) were calculated to compare the dissolution profile. Difference factor f_1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. The following equations were used to calculate difference factor f_1 and similarity factor f_2 .

$$f_1 = \left\{ \frac{\sum_{t=1}^n |R_t - T_t|}{\sum_{t=1}^n R_t} \right\} \times 100$$

$$f_2 = 50 \log \left\{ \left(1 + \frac{1}{n} \sum_{i=1}^n (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

Where n is the number of time points, R_t is the dissolution value of reference product at time t and T_t is the dissolution value for the test product at time t .

Similarity factor f_2 has been adopted by FDA (1997) and the European Agency for the Evaluation of Medicinal Products (EMA, 2001) by the Committee for Proprietary Medicinal Products (CPMP) to compare dissolution profile. Two dissolution profiles are considered similar and bioequivalent, if f_1 is between 0 and 15 and f_2 is between 50 and 100 (FDA, 1997).

Table 3.8 shows the name of products that are similar with the innovator brand in respect of f_2 values (> 50) at different dissolution media. Not a single brand was found similar with the innovator brand in all the dissolution media. Out of 36 dissolution run, only 10 (27.8%) dissolution profiles (1 in pH 1.2, 3 in pH 4.5 and 6 in pH 6.8) were found similar with the innovator brand. Similar study was also reported previously in case of atorvastatin and simvastatin market products (Popy *et al.*, 2012; Fatima *et al.*, 2013).

Table 3.8: List of similar marketed brands according to their similarity factor ($50 > f_2$)

Product	pH1.2	pH4.5	pH6.8
ATV	ATV MP-4	-	-
ROS	ROS MP1-2	ROS MP-2	-
SIM	SIM MR-3	SIM MR-2	SIM MR 1-4

Dissolution efficiency (DE) was also employed to compare the drug release from various brands. Dissolution efficiency is the area under the dissolution curve within a time range ($t_1 - t_2$). %DE was calculated by using the following equation:

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

where y is the percentage dissolved at time t

Dissolution efficiency of API was comparatively lower than reference product (REF) or local product (12.73% for SIM powder). Dissolution efficiency of reference product (REF) was comparatively higher than local product (92 % for ATV REF in pH 6.8). In case of locally produced product higher dissolution efficiency was found in case of brand ATV MP-3 (81.11%) in pH 6.8. On the other hand lowest dissolution efficiency was found in case of brand SIM MP-1 (35.20%) in pH 4.5. Out of 36 dissolution run, dissolution efficiencies of 12 run were more than 70% and may be considered as quality products.

Table 3.9: List of similar marketed brands according to their dissolution efficiency (% DE)

%DE	1.2	4.5	6.8
ATV	ATV MP-4	ATV MP-4	-
ROS	ROS MP-1, MP-2	ROS MP-2	ROS MP-2
SIM	SIM MR-3	SIM MR-2	SIM MR 1-4

The reference and the test product can be said to be equivalent if the difference between their dissolution efficiencies is within appropriate limits ($\pm 10\%$, which is often used)

(Anderson *et al.*, 1998). Similar products according to dissolution efficiency (% DE) are shown in Table 3.9. They are equivalent to innovator brand as difference of % DE (test product – reference product) is less than 10.

From Table 3.8 and Table 3.9 it is clear that, all the products that are similar in respect of f_2 are also similar in respect of % DE. Additionally product ATV MP-4 and ROS MP-2 were also found similar with the innovator brand in respect of % DE. This implies that both the similarity test methods are almost of equal value to test the dissolution similarity.

Poor solubility is the main reason for inter-brand (brand to brand) and intra-brand (within a brand) variations in dissolution profiles. So, different researchers tried different ways to increase solubility of poorly water soluble drugs to enhance bioavailability (Kamble *et al.*, 2014; Kapure *et al.*, 2013).

3.6 Comparative Quality Assessment of Commercially Available Tablet

The results of uniformity of weight, hardness, friability, disintegration time and assay of different marketed brands are shown in Figure 3.11. Uniformity of weight serves as a monitor to good manufacturing practices (GMP) as well as amount of the active pharmaceutical ingredient (API) contained in the formulation. Out of fifteen brands thirteen brands complied with the compendial specification for uniformity of weight which states that for tablets having 80-250 mg weight, not more than 2 tablets should differ from the average weight by more than 7.5% and none will deviate by 15% of average weight. SIM MP-3 did not comply in uniformity of weight test as 4 tablets crossed the limit of 7.5%. On the other hand, ATV MP-2 did not comply with uniformity of weight test as 1 tablet crossed the limit of 15%.

Hardness is referred to as non-compendial test. It can also influence other parameters such as friability and disintegration. Tablet hardness was found 56.3 - 127.1 N. A force of about 40 N is the minimum requirement for a satisfactory tablet (Allen *et al.*, 2004). Hence the tablets of all brands were satisfactory for hardness.

Friability test is now included in the United States Pharmacopeia (USP, 2012) as a compendia test. The compendial specification for friability is 1%. Friability for all the brands was below 1%.

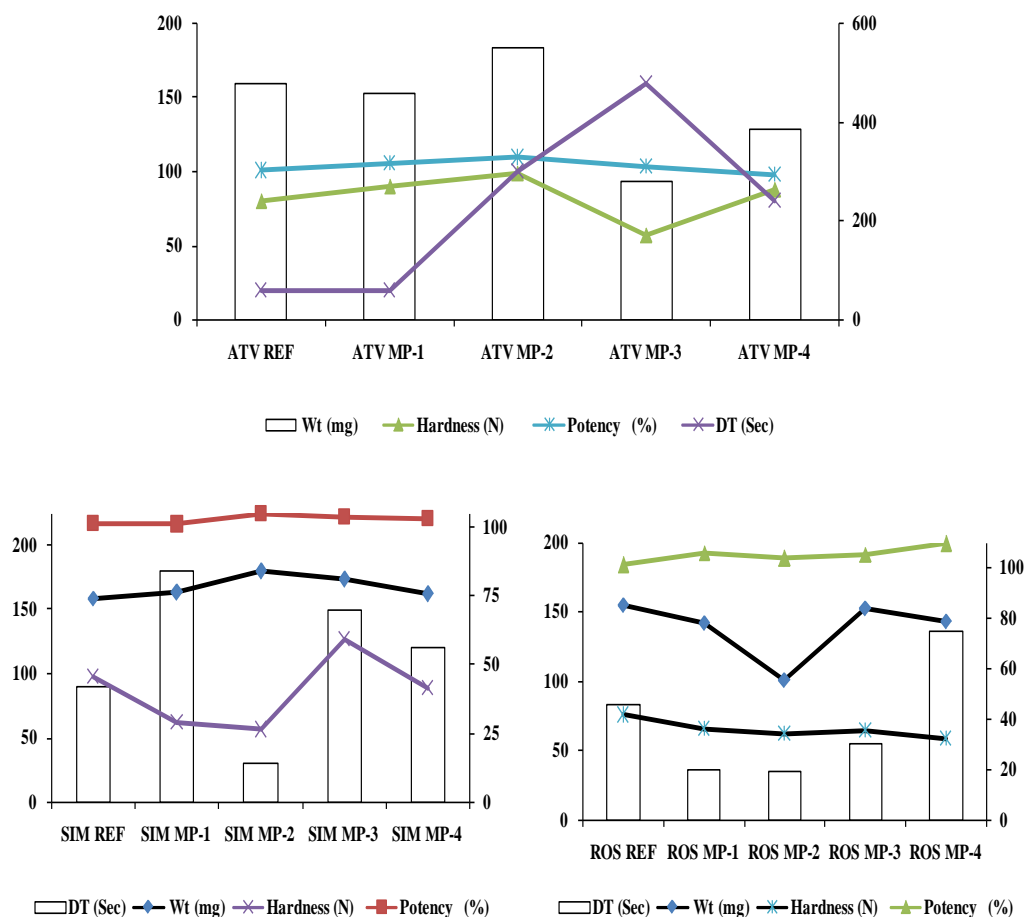


Figure 3.11: Comparative quality assessment of commercially available atorvastatin, simvastatin and rosuvastatin tablets: DT (atorvastatin tablet) and potency (simvastatin and rosuvastatin tablets) are mapped as per secondary vertical axis value)

Disintegration time of all the brands was within limit. BP (2014) specifies that uncoated tablets should disintegrate within 15 min and film coated tablets in 30 min while USP (2012) specifies that both uncoated and film coated tablets should disintegrate within 30 min. All the tested tablets were film coated and maximum time for disintegration was found 6 min in case of ATV MR-3. Potency of all the brands was found within 98.24%-109.45%. As per USP (2012) specification, of simvastatin tablet, (potency limit: 90%-110%) we can say that potency was within limit.

3.7 SEDDS: Formulation Development

Apparent solubility of three drugs (atorvastatin, simvastatin and rosuvastatin) was determined in different oils, surfactants and co-surfactants at ambient temperature. Based on solubility data, excipients were selected and formulated in SEDDS with varying ratios of surfactant and co-surfactant by mixing the components in sealed glass vials. These systems were titrated with water and phase clarity & quality of emulsion were observed.

3.7.1 Solubility Analysis

The solubility of atorvastatin, simvastatin and rosuvastatin in various lipids, surfactants and co-surfactants was determined by using shake flask method. drug was added in 1 ml of the selected vehicle and was mixed using a cyclomixer for 10 min, then shaken for 48 h in a water bath shaker and centrifuged at 3000 rpm for 5 min followed by filtration through membrane filter 0.45 μm . Filtrate was suitably diluted with mobile phase and concentration of drug was determined by HPLC analysis and presented in Figures 3.12-3.14.

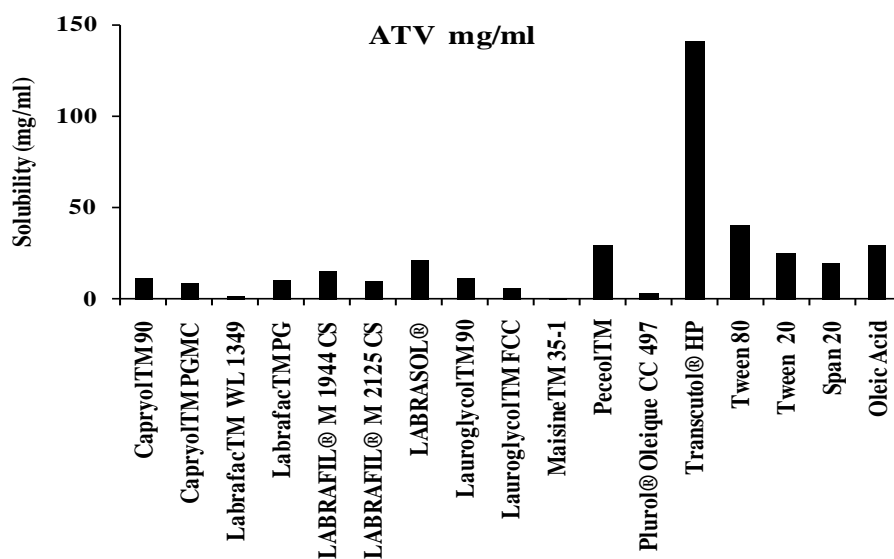


Figure 3.12: Solubility of atorvastatin in various excipients

Solubility of atorvastatin in lipid vehicles, surfactants and co-surfactants was higher than aqueous media. Oleic acid (30 mg/ml), Tween 80 (40 mg/ml), and Transcutol[®] HP (141 mg/ml) exhibited higher solubility than other vehicles. These three excipients were selected

for further studies, where oleic acid was chosen as the oil phase, Tween 80 as the surfactant and Transcutol® HP as the co-surfactant. Oleic acid is an amphiphilic compound with surfactant properties, which is progressively and effectively replacing the regular medium chain triglyceride oils in SEDDS (Constantinides, 1995). Tween 80 is one of the most widely recommended nonionic hydrophilic surfactants due to its relatively high hydrophilic-lipophilic balance value (HLB 15) and safety profile.

Solubility of simvastatin was comparatively higher in different oil, surfactant and co-surfactants than atorvastatin and rosuvastatin. On the other hand its aqueous solubility was lower than atorvastatin and rosuvastatin. It was found more lipophilic. Among the oil vehicle, solubility in Capryol™ 90 was found higher (105 mg/ml) and about same solubility was in Capryol™ PGMC (100 mg/ml). Solubility in Lauroglycol™ FCC and Maisine™ was found 50 mg/ml.

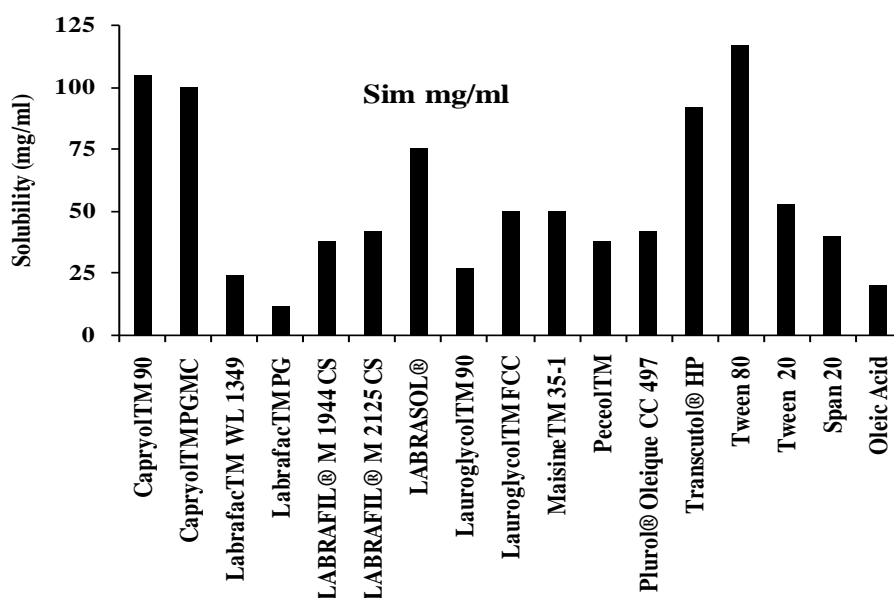


Figure 3.13: Solubility of simvastatin in different excipients

Among the surfactants 40 mg/ml and 53 mg/ml solubility were found in Span 20 and Tween 20 respectively and highest solubility was found in Tween 80 (117 mg/ml). Transcutol® HP exhibited higher solubility (92 mg/ml) than other co-surfactants such as PEG 400 (44 mg/ml) and propylene glycol (20 mg/ml). Depending on the solubility Capryol™ 90, Tween 80 and Transcutol® HP were selected for further studies, where

Capryol™ 90 was chosen as the oil phase, Tween 80 as the surfactant and Transcutol® HP as co-surfactant.

Among the three drugs rosuvastatin was found less lipophilic. Its solubility in different oil vehicles was comparatively lower (15.87 mg/ml in Capryol™ 90, 3.83 mg/ml in LABRAFIL® M 1944 CS). Among the oil higher solubility was found in Peceol™ (25.72 mg/ml) and it was chosen as oil phase. Labrasol® exhibited higher solubility (30.09 mg/ml) than other surfactants and Transcutol® HP exhibited higher solubility (122.50mg/ml) than other co-surfactants. These three excipients were selected for further studies.

A wide variety of lipids are available for the development of oral lipid-based formulations including long chain and medium chain triglycerides, propylene glycol esters, mono and diglycerides of medium chain and long chain fatty acids, various lipid mixtures etc. Adding to the diversity, the fatty acid components of the lipids can be either saturated or unsaturated.

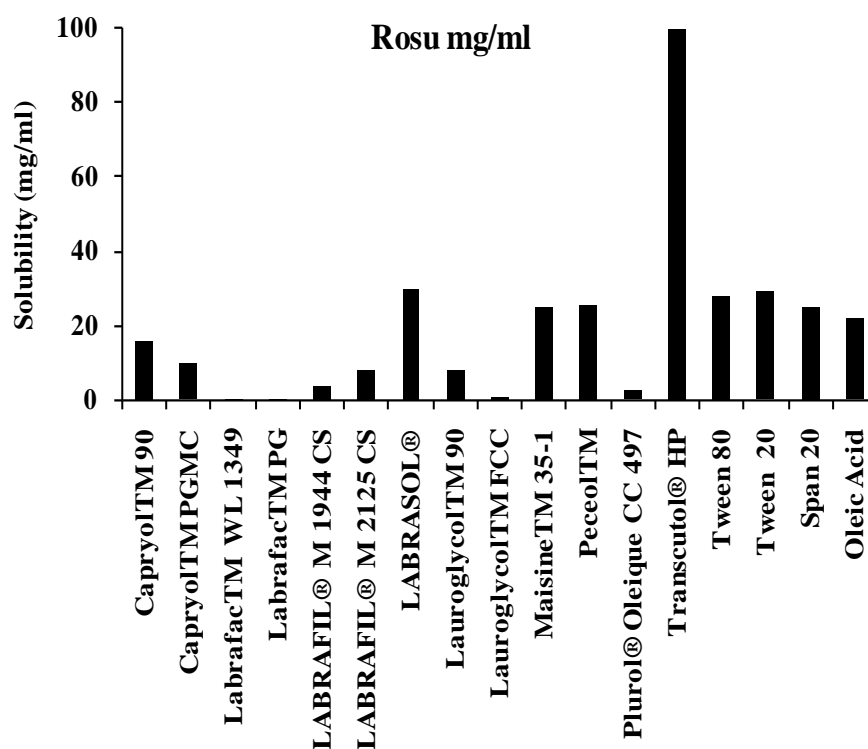


Figure 3.14: Solubility of rosuvastatin in various excipients

According to Cannon and Long (2008), lipids that have fatty acid chains of 14-20 carbons are considered long chain, while those with 6-12 carbons are medium chain. Unless they consist of unsaturated fatty acid chains, the long-chain glycerides are usually solid at room temperature and, therefore, may not be suitable for dissolving drugs. Further, long chain glycerides which exist as liquids at room temperature (e.g., corn oil, sesame oil, peanut oil, olive oil, soybean oil, etc.) have been reported to have lower drug solubility than medium-chain glycerides. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification system with a large number of surfactants approved for oral administration and exhibit better drug solubility properties (Constantinides, 1995; Hauss *et al.*, 1998). But natural long chain derivatives like oleic acid, which can be defined as amphiphilic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride lipids in the self-emulsifying formulations (SEFs) (Constantinides, 1995; Karim *et al.*, 1994).

3.7.2 Emulsifying Ability of Selected Surfactants and Co-Surfactants

Emulsification ability of selected surfactants and co-surfactants were determined and compared with other surfactants and co-surfactants by observing the ease of formation of emulsions and by measuring the transmittance at 638.2 nm. The %transmittance values of various dispersions containing selected oil and surfactants (PeceolTM - LABRASOL[®], CapryolTM 90 - Tween 80 and Oleic acid-Tween 80) were 90-93% which indicates good emulsifying ability of the selected surfactants. Addition of Transcutol[®] HP as co-surfactant to the above systems further increased the transmittance (up to 99%).

3.7.3 Construction of Solubility Phase Diagrams

Solubility phase diagrams were constructed in order to generate some solubility data of drugs in lipid/surfactant mixture. In the ternary phase diagram, individual point (X, Y, Z) representing the weight percentage of lipid, drug, and surfactant-co surfactant was considered to check whether drug is soluble/insoluble at that particular point. The major solubility points of the drug and those are plotted to construct the solubility phase diagrams.

Figure 3.15 shows the solubility phase diagrams for ATV. Tween 80 (surfactant) and Transcutol[®] HP (co-surfactant) were used in different ratio (1:2, 1:1, 2:1 and 3:1 as w/w) in

different systems. In the solubility phase diagrams (Figure 3.15), darker region indicates soluble area for ATV. Solubility of atorvastatin was found to depend on the solubility of ATV in individual component. Better solubility (3.9% ATV to 9.7% ATV) was found in S_{mix} (1:2) system. This is due to the higher amount of Transcutol[®] HP that exhibited higher solubility of ATV (141 mg/ml). On the other hand S_{mix} (3:1) exhibited lower solubility of ATV due to lower amount of Transcutol[®] HP (3.8% ATV to 4.8% ATV).

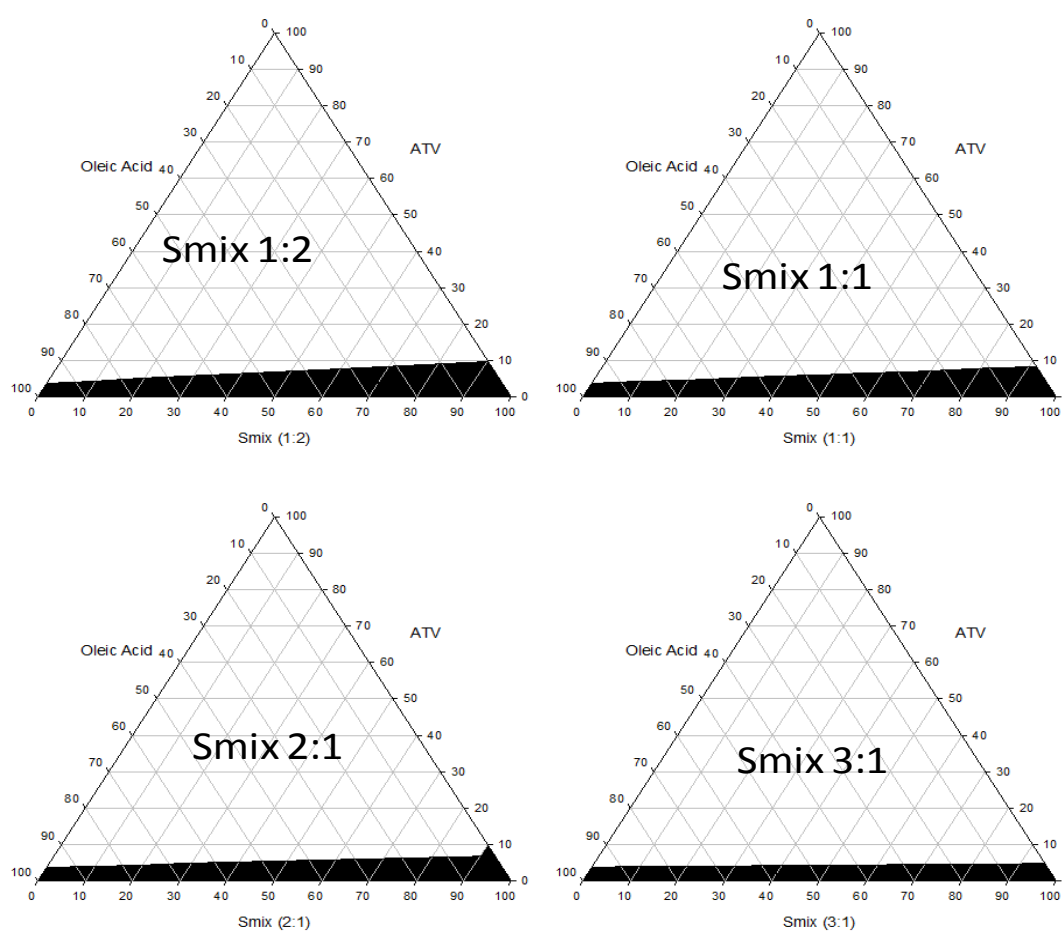


Figure 3.15: Solubility phase diagram of ATV in Oleic Acid:Tween 80: Transcutol[®] HP. S_{mix} means surfactant (Tween 80) and co-surfactant (Transcutol[®] HP) mixture. 1:2, 1:1, 2:1 and 3:1 indicate the weight ratio (w/w) of surfactant (Tween 80) and co-surfactant (Transcutol[®] HP). The shadow area represents soluble region

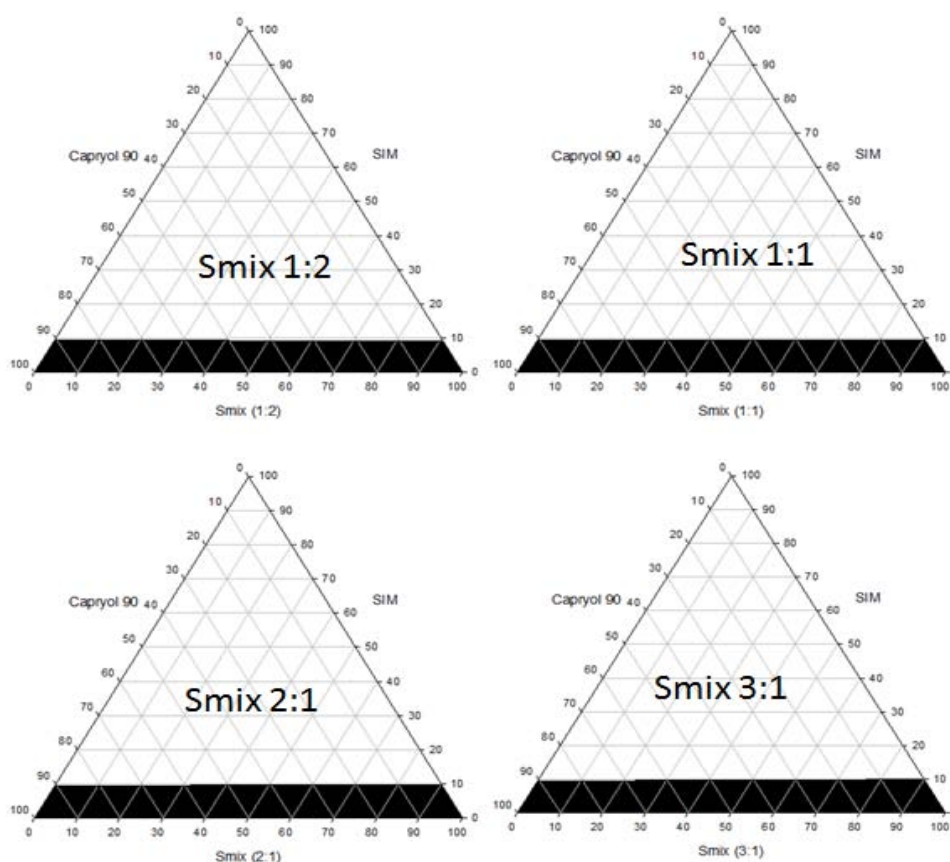


Figure 3.16: Solubility phase diagram of SIM in Capryol 90:Tween 80: Transcutol[®] HP. S_{mix} means surfactant (Tween 80) and co-surfactant (Transcutol[®] HP) mixture. 1:2, 1:1, 2:1 and 3:1 indicate the weight ratio of surfactant (Tween 80) and co-surfactant (Transcutol[®] HP) (w/w). The shadow area represents soluble region.

Figure 3.16 shows the solubility phase diagrams for simvastatin. In the solubility phase diagrams darker region indicates soluble area for SIM. Simvastatin is a highly lipophilic drug (Log P is 4.6). Lipid solubility of SIM is much higher than other statins. Capryol 90:Tween 80: Transcutol[®] HP system was used to construct these solubility phase diagram. Unlike the ATV amount of SIM dissolved in all system (S_{mix} 1:2, 1:1, 2:1 and 3:1) was found to be uniform (9.12% - 9.5% for S_{mix} 1:2, 9.46% - 9.50 for S_{mix} 1:1, 9.5% - 9.8% for S_{mix} 2:1 and 9.5% - 9.7% for S_{mix} 3:1). This is due to the similar solubility profile of SIM in Capryol[™] 90 (105 mg/ml, Tween 80 (117 mg/ml) and in Transcutol[®] HP (92 mg/ml).

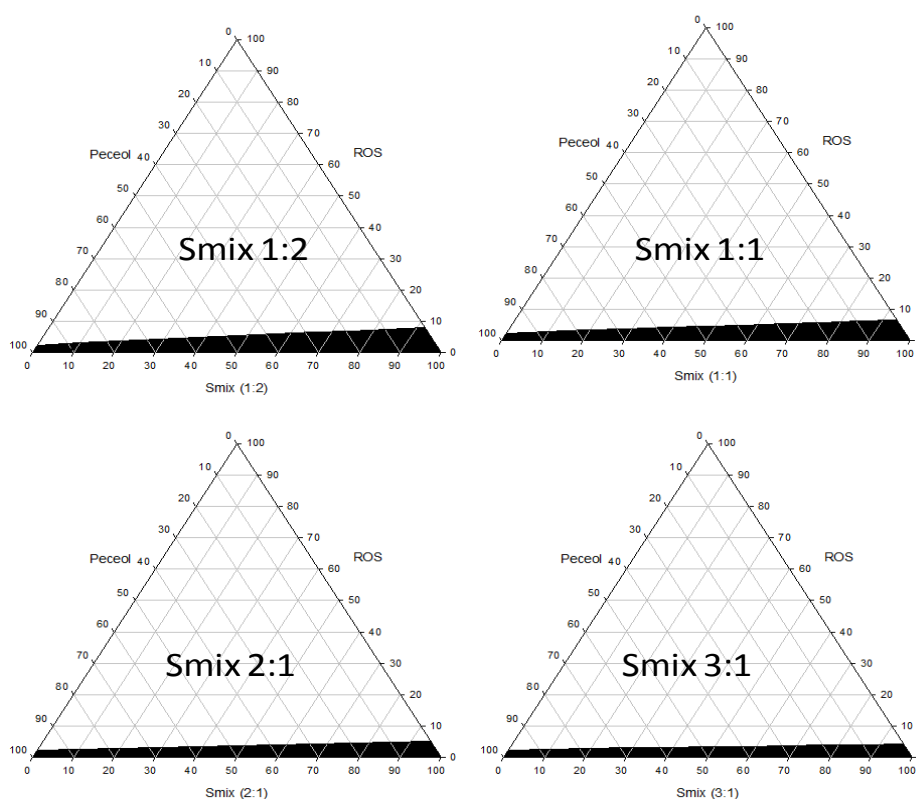


Figure 3.17: Solubility phase diagram of ROS in PeceolTM :Labrasol[®]: Transcutol[®] HP. S_{mix} means surfactant (Labrasol[®]) and co-surfactant (Transcutol[®] HP) mixture. 1:2, 1:1, 2:1 and indicate the weight ratio of surfactant (Labrasol[®]) and co-surfactant (Transcutol[®] HP) (w/w). The shadow area represents soluble region

Figure 3.17 shows the solubility phase diagrams for rosuvastatin. In the solubility phase diagrams darker region indicates soluble area for ROS. PeceolTM : Labrasol[®]: Transcutol[®] HP system was used to construct these solubility phase diagrams. Comparatively lower solubility region was found in the diagrams. Solubility of ROS in Transcutol[®] HP (122.50mg/ml) was found higher than PeceolTM (25.72 mg/ml) and Labrasol[®] (30.09 mg/ml). So system containing higher amount of Transcutol[®] HP (S_{mix} 1:2) exhibited higher solubility (2.5% ROS to 8.0% ROS) than system (S_{mix} 3:1) containing less amount Transcutol[®] HP (2.5% ROS to 4.3% ROS).

3.7.4 Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed to determine the area of the microemulsion region. Self-microemulsifying system forms fine oil-water emulsions after a gentle agitation, upon their introduction into aqueous media. Surfactant and co-surfactant are preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence.

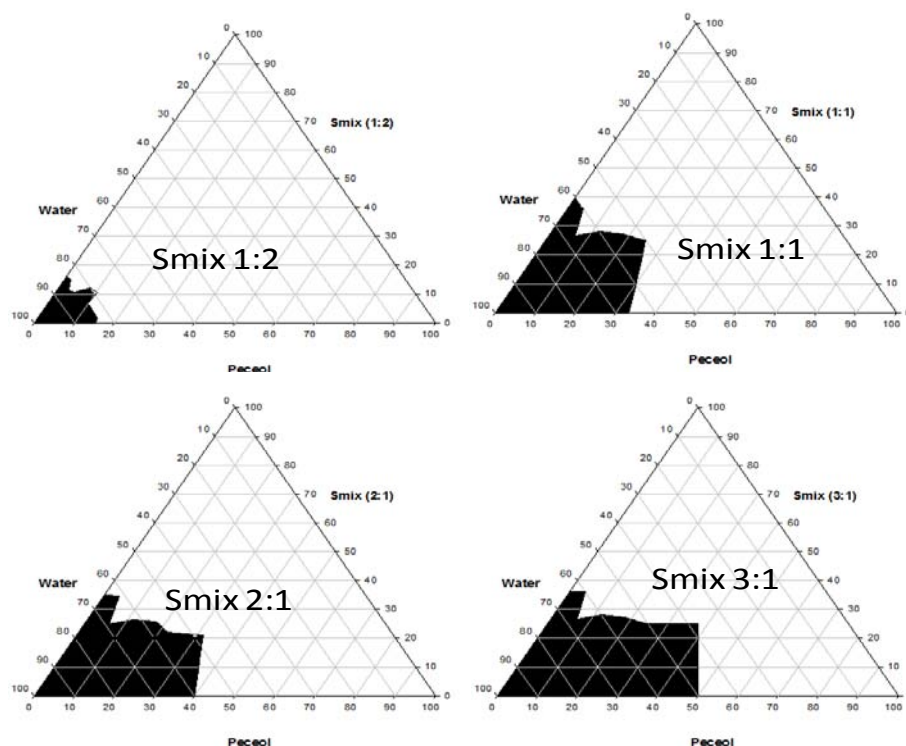


Figure 3.18: Pseudo-ternary phase diagrams of ROS-SEDDS where oil = Peceol, surfactant = Labrasol[®], co-surfactant = Transcutol[®] HP, S_{mix} means surfactant (Labrasol[®]) and co-surfactant (Transcutol[®] HP) mixture. 1:2, 1:1, 2:1 and 3:1 indicate the weight ratio (w/w) of surfactant (Labrasol[®]) and co-surfactant (Transcutol[®] HP). The shadow area represents microemulsion region.

The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the

formation of the microemulsion. In the present study, four ratios of S/CoS were considered preliminary.

Figure 3.18 shows the phase diagrams using four different ratios of S/CoS. Darker region indicates the microemulsion area. A wider microemulsion area was observed with a S/CoS ratio of 3:1 (Figure 3.18). As the S/CoS ratio was changed from 3:1, the microemulsion area became smaller and this narrowing of area was more distinguishable while the ratio was 2:1 and 1:1. Finally, the S/CoS mixture of the 2:1 ratio was selected for the formulation of SEDDS, as a larger microemulsion area indicates greater self-microemulsification efficiency, and a 2:1 ratio of S/CoS formed the largest microemulsion area.

3.7.5 Formulation of SEDDS

Various formulations were prepared with a constant amount of drug (10% SIM, 5% ATV and 2.5% ROS *m/m*) and varying ratios of surfactant to co-surfactant (Table 3.10). In brief, drug was dissolved in the oil phase in glass vials. Required amount of S_{mix} (S/CoS) was added to the mixture and mixed well. These systems were warmed to 40 °C using a water bath for 30 min with intermittent shaking to ensure complete mixing. The prepared formulations were stored at ambient conditions until further use.

Table 3.10: Composition of SEDDS formulations

Product	Drug	Oleic Acid	Peceol	Capryol 90	Tween 80	Labrasol	Transcutol
ATV SEDDS F-1	0.1	1			0.33		0.66
ATV SEDDS F-2	0.1	1			0.5		0.5
ATV SEDDS F-3	0.1	1			0.66		0.33
ATV SEDDS F-4	0.1	1			0.75		0.25
ROS SEDDS F-1	0.05		1			0.33	0.66
ROS SEDDS F-2	0.05		1			0.5	0.5
ROS SEDDS F-3	0.05		1			0.66	0.33
ROS SEDDS F-4	0.05		1			0.75	0.25
SIM SEDDS F-1	0.2			1	0.33		0.66
SIM SEDDS F-2	0.2			1	0.5		0.5
SIM SEDDS F-3	0.2			1	0.66		0.33
SIM SEDDS F-4	0.2			1	0.75		0.25

3.8 Characteristics of Optimized SEDDS

Optimized SEDDS were evaluated for emulsification time, optical clarity, droplet size, drug content, robustness to dilution, stability and *in-vitro* dissolution profile.

3.8.1 Emulsification Time

Emulsification time is an important index for the assessment of the efficiency of emulsion formation. SEDDS should disperse completely and rapidly when subjected to aqueous dilution under mild agitation. Emulsification time of the optimized SEDDS formulations was 1.32-1.8 min, 1.65-2.01 min and 1.05-1.84 min for ATV SEDDS, SIM SEDDS and ROS SEDDS, respectively. The formulation containing a higher amount of surfactant took less time to be emulsified. Emulsification time decreased from 1.8 min to 1.32 min while the surfactant amount was increased from 0.33 mg to 0.66 mg. It might be due to the presence of a higher concentration of surfactant, which facilitated the self-emulsification process that eventually led to a high emulsification rate (Gursoy and Benita, 2004; Patil *et al.*, 2004). Regression analysis revealed that surfactant and co-surfactant ratio had a significant effect on emulsification time ($P < 0.0001$). The response surface plot showed that formulations containing a higher amount of surfactant exhibited a lower emulsification time (Figure 3.19 A).

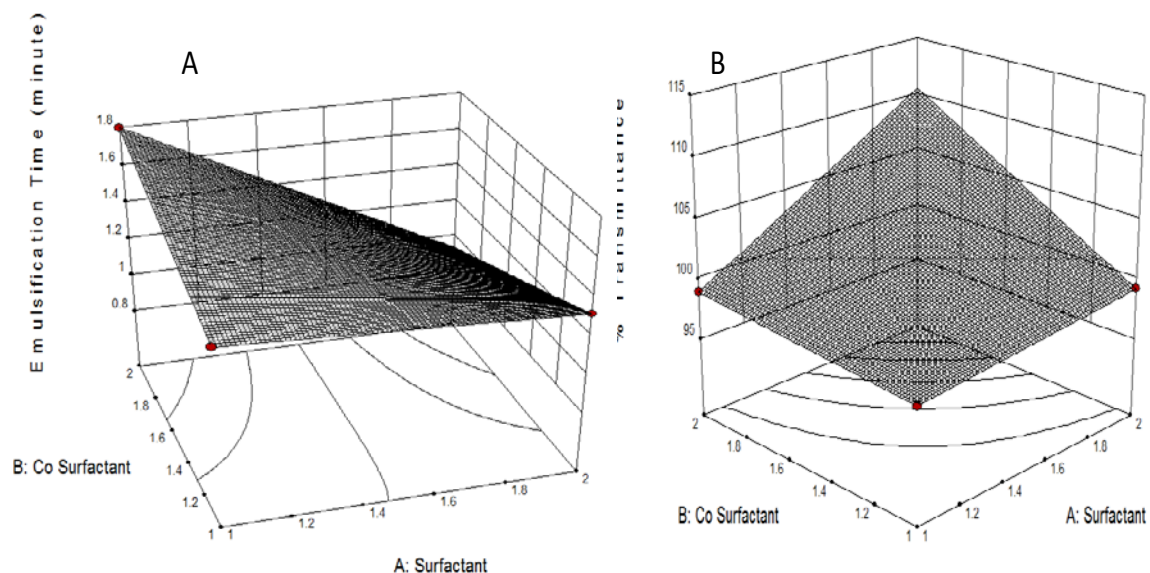


Figure 3.19: Response surface plot (3D) for the effects of surfactant and co-surfactant on emulsification time (A) and % transmittance (B)

3.8.2 Optical Clarity of the Optimized SEDDS

UV-visible spectrophotometer was used to measure the amount of light of a given wavelength transmitted by the emulsion. Since cloudier solutions will scatter more of the incident light, resulting in lower transmittance values, higher transmittance should be obtained with optically clear solutions. All formulated batches were transparent (transmittance > 93%). The maximum transmittance value was found for ATV SEDDS F-3 (99.19%), indicating the formation of the microemulsion of the finest droplets. Figure 3.19 (B) shows the 3D response surface plot of transmittance. Percent transmittance values of SEDDS were also measured at 10, 20, and 30 min after dilution. The transmittance values remained unchanged even after 30 min of dilution which may be considered as a primary indication about the fact that the optimized SEDDS were stable.

3.8.3 Emulsion Droplet Size Analysis

SEDDS formulations (equivalent to 10 mg drug) were diluted with 50 ml distilled water and thereafter, visual observations were made immediately after dilution for assessment for self-emulsification efficiency, appearance (transparency), phase separation and precipitation of drug. The mean droplet size of the resulting emulsions was determined by Malvern particle size analyzer (Mastersizer 2000, Malvern, UK).

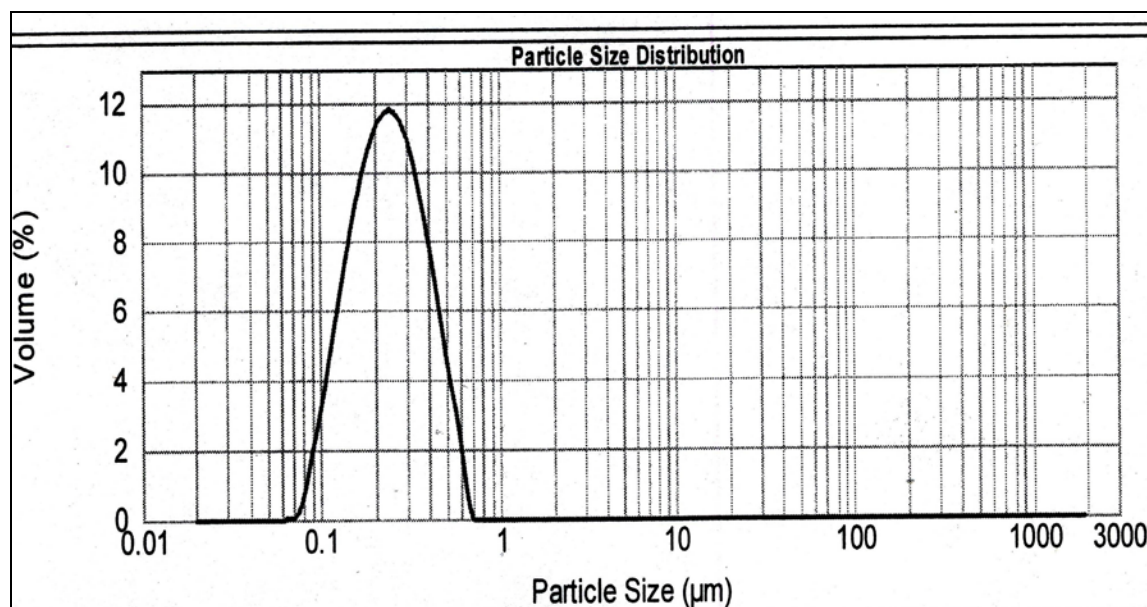


Figure 3.20: Droplet size distribution of ROS SEDDS F-3

Table 3.11: Droplet size (average \pm SD) of different SEDDS at D= 0.5 (nm)

Drug	SEDDS F-1	SEDDS F-2	SEDDS F-3	SEDDS F-4
Atorvastatin	249.94 \pm 2.25	210.58 \pm 3.05	177.83 \pm 2.48	223.32 \pm 7.65
Rosuvastatin	274.93 \pm 2.48	238.66 \pm 3.45	121.88 \pm 1.7	281.11 \pm 7.17
Simvastatin	251.70 \pm 3.65	241.78 \pm 3.39	191.23 \pm 2.82	367.24 \pm 10.1

Emulsion droplet size distribution was characterized with the help of droplet size distribution of 10% particles – d (0.1), droplet size distribution of 50% droplet – d (0.5), droplet size distribution of the 90% particles – d (0.9). 50% droplet – d (0.5) size range of different SEDDS formulation were shown in Table 3.11 and droplet size distribution of ROS SEDDS F-3 is shown in Figure 3.20.

Smallest droplets (121-191 nm) were found in SEDDS F-3 for all the drugs. This system contains higher amount of surfactants which facilitate easy emulsification and smallest droplet size. On the other hand SEDDS F-1 containing lower amount of surfactant showed larger droplet size. Droplet size was found to decrease with the increase of surfactants. Exception was found in SEDDS F-4 where increase of surfactants decreased the droplet size. This may be due to the lower amount of co-surfactants. As solubility of drugs in co-surfactants is higher than surfactants, lower amount of co-surfactants may produce precipitation of drug with higher droplet size.

Effect of Surfactant and Co-Surfactant on Droplet Size of SEDDS

In order to ascertain the effect of surfactant and co-surfactant on droplet size droplet size was subjected to multiple regression analysis and the response surface plots (Figure 3.21) were constructed using Design Expert 9.0 (Stat-Ease Inc., USA).

From the Figure 3.21 it is clear that droplet (DP) size decreases with the increase of surfactants and co-surfactants. This may be due to higher emulsification activity for larger amount of surfactant and co-surfactant.

Effect of Drug Loading and pH of Dilution Media on Droplet Size of SEDDS

The increase or decrease in the amount of rosuvastatin would influence the droplet size of the resultant SEDDS if ROS participates at the interface of emulsion. In order to investigate

role of rosuvastatin, various formulations were prepared containing varying amount of ROS from 1 to 3% (w/w). SEDDS formulations (equivalent to 10 mg drug) were diluted with 50 ml pH 1.2 and phosphate buffer pH 6.8 and the mean droplet size of resulting microemulsions were determined by Malvern particle size analyzer (Mastersizer 2000, Malvern, UK).

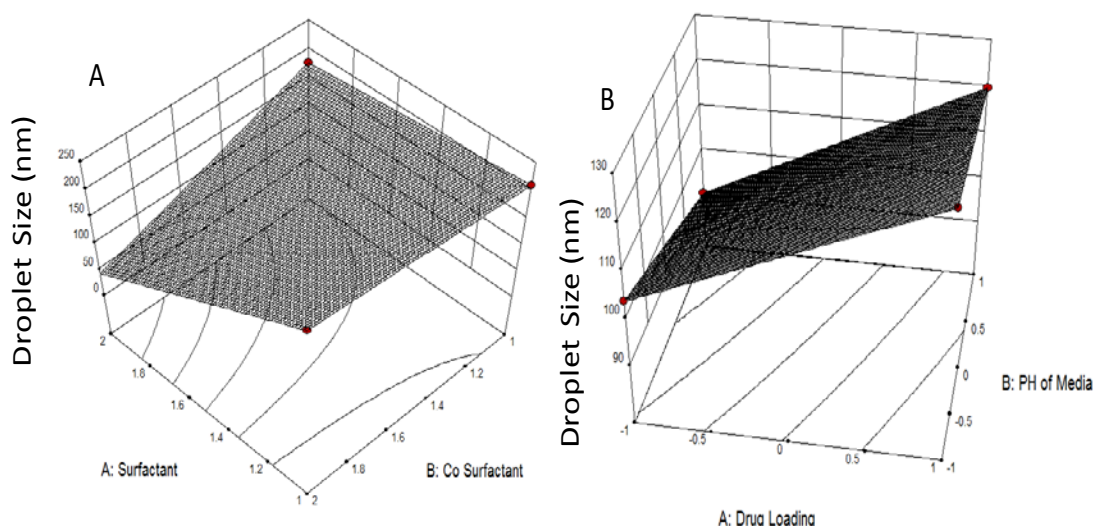


Figure 3.21: Response surface plot (3D) for the effects of surfactant and co-surfactant (A) pH of media and drug loading (B) on emulsion droplet size

Table 3.12: 2^2 factorial designs of ROS-SEDDS: Independent (% Drug loading, pH) and Dependent variables (Droplet Size)

Drug	% Drug loading	pH	Droplet Size (nm)
Rosuvastatin	1	1.2	104
Rosuvastatin	1	6.8	90
Rosuvastatin	3	1.2	130
Rosuvastatin	3	6.8	120

The droplet size was found increased with the increase in % rosuvastatin loading. Rosuvastatin, due to its low aqueous solubility, is likely to participate in the microemulsion by orienting at the interface. Dilution media has also effects on droplet size of emulsion.

Dilution media of higher pH value produce smaller droplet size. This may be due to pH dependant solubility of drug.

3.8.4 Drug Content Analysis of SEDDS

Drug content of SEDDS was determined by a validated HPLC method. Drug was first extracted from pre weighed SEDDS by dissolving it in 25 ml methanol. Drug content was then determined by injecting methanolic extract of SEDDS and standard solution of drug in HPLC system. Drug content in the SEDDS were within limit (97.45%- 99.67%)

3.8.5 Robustness to Dilution of SEDDS

Robustness of SEDDS to dilution was studied by diluting it 50, 100 and 1000 times with various dissolution media viz. water, SGF pH 1.2 and Phosphate buffer pH 6.8 The diluted SEDDS were stored for 12 h and observed for any signs of phase separation or drug precipitation. SEDDS resulting from dilution with various dissolution media were robust to all dilutions and did not show any separation even after 12 h of storage.

3.8.6 Stability Study of SEDDS

There was no obvious change in the droplet size of SEDDS formulations dispersed with the medium after standing for 0.2, 1, 10, and 24 h . After 1 year's storage, there was no major change in the content of drug and droplet size of microemulsion.

3.8.7 *In-vitro* Dissolution Study of SEDDS

SEDDS formulations of ATV, SIM and ROS were tested for drug release. *In- vitro* release profile of SEDDS was determined using USP XXIII apparatus II at $37 \pm 0.50^{\circ}\text{C}$ with a rotating speed of 75 rpm in dissolution medium (pH 1.2 hydrochloric acid buffer). At designated time intervals (5, 10, 15 and 20 min) 5 ml of release medium was collected and concentrations of drugs were analyzed by a validated HPLC method. Release percentages were calculated as the ratio of drug released to total drug. All the operations were carried out in triplicate.

In-vitro dissolution profiles of optimized ATV, SIM and ROS SEDDS, commercial tablets and drug powder are reference are presented in Figure 3.22. *In-vitro* dissolution

experiments demonstrated a marked increase in the release percentage for the SEDDS formulation as compared with the commercial tablet. From ATV SEDDS F-3 and ROS SEDDS F-3 100% drug was released within 20 min and SIM SEDDS F-3 released 93% within 20 min. Whereas ATV POWDER, SIM POWDER and ROS POWDER released only 24%, 9% and 30% drug respectively. Drug release from REF TAB was also lower than the SEDDS formulations (68% from ATV REF, 57% from SIM REF and 78% from ROS REF within 20 min). So it could be concluded that SEDDS formulation permits a faster rate of drug release into aqueous phase, faster than reference and it could enhance bioavailability.

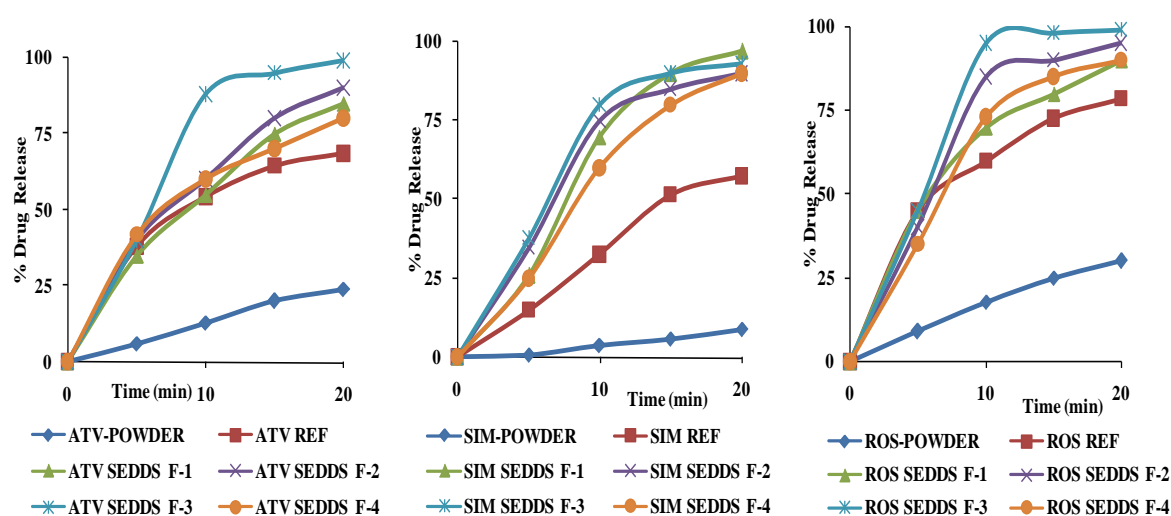


Figure 3.22: Comparative release profile of SEDDS formulation

Statistical Analysis of Dissolution Data

Difference factor (f_1), similarity factor (f_2), dissolution efficiency (%DE) and $T_{50\%}$ were calculated to compare the dissolution profile (Table 3.13). In all the cases REF products were not found similar with any SEDDS formulation as f_2 was less than 50. % DE for ROS SEDDS F-3 was found higher than any other product which indicates the enhancement of dissolution and bioavailability by SEDDS technology. By comparing % DE we found that 1.13-1.36 fold dissolution was increased in case of rosuvastatin, 1.64 to 1.95 fold dissolution was increased in case of simvastatin and 1.09 to 1.46 fold dissolution was increased in case of atorvastatin. When we calculate $T_{50\%}$ value by model dependent analysis, we found that 1.5 – 1.95 fold more time was required to dissolve 50% drug from REF TAB than SEDDS.

Table 3.13: Similarity factor (f_2), dissolution efficiency (%DE_{20min}) and T_{50%} of SEDDS formulation

Product	Atorvastatin			Simvastatin			Rosuvastatin		
	%DE _{20min}	f_2	T _{50%}	%DE _{20min}	f_2	T _{50%}	%DE _{20min}	f_2	T _{50%}
REF TAB	57.44		8.90	40.13		15.86	64.74		6.42
SEDDS F-1	62.83	47.77	7.92	73.83	23.56	14.20	74.17	49.45	6.04
SEDDS F-2	66.67	45.48	7.34	74.14	21.50	6.51	80.83	31.01	4.88
SEDDS F-3	84.17	28.17	5.90	78.50	24.02	7.24	88.33	37.78	5.99
SEDDS F-4	65.33	53.52	6.98	65.83	29.17	9.57	73.50	46.31	7.35

Table 3.14: Results of analysis of variance of %DE_{20 min} for ROS SEDDS formulations and ROS REF

	Sum of Squares	DF	Mean Square	F	Sig.
Between Groups	1901.54	4	475.384		
Within Groups	27.373	25	1.095	434.169	0.000
Total	1928.91	29			

% DE for ROS SEDDS formulations and ROS REF were further tested statistically using SPSS software (version 16.0; SPSS Inc., USA) to ascertain differences among formulation using one-way analysis of variance (ANOVA) while Bonferroni test was employed to ascertain where the difference arose. The analyses were undertaken for % DE_{20min}. The results of ANOVA as shown in Table 3.14 indicate that ROS SEDDS formulations and ROS REF was significantly different at 0.05 level.

In order to ascertain the source of the difference, pair wise comparisons of ROS REF and SEDDS formulation of ROS were performed by multiple comparisons using Bonferroni test and the outcome at 0.05 level. Result is shown in Table 3.15. Consequently, it can be inferred that the ROS REF is significantly difference from any SEDDS formulation. On the other hand SEDDS F-1 was found to similar with SEDDS F-4 but their % DE was lower than other SEDDS formulations.

Table 3.15: Multiple comparisons analysis result of SEDDS by Bonferroni test

(I) ITEM	(J) ITEM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper Bound
ROS SEDDS F1	ROS SEDDS F2	-6.69368*	0.60413	.000	-8.5533	-4.834
	ROS SEDDS F3	-14.39476*	0.60413	.000	-16.254	-12.535
	ROS SEDDS F4	0.49925	0.60413	1	-1.3604	2.3589
	ROS REF	9.45740*	0.60413	.000	7.5978	11.317
ROS SEDDS F2	ROS SEDDS F1	6.69368*	0.60413	.000	4.834	8.5533
	ROS SEDDS F3	-7.70108*	0.60413	.000	-9.5607	-5.8414
	ROS SEDDS F4	7.19293*	0.60413	.000	5.3333	9.0526
	ROS REF	16.15108*	0.60413	.000	14.2914	18.0107
ROS SEDDS F3	ROS SEDDS F1	14.39476*	0.60413	.000	12.5351	16.2544
	ROS SEDDS F2	7.70108*	0.60413	.000	5.8414	9.5607
	ROS SEDDS F4	14.89402*	0.60413	.000	13.0344	16.7537
	ROS REF	23.85217*	0.60413	.000	21.9925	25.7118
ROS SEDDS F4	ROS SEDDS F1	-0.49925	0.60413	1	-2.3589	1.3604
	ROS SEDDS F2	-7.19293*	0.60413	.000	-9.0526	-5.3333
	ROS SEDDS F3	-14.89402*	0.60413	.000	-16.754	-13.034
	ROS REF	8.95815*	0.60413	.000	7.0985	10.8178
ROS REF	ROS SEDDS F1	-9.45740*	0.60413	.000	-11.317	-7.5978
	ROS SEDDS F2	-16.15108*	0.60413	.000	-18.011	-14.291
	ROS SEDDS F3	-23.85217*	0.60413	.000	-25.712	-21.993
	ROS SEDDS F4	-8.95815*	0.60413	.000	-10.818	-7.0985

*The mean difference is significant at the 0.05 level.

Effect of Droplet Size of SEDDS on Dissolution Efficiency (%DE)

Dissolution efficiency was found to depend on droplet size. SEDDS having lower droplet size shows maximum dissolution efficiency (%DE). ROS SEDDS F-3 having droplet size 121.88 ± 1.7 nm have highest value of dissolution efficiency (%DE 88.33). Relationship between droplet size and % DE is shown in Figure 3.23.

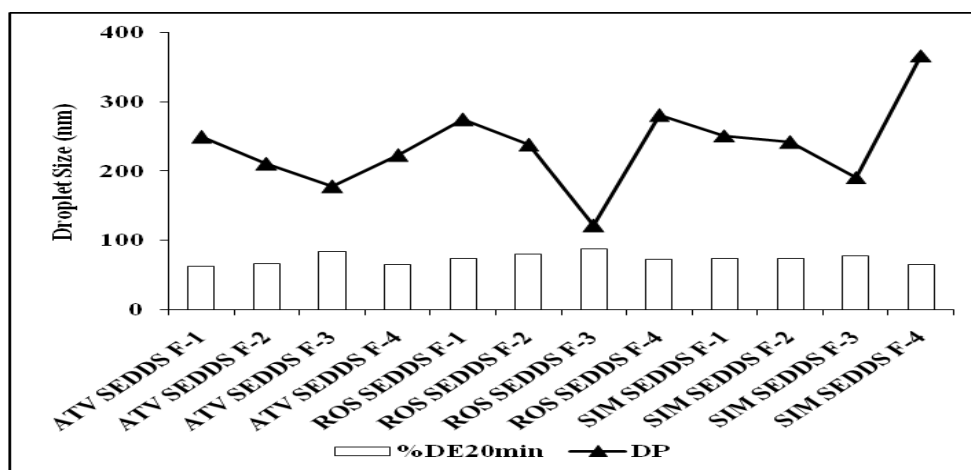


Figure 3.23: Effect of droplet size on dissolution efficiency (% DE)

Effect of Surfactant and Co-Surfactant on Dissolution Efficiency (%DE)

In order to ascertain the effect of surfactant and co-surfactant on dissolution efficiency, dissolution data were subjected to multiple regression analysis and the response surface plots (Figure 3.24) were constructed using Design Expert 9.0 (Stat-Ease Inc., USA). From the Figure 3.24 it is clear that % DE increased with the increased of surfactants and co-surfactants. This may be due to the emulsification activity due to the higher amount of surfactant and co-surfactant.

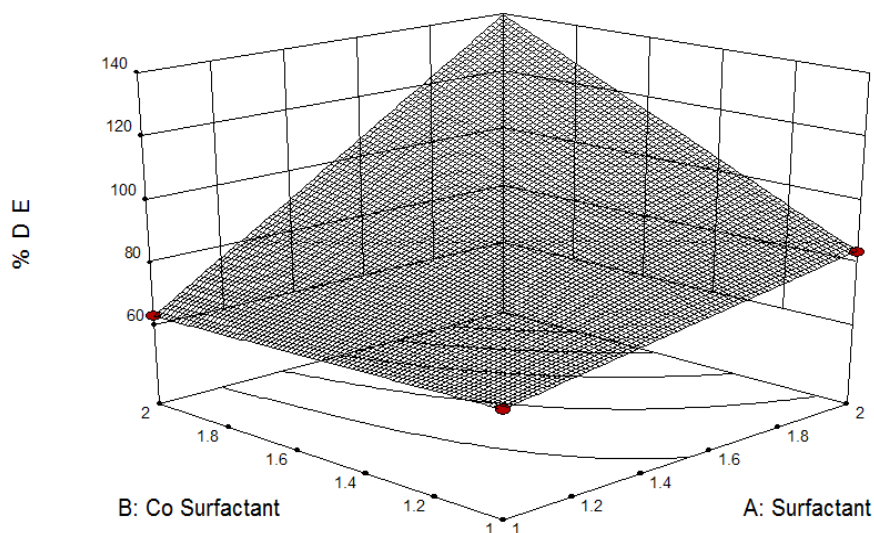


Figure 3.24: Response surface plot (3D) for the effects of surfactant and co-surfactant) on dissolution efficiency (% DE)

3.9 Preparation and Evaluation of SEDDS Capsules

SEDDS that showed maximum drug release (SIM SEDDS F-3, ATV EDDS F-3 and ROS SEDDS F-3) was further formulated in capsules containing 10 mg drug. Volume of SEDDS for a single dose of drug was 0.1 ml for simvastatin, 0.2 ml for atorvastatin and 0.4 ml for rosuvastatin. So 3 size EGHCS (volume 0.3 ml) were used to prepare simvastatin and atorvastatin capsule. On the other hand 0 size (volume 0.5 ml) was used to prepare rosuvastatin capsule. The optimal SEDDS formulations filled in capsules were tightly sealed for storage at room temperature. The capsules were tested for drug release and stability study for one year. Content and droplet size were determined at predetermined intervals.

Drug release from liquid SEDDS and SEDDS capsules formulation are shown in Figure 3.25. Drug release from capsules was lower initially due to the dissolution of capsule shell but 100 % drug was released within 25 min from all SEDDS capsules. By comparing the % DE_{20 min} of liquid SEDDS and SEDDS capsule we can conclude that there is no significance difference between liquid SEDDS and SEDDS capsule.

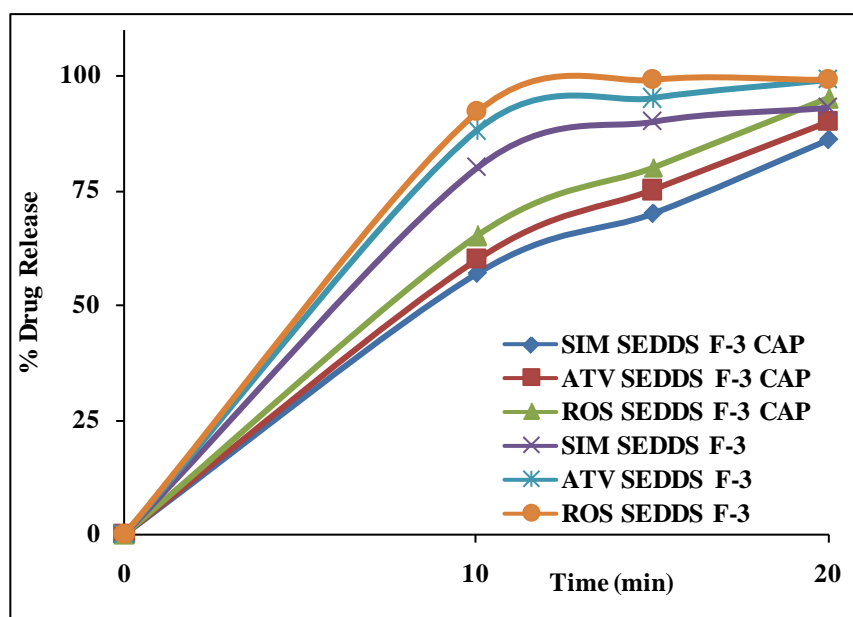


Figure 3.25: Comparative release profiles of liquid SEDDS and SEDDS capsules

Table 3.16 illustrates the result. According to Anderson *et al* two product can be said equivalent if the difference between their dissolution efficiencies is within appropriate

limits ($\pm 10\%$, which is often used) (Anderson *et al.*, 1998). Difference of % DE between liquid SEDDS and SEDDS capsule was < 10 (Table 3.16).

Table 3.16: Comparison of % DE between liquid SEDDS and SEDDS capsule

Code	% DE 20 min		
	Liquid SEDDS	SEDDS Capsule	Def.
SIM SEDDS F-3	78.5	70.5	8
ATV SEDDS F-3	84.17	75	9.17
ROS SEDDS F-3	88.33	81.6	6.73

There was no obvious change in the droplet size of SEDDS formulations dispersed with different media after standing for 24 h. The data of droplet size after standing for 0.15 h and 10 h are unchanged. After 1 year's storage, there was no major change in the content of drug and droplet size of SEDDS dispersed from the above formulations.

3.10 Solid Dispersion

Solid dispersions (SDs) of atorvastatin, simvastatin and rosuvastatin were prepared by solvent evaporation technique. In primary solid dispersions drug carrier weight ratio was 1:1, 1:3 and 1:5. Physical mixtures, secondary and tertiary solid dispersions were also prepared and compared with the primary solid dispersions.

3.11 Selection of Career for SD and their Effect on Drug Release Behavior

Primary Solid Dispersions (1:1 PSD): 1:1 Primary solid dispersions (1:1 PSD) were prepared with poloxamer 407 (POL), croscarmellose sodium (CCS), sodium starch glycolate (SSG), hydroxypropylmethylcellulose (HPMC 5 cps) and povidone K-30 (POV). Initially solid dispersion of drugs (atorvastatin, simvastatin and rosuvastatin) was prepared in 1:1 ratio of drug and polymer by solvent evaporation method. Respective amount of carrier was dissolved in glass beaker containing ethanol and drug was added in parts with continuous stirring. Then the solvent was removed by evaporation at 40°C under vacuum.

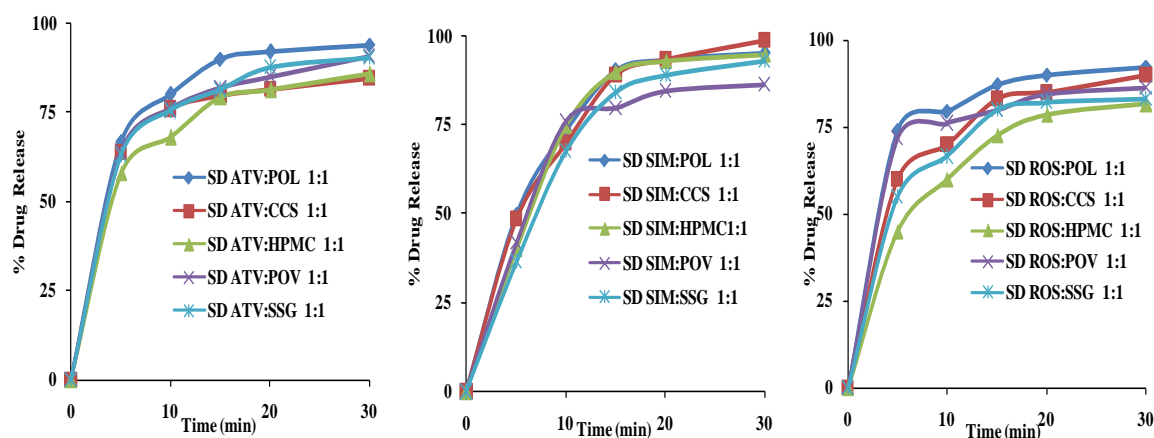


Figure 3.26: Comparative drug release profile of primary solid dispersions (1:1 PSD)

All the SDs showed significant increase in drug release in dissolution study. After one hour of dissolution, atorvastatin, simvastatin and rosuvastatin pure drug powder released only 44%, 20% and 52% drug respectively as reported in section 3.4, whereas 90%, 91% and 87% drug was released from SD ATV:POL 1:1, SD SIM:POL 1:1 and SD ROS:POL 1:1 respectively within 15 min (Figure 3.26). Dissolution rate has also been improved markedly. SD containing poloxamer 407 showed 100% dissolution after 40 minutes of dissolution. This enhancement of dissolution by poloxamer 407 might be due to the

improvement of drug wetting by the surface active property and micellar solubilization of the carrier (Masum *et al.*, 2013).

The order of carrier efficacy to improve dissolution rate of 1:1 PSD was found to be as: POL 407 > SSG > POV > CCS > HPMC (for atorvastatin). POL 407 > CCS > HPMC > SSG > POV (for simvastatin). POL 407 > POV > CCS > SSG > HPMC (for rosuvastatin). Comparative effectiveness of carrier for different drugs was determined from %DE_{30min} as shown in Figure 3.27.

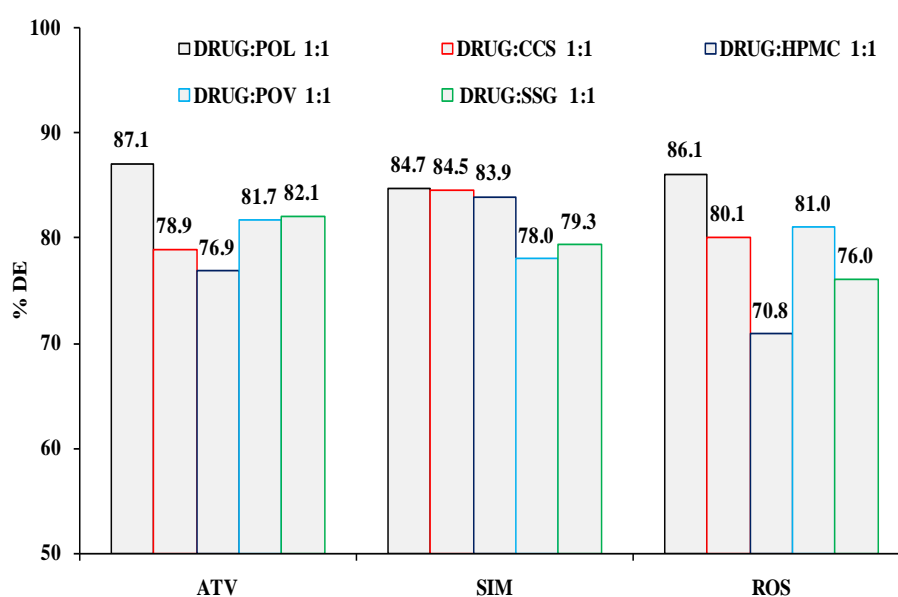


Figure 3.27: Effect of carrier on % dissolution efficiency (%DE)

Primary Solid Dispersions Containing Higher Amount of Carrier (1:3 and 1:5 PSD)

Primary solid dispersions (1:3 PSD and 1:5 PSD) containing higher drug-polymer ratio (1:3, 1:5) were prepared with the best performed polymer [poloxamer 407 (POL) and sodium starch glycolate (SSG) for atorvastatin, poloxamer 407 (POL) and croscarmellose sodium (CCS) for simvastatin, poloxamer 407 (POL) and povidone K-30 (POV) for rosuvastatin]. Dissolution profiles are shown in Figure 3.28. 98%, 97% and 99% drug was released from the preparation of SD ATV: POL 1:5, SD SIM: POL 1:5 and SD ROS: POL 1:5 respectively within 30 min. Further improvement of drug release and dissolution rate was observed due to addition of more carriers in solid dispersions. But the increase of drug release rate was not proportional to the amount of carrier. Similar results were also reported in case of nifedipine (Alam *et al.*, 2013).

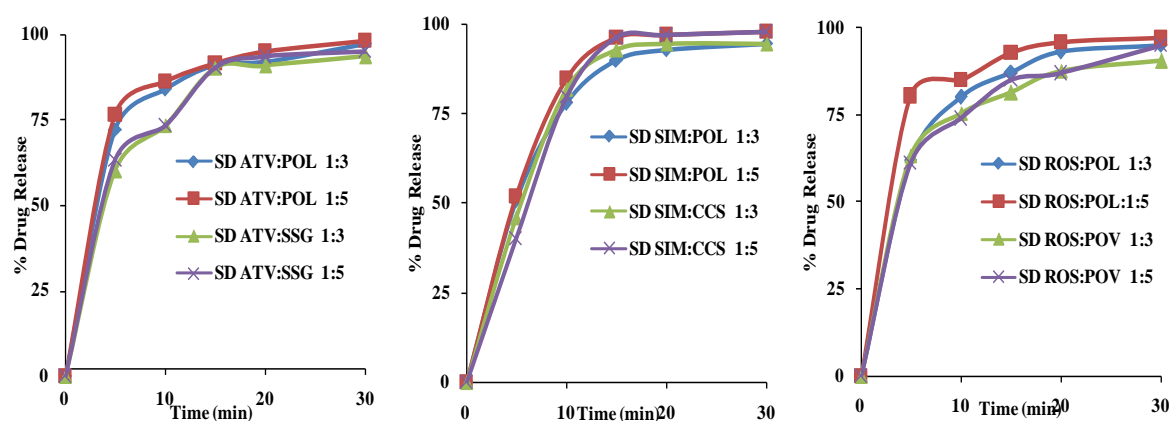


Figure 3.28: Comparative drug release profile of primary solid dispersions (1:3 and 1:5 PSD)

Secondary Solid Dispersions (SSD): Secondary solid dispersions were also prepared by solvent evaporation method using two best performed carriers. Carriers were selected depending on their performance in primary solid dispersions. The following factorial design was used to formulate different secondary solid dispersions (Table 3.17).

Table 3.17: 2^2 factorial designs of SD: Independent (Carrier A, Carrier B) and Dependent variables (%DE)

Drug level	Carrier	Lower level	Higher level
1	Carrier A	1	3
1	Carrier B	1	3

The data were fitted in equation 1,

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 \text{ ----- (1)}$$

where the independent variables (X) were the amounts of carrier A and carrier B mixture, while the dependent variables (Y) was % dissolution efficiency (%DE)

All the SDs showed significant increase in drug release in the *in-vitro* dissolution study (Figure 3.29). 97%, 95% and 97% drug was released from the preparation of SD ATV: POL: SSG 1:3:3, SD SIM: POL: CCS 1:3:3 and SD ROS: POL: POL:POV 1:3:3 respectively with in 30 min.

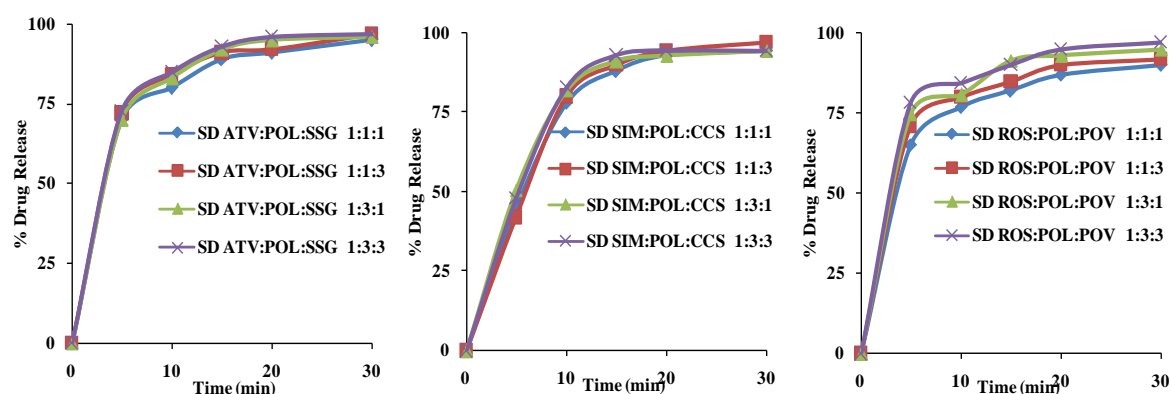


Figure 3.29: Comparative drug release profile of secondary dispersions (SSD)

The data were subjected to multiple regression analysis and the response surface plots were constructed using Design Expert 9.0 (Stat-Ease Inc., USA). Then the effect of carrier on drug release was determined by regression equation. Response surface methodology (RSM) is used when only a few significant factors are involved. There are a good number of approaches available to achieve RSM, namely a three-level factorial design (Boza *et al.*, 2000), central composite design (CCD) (Box and Wilson, 1951), Box-Bekhen design (Singh *et al.*, 1995) and D-optimal design (Sánchez-Lafuente *et al.*, 2002; Singh *et al.*, 2011), etc.

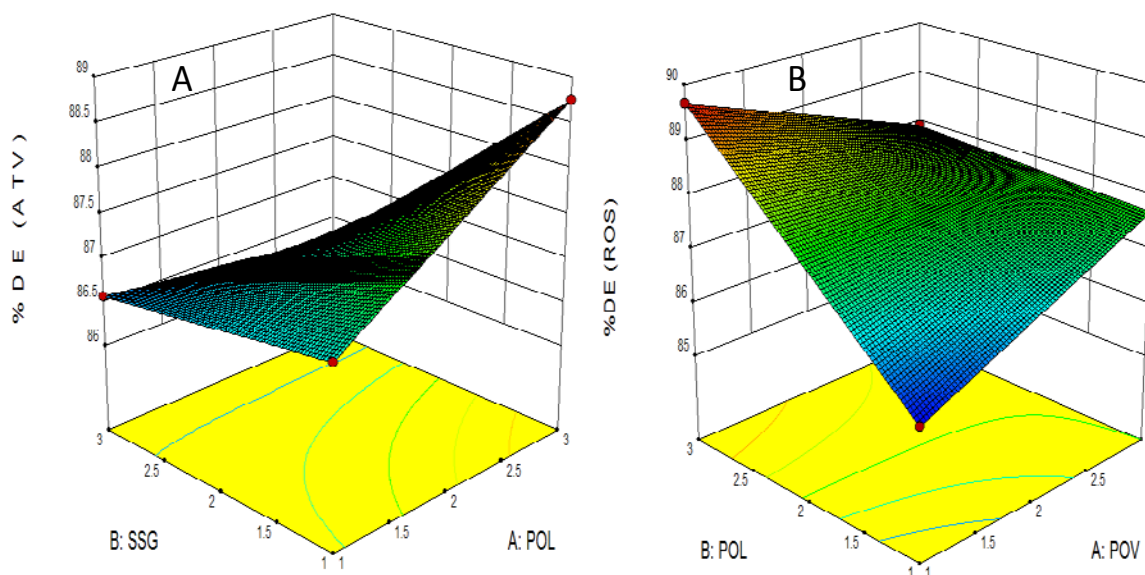


Figure 3.30: Response surface plot (3D) for the effects of sodium starch glycolate (SSG), povidone (POV) and poloxemar 407 (POL) on dissolution efficiency (% DE)

Reduced 2² factorial design (without replicate) is also an established method to study the effect of selected parameters, and the rationale of using this method has been depicted previously (Rawat *et al.*, 2007; Patil *et al.*, 2004). We used a reduced 2² factorial design (without replicate) to characterize the SD, where the two independent variables and one dependent variables were considered.

Figure 3.30 shows the % Dissolution Efficiency versus carrier A/ carrier B level. Evidently, the effects of the carrier are non-linear and antagonistic. Statistical analysis of the data (Design Expert 9.0 (Stat-Ease Inc., USA).) revealed that % Dissolution Efficiency for ATV, SIM, ROS can be best described by following cubic model:

$$\%DE (ATV) = 87.13 + 1.391 POL + 0.2977 SSG - 0.516 POL * SSG$$

$$\%DE (SIM) = 87.13 - 1.5324 * CCS - 1.93838 * POL + 0.6655 CCS * CCS$$

$$\%DE (ROS) = 81.8618 + 1.9128 * POV + 2.8969 * POL - 0.9287 * POV * POL$$

The corresponding response surface plot (Figure 3.30) revealed that Carrier A and Carrier B significantly influenced the drug dissolution rate ($P < 0.0001$) in different ways. In case of atorvastatin and rosuvastatin, it was found that poloxamer 407 have greater influence than the second carrier (SSG and POV).

Tertiary Solid Dispersions

Tertiary solid dispersions contain three components. Lactose and aerosil-200 were used as a third carrier along with the carriers that were used in secondary solid dispersion. At first tertiary solid dispersions containing three carriers at the ratio of 1:1:1 were prepared. About 100% drug was released from all tertiary solid dispersions within 15 minutes (Figure 3.31). Then TSDs containing three carriers at the ratio of 1:1:3 and 1:3:3 were also prepared and evaluated by *in-vitro* drug release study. Drug release almost remain same as lower ratio of carriers(1:1:1). So drug release was not proportional to the amount of carrier

The therapeutic efficacy of a drug depends on rate and extent of drug absorption by the gastrointestinal tract. The dissolution rate of poorly water-soluble drugs is often a rate-limiting step in their absorption from the GI tract (Chiba *et al.*, 1991). Such drugs suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability. All the drugs included in this study are also suffer limited oral bioavailability

and are often associated with high intra subject and inter subject variability. Significant increases (2 to 3-fold) in drug releases were observed ($p < 0.01$) in tertiary solid dispersion.

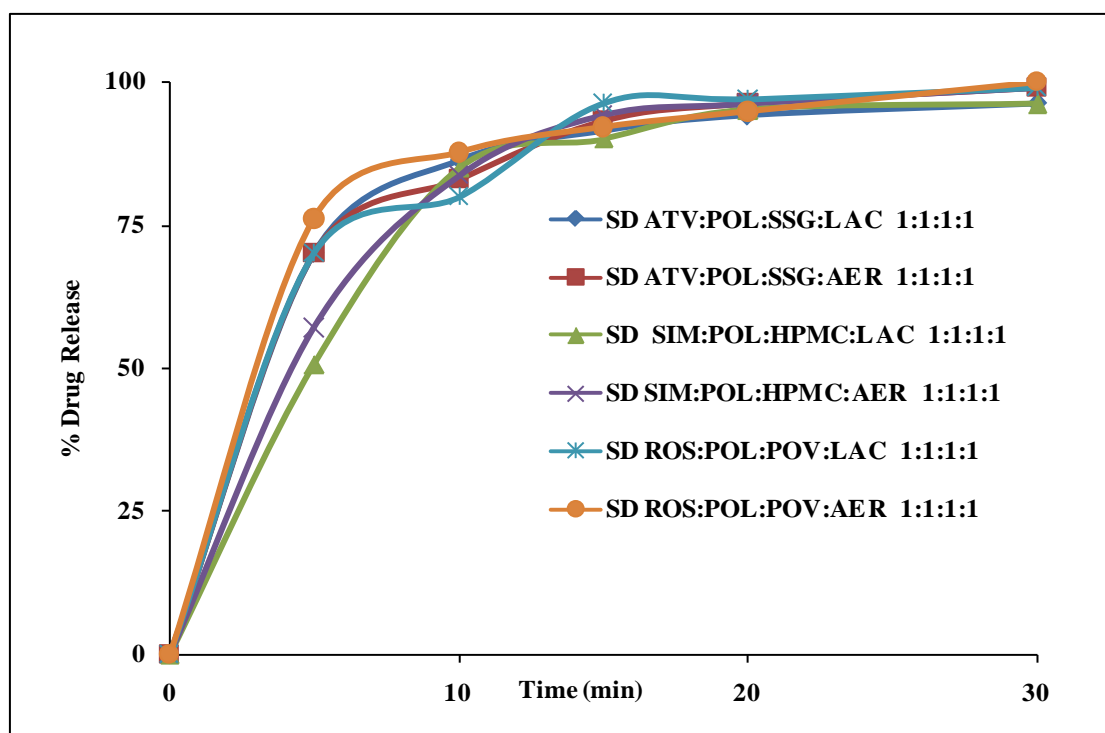


Figure 3.31: Comparative drug release profile tertiary solid dispersions (TSD)

Statistical Analysis of Dissolution Data

Difference factor (f_1) and similarity factor (f_2) were calculated to compare the dissolution profile of solid dispersion with API and ref product. In all the cases API powder and Ref Products were not similar with any solid dispersion (PSD, SSD and TSD) as f_2 was less than 50 and f_1 was more than 15.

Dissolution efficiency (%DE) was also calculated to compare the dissolution profile. From % DE we found that drug release was increased for all the drugs (1.9-2.7 fold in case of rosuvastatin SD, 1.4 to 2.4 fold in case of simvastatin SD and 3.0 to 3.6 fold in case of atorvastatin SD). According to Anderson *et al* API and the SD may be said equivalent if the difference between their dissolution efficiencies is within appropriate limits ($\pm 10\%$, which is often used) (Anderson *et al.*, 1998). But in all the case %DE for API is much lower than that of SD and difference are more than 50. By ANOVA analysis we found that % DE for SD are significant than that of API ($0.001 < p$)

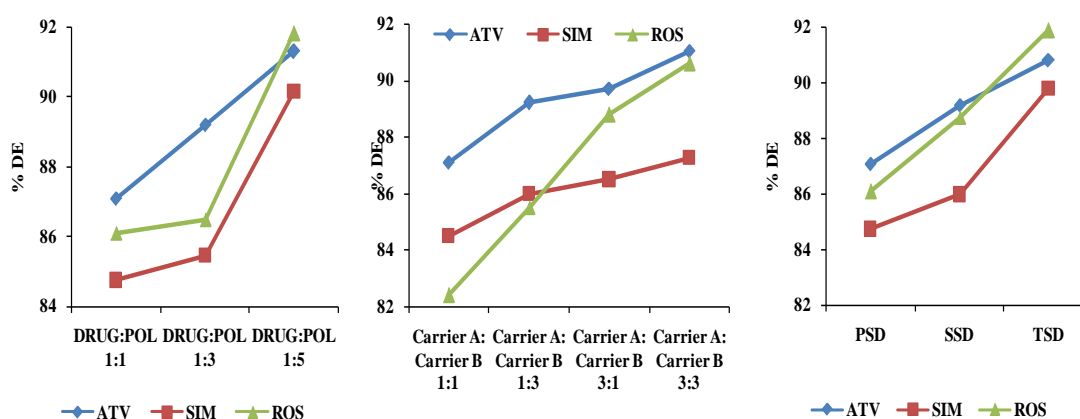


Figure 3.32: Comparative % Dissolution Efficiency as a function of Carrier level, type of solid dispersion

Figure 3.32 showed % dissolution efficiency (%DE) of different types solid dispersions (PSD, SSD and TSD) prepared from different carrier at different levels. %DE was increased with increase of carrier for all drugs and for all types of SD.

Poor solubility is the main reason for inter-brand (brand to brand) and intra-brand (within a brand) variations in dissolution profiles. Here all SD increased solubility and dissolution rate and hence they will decrease inter-brand (brand to brand) and intra-brand (within a brand) variations in dissolution profiles

$T_{25\%}$, $T_{50\%}$, $T_{80\%}$, and MDT values were also calculated by model dependent technique, we found that 3.0-3.2 fold more time was required to dissolve 80% drug from powder API than SD.

3.12 Characterization of Solid Dispersion

3.12.1 Physical Appearance and Potency of Prepared Solid Dispersion

Solid dispersions were prepared employing solvent evaporation method. All solid dispersions were white fine powders. No discoloration was observed during preparation of SD. Prior to *in-vitro* dissolution study the prepared solid dispersion was subjected to potency test. Validated reversed phase High Performance Liquid Chromatographic (RP-HPLC) method as discussed in section 2.3.2 was used to determine the potency of SDs. Potency of SD was between 98.5-99.7%.

3.12.2 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

FTIR studies of pure drug, poloxamer 407, physical mixture (PM 1:1) and solid dispersion (SD 1:1) were conducted and are shown in Figures 3.33-3.35. The prominent peaks of atorvastatin was observed (Figure 3.33) in the region of 3369.64 cm^{-1} (OH-stretching), 2953.02 cm^{-1} (CH –stretching), 1649.14 cm^{-1} (C=O – stretching), 1573.91 cm^{-1} (C=C -bending), 1315.45 cm^{-1} (C-N – stretching), 1226.73 cm^{-1} (C-F -stretching). The FTIR spectrum of poloxamer 407 is characterized by principal absorption peaks at 2885.51 cm^{-1} (C-H stretching aliphatic), 1379.1 cm^{-1} (in-plane O-H bending) and 1111 cm^{-1} (C-O stretching).

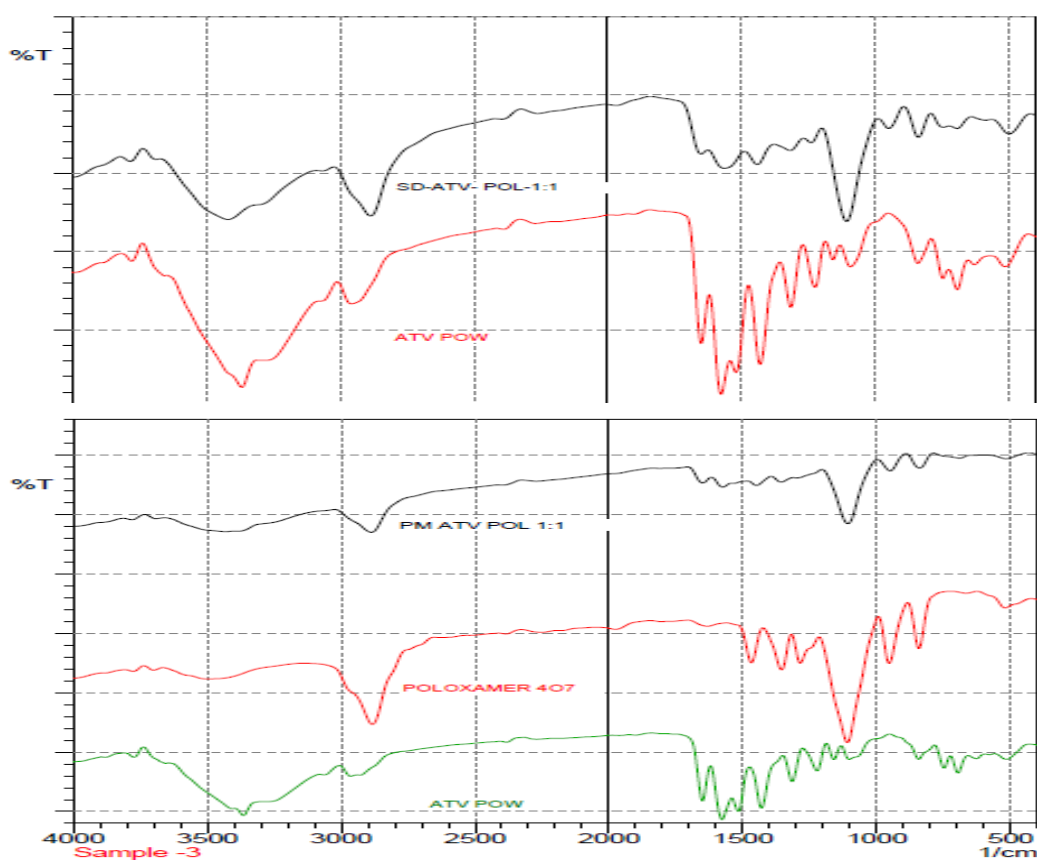


Figure 3.33: FTIR spectra of poloxamer 407, pure drug, physical mixture and solid dispersion of atorvastatin

All peaks of atorvastatin and poloxamer 407 were found in solid dispersion with a slight shifting of peaks. The FTIR spectrum of the physical mixture (Figure 3.33) displayed the superimposition pattern of atorvastatin and polymer peaks with decreased peak intensity and little shifting of the peaks. The peak of OH stretching (3369.64 cm^{-1}) was also shifted to

higher wavelength (3417.86 cm^{-1}) which may be due to presence of higher O-H groups in poloxamer 407. Other peaks related to C-H, C-O, C-N, stretching, remain unchanged.

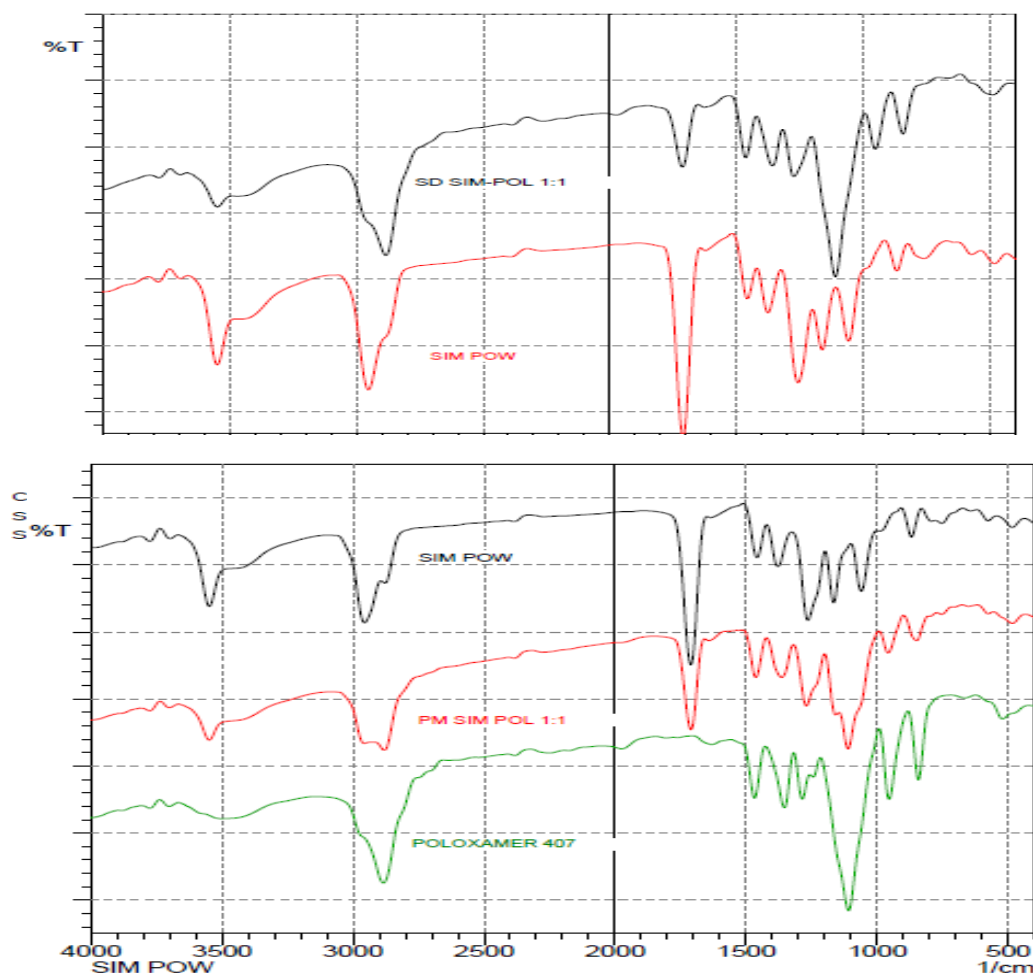


Figure 3.34: FTIR spectra of poloxamer 407, pure drug, physical mixture and solid dispersion of simvastatin

FTIR spectroscopy was also used to study the possible interactions between SIM and POL in SD. There was no significant difference in the FTIR spectra of physical mixture and SD (Figure 3.34). All major peaks of SIM observed at wave numbers 3553 cm^{-1} (alcohol O-H stretching vibration), 2951.09 cm^{-1} (methyl and methylene C-H asymmetric and symmetric stretching vibration), 1712.79 cm^{-1} (Lactone C=O stretching) 1058.92 cm^{-1} (C-O stretching) were retained in physical mixtures and SDs, which clearly indicate that no interaction exists between pure drug and POL in SD.

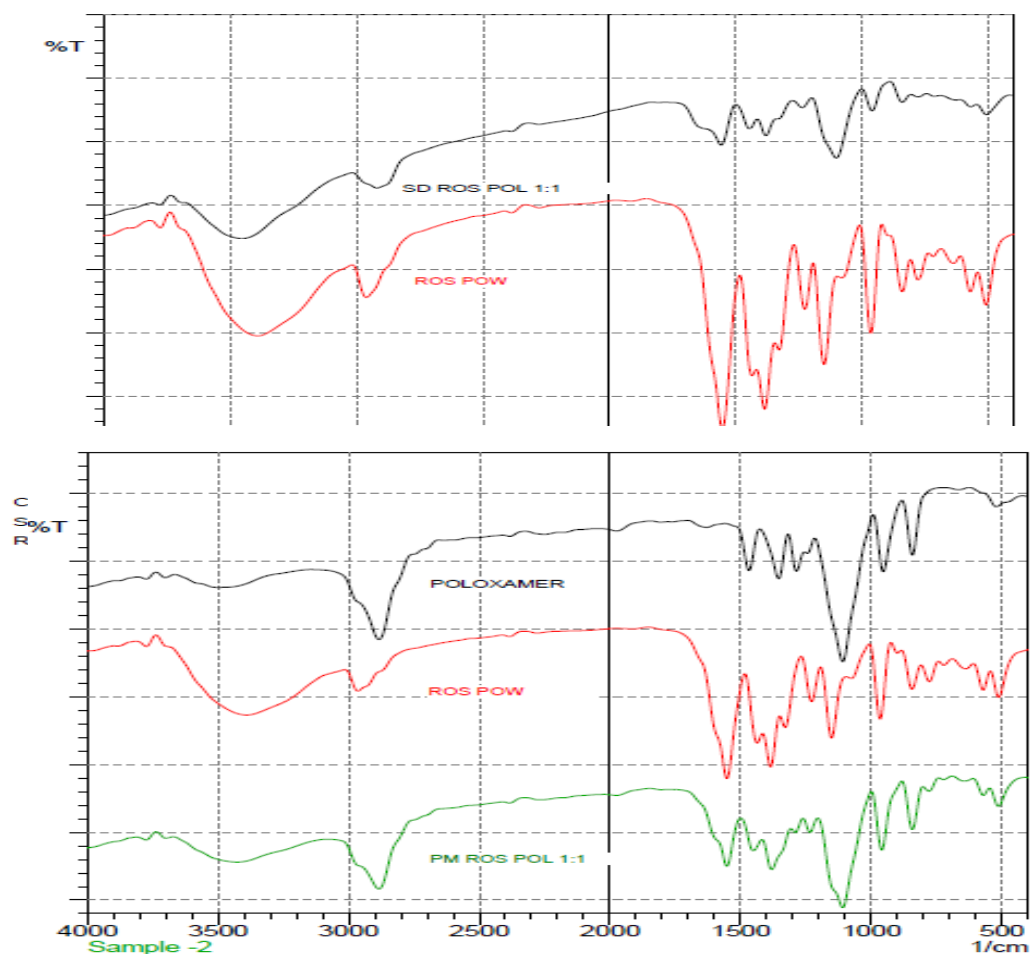


Figure 3.35: FTIR spectra of poloxamer 407, pure drug, physical mixture and solid dispersion of rosuvastatin

In case of rosuvastatin there was no significant difference in the FTIR spectra of physical mixture and SD (Figure 3.35). All major peaks of ROS observed at wave numbers 3390.86 cm^{-1} (OH-stretching), 2968.45 cm^{-1} (C-H-stretching), 1548.84 cm^{-1} (C=C-stretching), 1382.96 cm^{-1} (C-N-stretching), 1330.88 cm^{-1} (S=O-asymmetric), 1153.43 cm^{-1} (C-F-stretching) were retained in physical mixtures and SDs, which clearly indicate that no interaction exists between pure drug and POL in SD. Principal absorption peaks of poloxamer 407 [2885.51 cm^{-1} (C-H stretching aliphatic), 1379.1 cm^{-1} (in-plane O-H bending) and 1111 cm^{-1} (C-O stretching)] were also retained both in SD and PM.

3.12.3 Differential Scanning Calorimetric (DSC) Analysis

DSC studies were carried out between the active ingredient and other excipients used in the preparation of SD formulation so as to check any kind of incompatibilities that may affect stability, solubility, dissolution rate and bioavailability of drug.

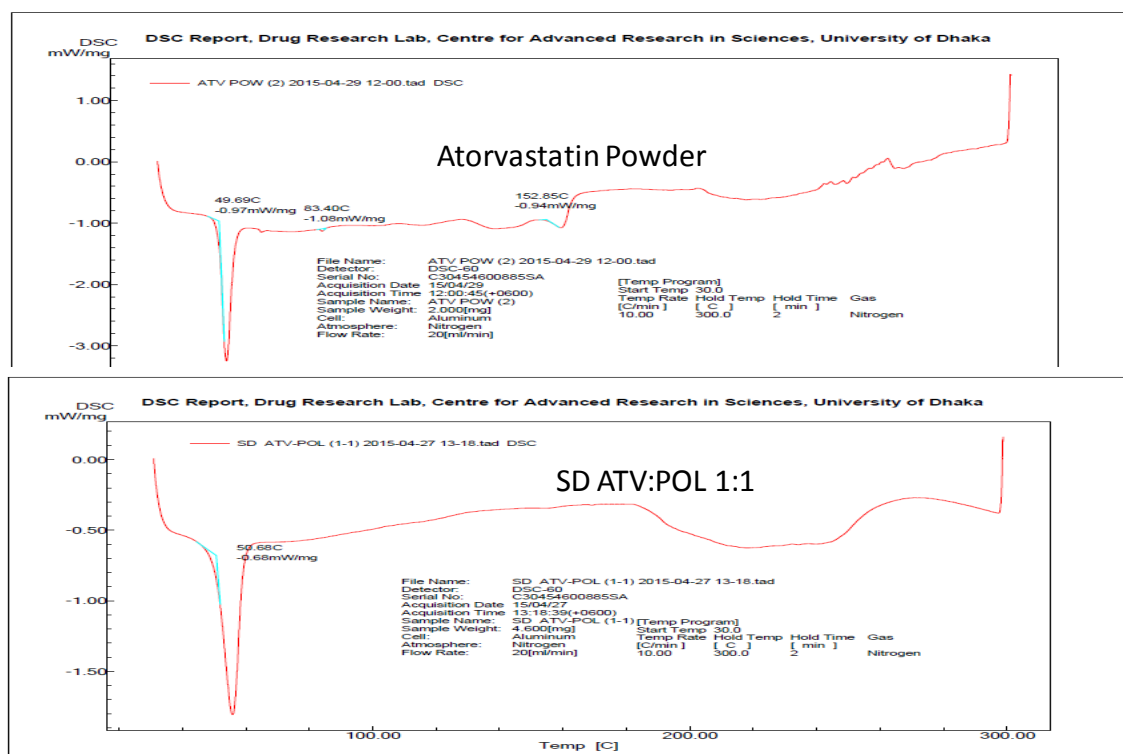


Figure 3.36: DSC curves of pure drug and solid dispersion of atorvastatin

DSC thermo gram of atorvastatin shows (Figure 3.36 upper part) two endothermic peaks one of which at 152.85°C corresponding to the melting point of atorvastatin and another at 49.69°C due to loss of water or dehydration. In case of solid dispersion (Figure 3.36 lower part) there is a single sharp peak 50.86°C for poloxamer and loss of water. Atorvastatin melting peak in solid dispersion was completely disappeared which attributed that the drug was completely miscible in the melted carrier and the drug was present in amorphous form. This disappearance of drug peaks upon formulation into SD system was in agreement with Mc Cauley and Brittain who declared that the complete suppression of all drug thermal features undoubtedly indicates the formation of an amorphous solid solution. In addition, Khaled (1998) found that the total disappearance of the drug melting peak indicates that drug amorphization had taken place (Khaled, 1998; Gubbi and Jarag, 2010).

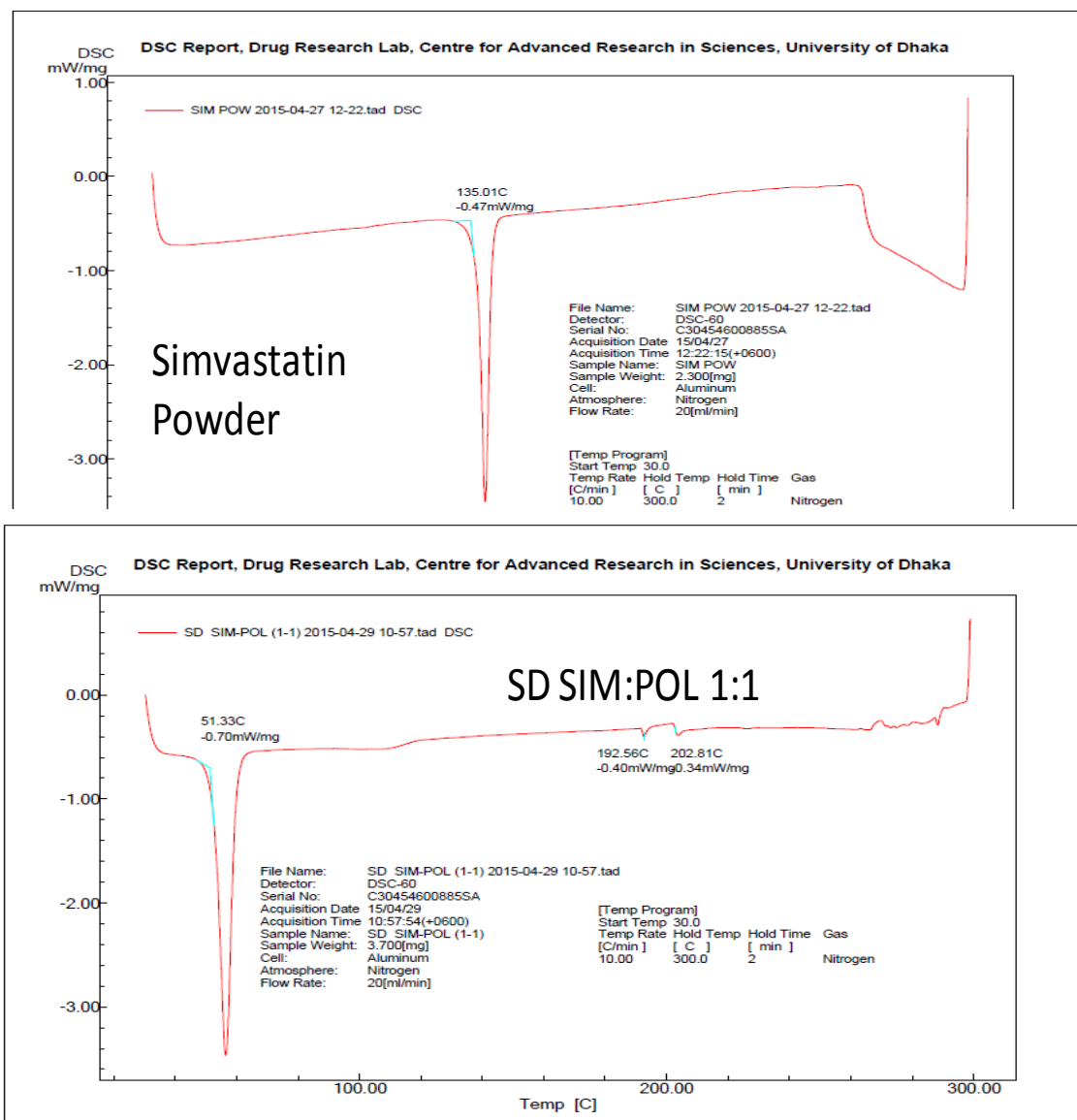


Figure 3.37: DSC curves of pure drug and solid dispersion of simvastatin

DSC thermogram of SIM exhibits a sharp melting endotherm at 135.01 °C (Figure 3.37 upper part) indicates the melting point of simvastatin. In SD prepared with POL, the melting endotherm of POL was found in the temperature range of 51.33-60.5°C. Melting peak of simvastatin in solid dispersion was completely disappeared which attributed that the drug was completely miscible in the melted carrier and the drug was present in amorphous form. Moreover peak at 192-202° C indicates thermal breakdown of compounds.

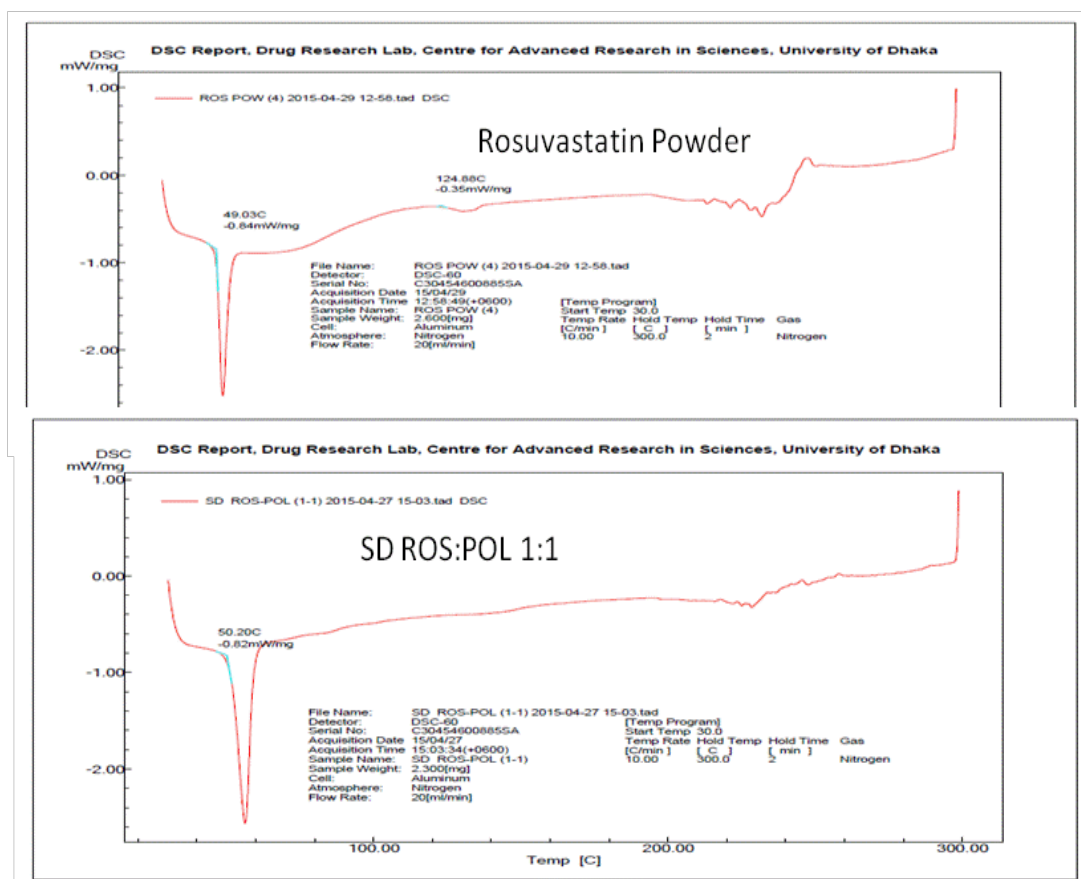


Figure 3.38: DSC curves of pure drug and solid dispersion of rosuvastatin

Like atorvastatin calcium DSC thermogram of rosuvastatin shows (Figure 3.38 upper part) two endothermic peaks one of which at 124.66°C corresponding to the melting point of rosuvastatin and another at 49.69 due to loss of water or dehydration. But solid dispersion (Figure 3.38 lower part) showed a single sharp melting peak 50.86°C, the rosuvastatin melting peak in solid dispersion was completely disappeared which attributed that the drug was completely miscible in the melted carrier and the drug was present in amorphous form.

3.12.4 SEM Studies

SEM photomicrographs that reveal the surface morphology of the API, PM and solid dispersion are shown in Figures 3.39-3.41. SEM study showed that pure drug particles were irregular in shape (Figures 3.39), while the physical mixture of the drug and carrier shows

that drug particle remains dispersed and physically adsorbed on the surface of carrier particles (Figure 3.40).

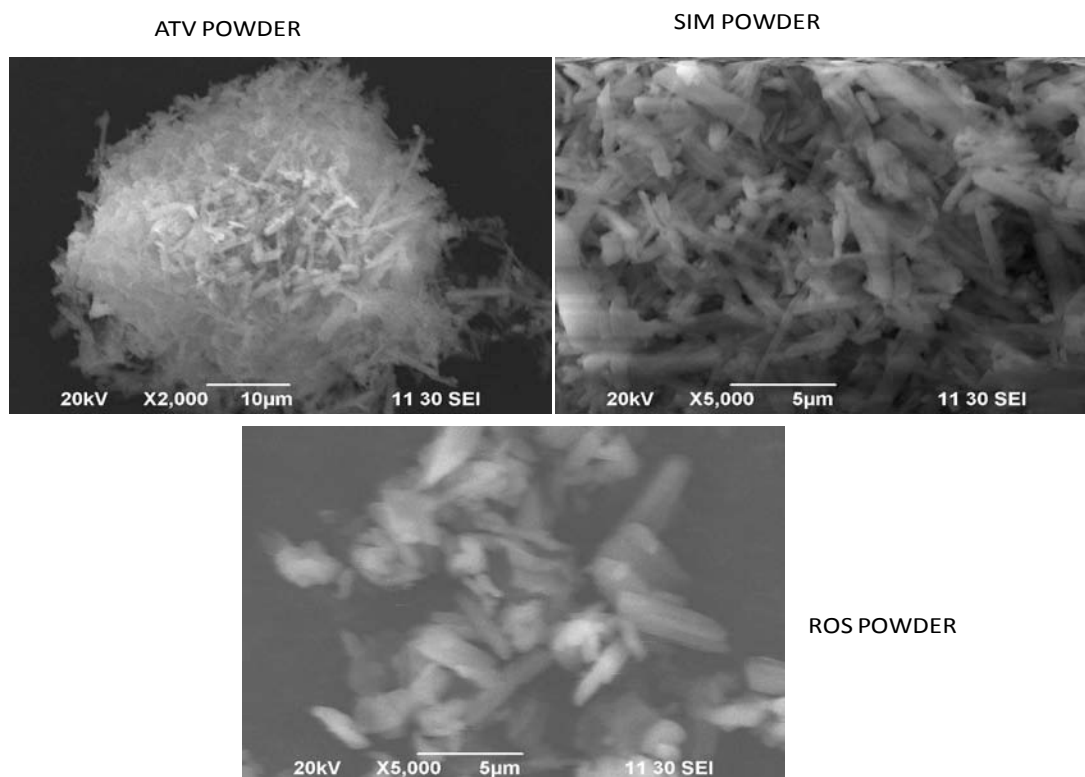


Figure 3.39: Scanning electron micrographs of atorvastatin, simvastatin and rosuvastatin pure drug

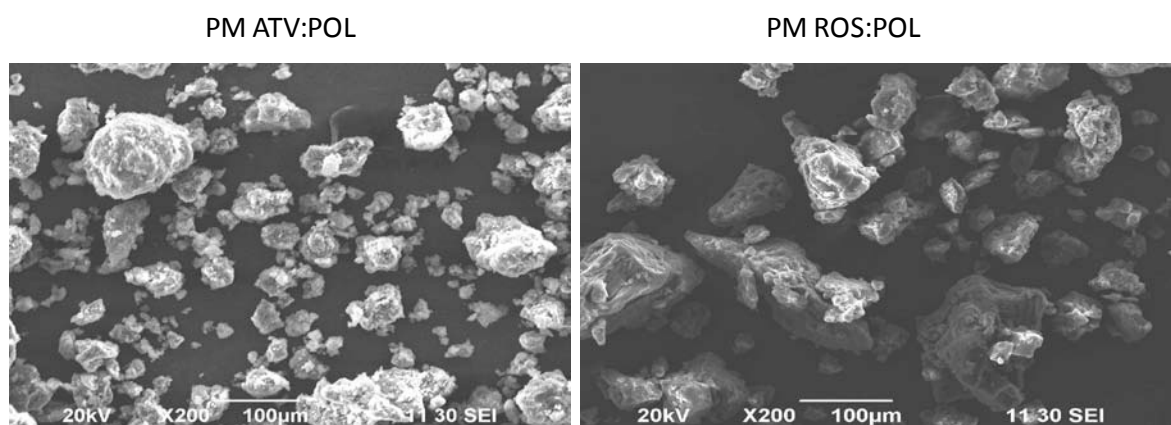


Figure 3.40: Scanning electron micrographs of atorvastatin and rosuvastatin in physical mixture with poloxamer 407

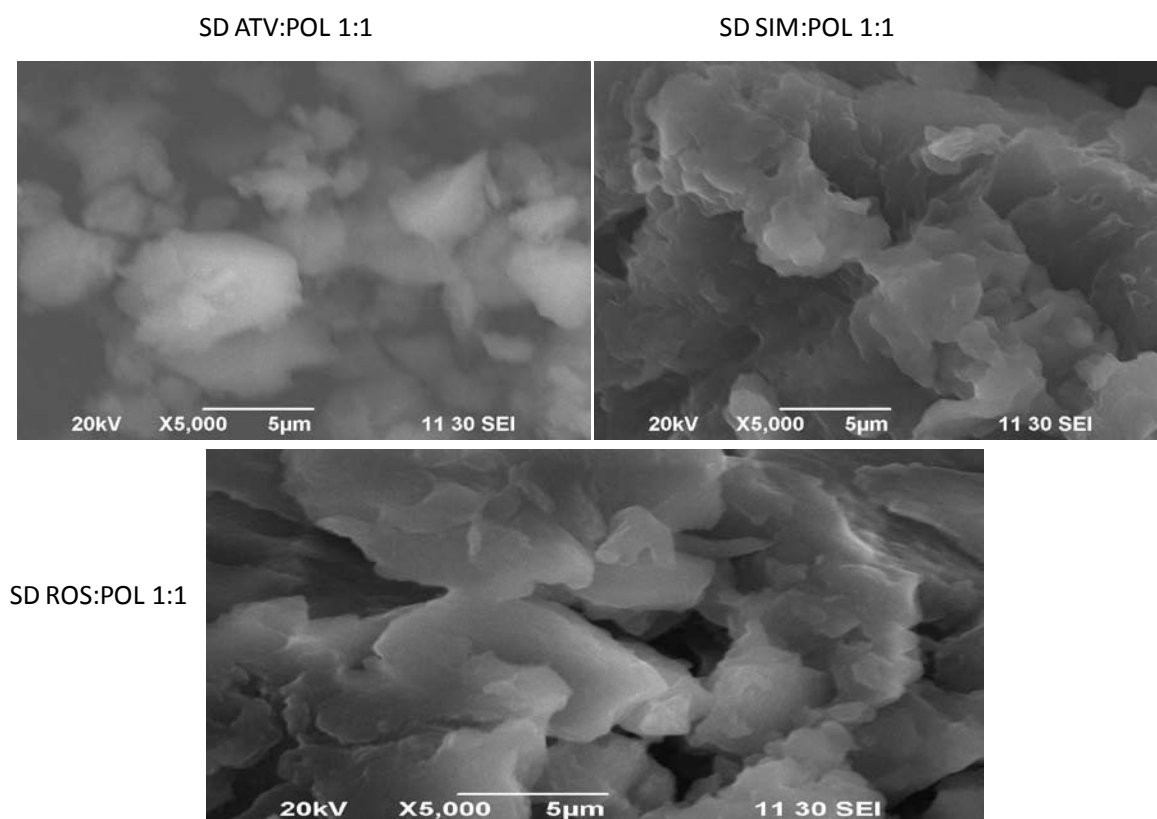


Figure 3.41: Scanning electron micrographs of SD of atorvastatin, simvastatin and rosuvastatin

Characteristic needle-shaped crystals of simvastatin were observed in the photomicrograph of simvastatin (Figure 3.39). On the other hand rosuvastatin photomicrograph showed microcrystal. Solid dispersion of drug with poloxamer 407 showed homogeneous dispersion, which indicates that, the drug molecules were uniformly dispersed into the polymer (poloxamer 407) matrices in the ratio of 1:1 (Figure 3.41). SEM of the SD reveals several microscopic cracks and crevices, which provide additional surface for deposition of the drug particles. There is no evidence of drug crystals, which confirms amorphous form in SD.

3.12.5 Powder X-ray Diffraction (PXRD) Analysis

The powder X-ray diffraction patterns were traced employing X-Ray diffractometer (XPRT-PRO PW3050/60, BCSIR) for the samples using Ni filtered Cu (K- α) radiation, a voltage of 40 KV, a current of 30 mA. The samples were analysed over 2θ range of 5-75° with scan step size of 0.020° (2θ) and scan step time 0.30 S.

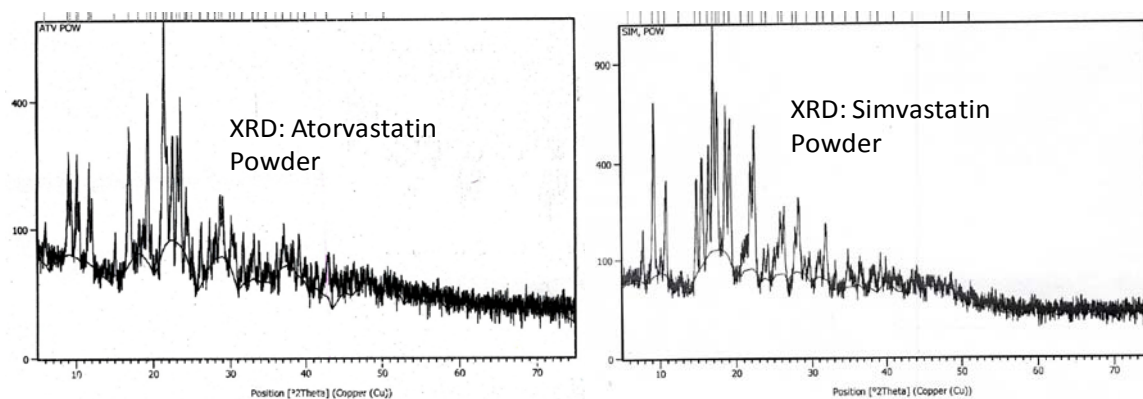


Figure 3.42: X-ray powder diffractograms of pure drugs (atorvastatin and simvastatin)

The diffraction patterns of pure atorvastatin, simvastatin and rosuvastatin showed that the drugs were of crystalline nature, as demonstrated by numerous distinct peaks (Figure 3.42). Atorvastatin's numerous diffraction peaks were observed at 2θ 6.00, 9.02, 10.13, 10.42, 11.71, 12.05, 13.75, 15.09, 16.90, 17.76, 18.15, 18.67, 19.33, 19.75, 20.47, 21.48, 21.85, 22.58, 23.18, 23.60, 24.27, 25.04, 26.25, 27.39 etc. (fingerprint region), indicating the crystalline nature of atorvastatin. Simvastatin's numerous diffraction peaks were observed at 9.35, 10.82, 14.94, 15.61, 16.55, 17.21, 17.68, 18.8, 19.3, 22.01 and 25.01 etc. On the other hand rosuvastatin powder showed diffractograms that represent crystal type C as mentioned in US Patent Application Publication (Blatter *et al.*, 2011) and characterized by X-ray powder diffraction peaks at about 4.7, 19.4 and 22.3° 2.

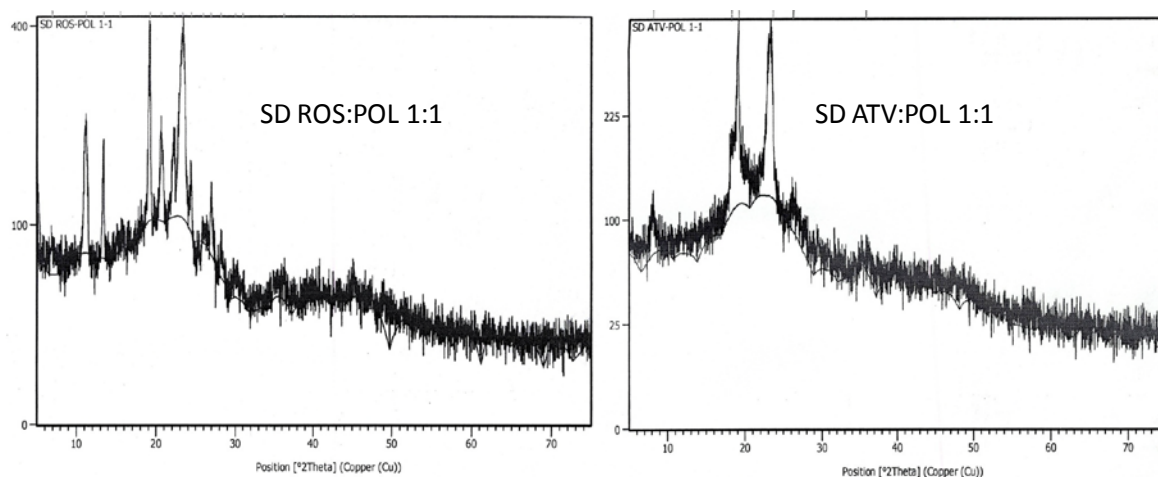


Figure 3.43: X-ray powder diffractograms of SD of rosuvastatin and atorvastatin

SDs contained poloxamer 407 showed two peaks with highest intensity at 2θ of 19.07 and 23.15. Poloxamer 407 peaks in solid dispersion were just superimposed, which ruled out the possibility of chemical interaction between drugs and poloxamer 407 (Figure 3.43). In the spectrum of solid dispersion most of the drug peaks were absent or the intensity of the drug's peaks was reduced when compared to that of the pure drug. This clearly indicates the tremendous reduction in the crystallinity of the drug. The absence of diffraction peaks in the spectrum of SD is characteristic of an amorphous compound. The results indicate that the drug in solid dispersion was amorphous as compared to the pure drug. Hence the dissolution of drugs was improved.

3.13 Effectiveness of Solid dispersion (SD) in Contrast to Physical Mixture (PM)

According to the dissolution profiles plotted below (Figure 3.44), all the prepared physical mixtures, solid dispersions were found capable of enhancing dissolution behavior of atorvastatin, simvastatin and rosuvastatin when compared with the pure drug. Physical mixtures showed 63% (atorvastatin), 40% (simvastatin) and 75% (rosuvastatin) drug release within 30 min minutes whereas pure drug powder showed only 20% -52% drug release within 60 min. This is due to the surface adsorption of the drug on the carriers and thereby increased wetting of the drug in physical mixtures than compared to the pure drug which floats on the surface in the form of aggregates leading to reduced effective surface area (Alam *et al.*, 2013).

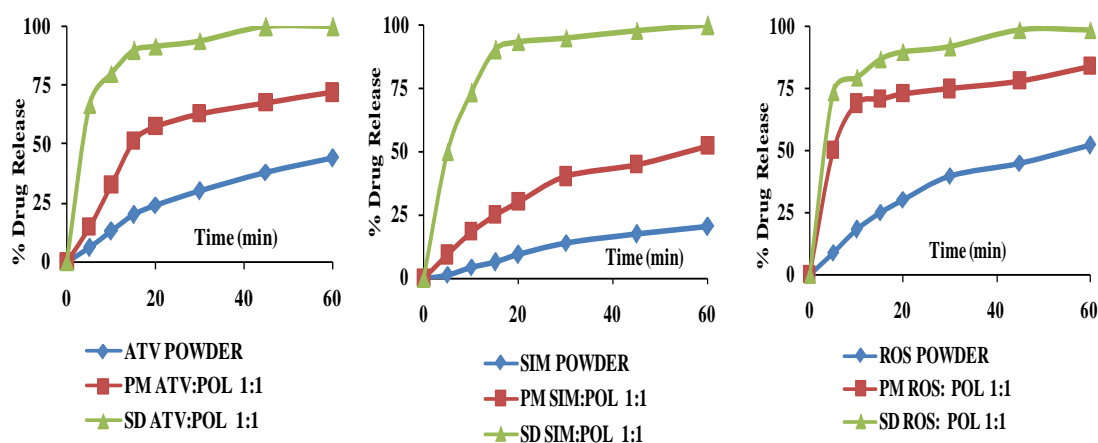


Figure 3.44: Comparative drug release profiles of SDs, and PMs of ATV, SIM and ROS for POL carrier (1:1)

On the other hand drug release was enhanced to a greater extent by SDs when compared to both pure drug and physical mixtures. For the water soluble carriers, the inherent differences are due to hydration, dissolution and possible complexation with drug that influence in the improvement of dissolution characteristics (Rupal *et al.*, 2009). Incorporation of such carriers in solid dispersions renders them more efficient in improving wettability of drug and hence dissolution has been improved. Water insoluble carriers become hydrated in presence of water and swell rapidly by water intake. Thus drug release enhances as the drug wetted and dissolved that was adsorbed on the carriers in a finer form or molecular form in solid dispersions.

Distribution of drug into carriers was thus the key factor of enhancing drug dissolution and this made the difference between release behavior of PMs and SDs. Drug was distributed at molecular level in SDs and undergone better wetting and hence, better dissolution. But the physical mixtures were unable to bring the drug dispersed at that finer level and as a result slight improvement of wetting characteristics may take place (Islam *et al.*, 2013).

3.14 Development of Tablets Containing SD

SDs that showed maximum drug release (SD SIM:POL 1:1, SD ATV:POL 1:1 and SD ROS:POL 1:1) was further formulated in tablet containing 10 mg drug. The physical properties of all selected SD samples were suitable for tablet formulation. Percentage compressibility was 14-15% and the angle of repose of samples was between 25⁰ and 30⁰. These values indicate good compressibility and flow properties, making these samples suitable for tablet formulation.

Tablets containing 10 mg of drug in SDs were made by direct compression using different formulation excipients such as directly compressible lactose, colloidal silicon dioxide, purified talc and magnesium stearate. Three disintegrating agents were used in three different formulations (Cross povidone in TAB-1, Ludiflash in TAB-2 and Pharmabust in TAB-3). The blend was compressed on an eight-station rotary machine (Shakti Pharmatech, India) using round-shaped, flat punches to obtain tablets.

Physical Characterization of Tablets

The tablets were evaluated for hardness, thickness, diameter, average weight and disintegration time for all the formulations. No significant difference was observed in the

weight of individual tablets from the average weight (150 mg). The hardness of tablets of all formulations was found uniform (40 to 50 N). No significant difference was observed in the thickness of individual tablet from the average 2.8mm. Disintegration time was found within 2-3 minutes for all the formulations.

***In-Vitro* Drug Release Study from Tablets**

Drug dissolution data revealed that the tablets of SDs were capable of releasing the drug much greater extent than API powder or marketed product (Figures 3.45). But drug release from tablet was found slightly lower than respective SDs. This may be due to the initial disintegration time that required to break down the tablets. But drug release was increased after initial slow release and after 45 min both the SDs and tablet released about 100% drug. Whereas the references product released only 81% at 45 min dissolution and API powder released only 45 % drug within same time. Thus the solid dispersions proved their efficacy to improve the dissolution characteristics when presented in finished dosage form.

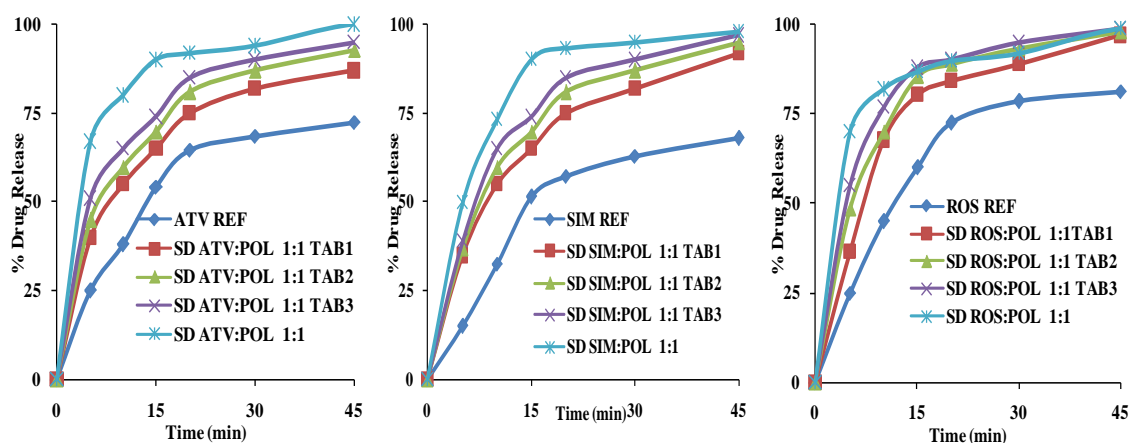


Figure 3.45: Comparative release profiles of SDs, SDs tablet of ATV, SIM and ROS for POL carrier (1:1)

Drug release from tablet was also found formulation dependant. TAB-3 formulation containing Pharmabust released higher amount of drug than tablet containing cross povidone (TAB-1) and ludiflash (TAB-2) for all the three drugs.

To evaluate the effect of disintegrating agent %DE was calculated for all the tablet formulation. %DE indicates the overall performance of the disintegrating agent in drug

release. The results indicate that solid dispersion is more effective to increase the dissolution rate than REF tab. % DE of SD was higher than REF tablet.

Table 3.18: %DE_{45min} and T_{80%} of different tablet formulations containing SD

Product	Atorvastatin		Simvastatin		Rosuvastatin	
	%DE45min	T _{80%}	%DE45min	T _{80%}	%DE45min	T _{80%}
REF TAB	60.25	40.78	54.66	40.96	68.09	31.50
SD DRUG:POL 1:1TAB1	73.50	29.04	74.13	27.33	82.59	21.01
SD DRUG:POL 1:1TAB2	78.88	23.87	78.75	23.69	86.80	17.44
SD DRUG:POL 1:1TAB3	82.44	19.05	82.06	19.17	89.23	13.71
SD DRUG:POL 1:1	90.06	10.61	92.10	14.13	94.28	4.42

% DE of TAB-3 formulation containing Pharmabust was higher than tablet containing cross povidone (TAB-1) and fuliflash (TAB-2) for all the three drugs although the differences were not significant (less than 10). According to the similarity factor (f_2), TAB1, Tab2 and TAB 3 are found similar for all the drugs ($50 > f_2$) but they are differ with the ref product or SDs ($50 < f_2$). T_{80%} value also calculated and compared. T_{80%} values when compared to SDs Tab and REF products, indicated the efficiency of solid dispersions to improve the dissolution behavior.

3.15 *In-Vitro* Diffusion Study of SEDDS and SD through Cellulose Dialysis Tubing

In-vitro diffusion studies were carried out using cellulose dialysis tubing for selected SEDDS (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3), SD (SD ATV:POL 1:1, SD SIM:POL 1:1, SD ROS:POL 1:1) along with API (atorvastatin, simvastatin, rosuvastatin) and ref products (ATV REF, ROS REF, SIM REF). Results of drug diffusion study are summarized in Figure 3.46 and Table 3.19.

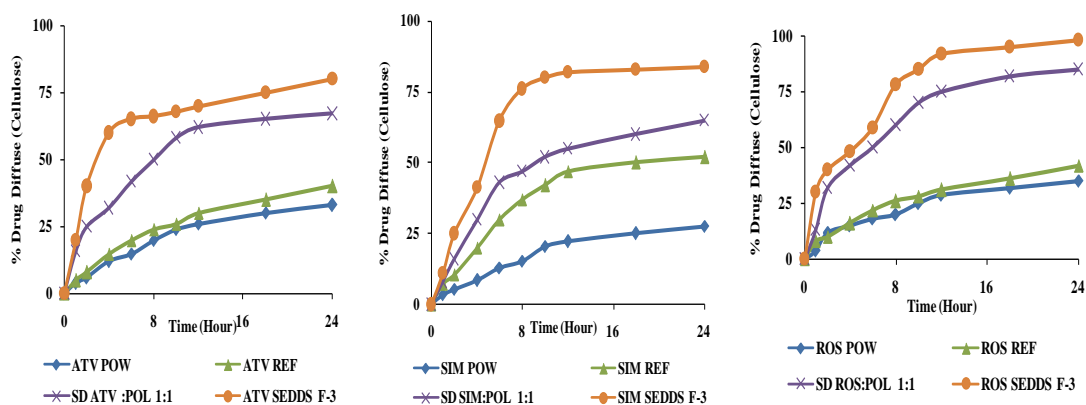


Figure 3.46: Diffusion profiles of atorvastatin, simvastatin and rosuvastatin through cellulose dialysis tubing

Drug diffusion studies using pretreated cellulose dialysis tubing have been well documented in literature (Kang *et al.*, 2005; Patil *et al.*, 2004; kim *et al.*, 2000). Drug diffusion studies through cellulose dialysis tubing were continued up to 24 hours. Drug diffusion profiles of all formulations did not show any significant differences during initial 1 h of study, which might be the lag period. However, at the end of 24 hours, all SEDDS formulations showed higher diffusion against API (75% diffusion for ATV SEDDS F-3, 99% diffusion for ROS SEDDS F-3, 78% diffusion for SIM SEDDS F-3). This clearly indicates the effect of SEDDS formulation on drug diffusion across dialyzing membrane.

% DE for all API powder was lower than that respective Ref Tab, SD and SEDDS for all the drugs. This is due to the lower solubility of drugs. For the same reason higher $t_{25\%}$ value was found for powder drug. Lowest % DE was 19.11 for simvastatin powder and for this drug $t_{25\%}$ was 18.30 hours. By calculating similarity factor (f_2) of diffusion profiles of three drugs, we found that drug diffusion from atorvastatin powder and rosuvastatin powder was also similar with simvastatin ($f_2=68.4$ for atorvastatin and 60.75 for rosuvastatin). But

significant differences were found when we compared the %DE and tested by ANOVA (Table 3.20)

Table 3.19: %DE_{45min} and T_{25%} (hour) of SEDDS and SD for *In-vitro* diffusion study through cellulose dialysis tubing

Product	Atorvastatin		Simvastatin		Rosuvastatin	
	% DE	t _{25%}	% DE	t _{25%}	% DE	t _{25%}
POWDER	23.35	11.40	19.11	18.30	25.24	10.30
REF TAB	27.65	9.40	40.00	5.10	29.09	7.40
SD	53.78	2.00	49.35	3.50	66.46	1.50
SEDDS	67.13	1.50	70.67	2.00	80.39	0.80

Table 3.20: ANOVA results for *In-vitro* diffusion study of API through cellulose dialysis tubing

	Sum of Squares	DF	Mean Square	F	Sig
Between Groups	118.93	2	59.47		
Within Groups	1.35	15	0.09	662.11	0.000
Total	120.28	17			

DF = degree of freedom

% DE for all SEDDS formulation was higher than that respective API, Ref Tab and SD in all the cases. This is due to the higher solubility of drugs from SEDDS formulation. For the same reason lowest t_{25%} value was found for SEDDS formulation. % DE for rosuvastatin SEDDS was 80.34 and for this SEDDS formulation t_{25%} was only 50 min. By calculating similarity factor (f₂) of diffusion profiles of SEDDS formulation of three drugs, we found that drug diffusion from different SEDDS was not similar (f₂=47.4 for atorvastatin SEDDS and 46.16 for rosuvastatin SEDDS). Significance differences were also found when we compared the %DE and tested by ANOVA. Similar results were also observed for SD. Drug diffusion from SD formulations was faster than that APT or Ref Tab in all the cases but inter products similarity was not found (f₂=64.28 for atorvastatin SD and 39.16 for rosuvastatin SD). Drug diffusion from REF Tablet was faster than that APT in all the cases.

3.16 *Ex-Vivo* Permeability Study of SEDDS and SD through Chicken and Rabbit Intestinal Sacs

Ex-vivo permeability study through intestinal sacs is one of the essential parts in the prediction of oral bioavailability (Ginski *et al.*, 1999). A number of methods for assessing the intestinal permeability for a given drug have been developed and reviewed (Ferrec *et al.*, 1999). Isolated intestinal sacs of several animal species including rat, rabbit, pig, dog, and monkey can be used in permeability studies (Tukker, 2000). Irvine *et al* (1999) reported that the chicken small intestine could be a useful model for intestinal absorption. Kale *et al* (2007) performed absorption studies of slow drug release formulations by using chicken intestine segment. Dias *et al* (2010) also used chicken intestine segment to investigate the effect of sodium lauryl sulfate as a permeation enhancer for muco-adhesive acyclovir tablets.

In this study we used chicken and rabbit intestinal segment for permeability study. Selected SEDDS formulations (ATV SEDDS F-3, SIM SEDDS F-3 and ROS SEDDS F-3) having lowest droplet size and highest % DE value, SD formulations (SD ATV:POL 1:1, SD SIM:POL 1:1, SD ROS:POL 1:1) having highest % DE value were included in this study. API (atorvastatin, simvastatin, rosuvastatin) and ref products (ATV REF, ROS REF, SIM REF) were also included in the study for comparison. Figures 3.47 and 3.48 showed the drug permeability results.

Drug permeability from SEDDS formulations was faster than SD, API, and ref product. In all the cases drug permeability followed the following sequences: SEDDS > SD > RP > API. It was estimated that drug was dissolved perfectly from SEDDS formulations with very small droplet size, so it released much more rapidly than the tablet.

Permeability study through the chicken intestinal sacs was continued up to six hours. Drug permeability from all the SEDDS formulations was significantly higher from the beginning of the study and drug release rate increased with time. At the end of 6 hours, ROS SEDDS F-3 released highest amount of drug (99%), whereas ATV SEDDS F-3, SIM SEDDS F-3 released 83% and 78% drug respectively. This may be due to the higher solubility and lower droplet size of ROS SEDDS F-3. This clearly indicates the effectiveness of SEDDS formulation on drug release across the chicken intestinal sacs.

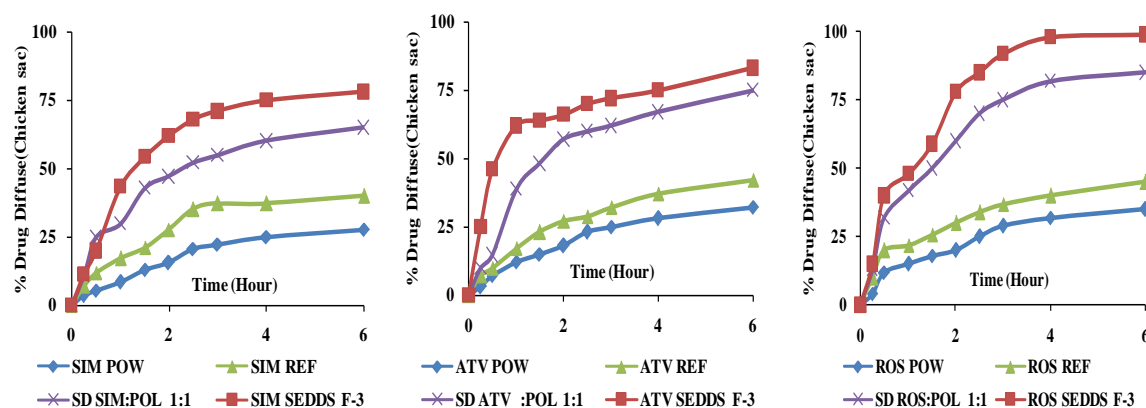


Figure 3.47: Permeability profiles of atorvastatin, simvastatin and rosuvastatin through chicken intestinal sacs

Drug release from SD formulation was faster than that APT and ref tablet in all the cases. SD ATV:POL 1:1, SD SIM:POL 1:1, SD ROS:POL 1:1 released 75%, 65% and 85% drug within six hours whereas ATV REF, ROS REF, SIM REF released only 42%, 40% and 45% drug. On the other hand ATV POW, SIM POW and ROS POW released only 32%, 27% and 35% drug within six hours.

Drug release from ref tablet was faster than that APT in all the cases. This is due to the presence of different excipients that facilitate drug dissolution than API.

By fitting the drug permeability data into f_1 and f_2 equation, we found that all API and ref tablets were similar in respect of drug release ($50 > f_2$). On the other hand, in case of SEDDS formulation, ATV SEDDS F-3 was found similar with SIM SEDDS F-3 ($f_1=5.32$, $f_2=65.27$) but was not similar with ROS SEDDS F-3 ($f_1=25.17$, $f_2=37.01$). Similarly, in case of SD formulation, SD ATV:POL 1:1 was found similar with SD SIM:POL 1:1 ($f_1=14.57$, $f_2=54.65$) but not with SD ROS:POL 1:1 ($f_1=31.06$, $f_2=37.88$). This may be due to the solubility differences among the drugs in the diffusion medium.

Similar results were found when %DE was compared and tested by ANOVA and t-test (Table 3.21).

Table 3.21: Summary of ANOVA results for *Ex-vivo* permeability study of SEDDS and SD through intestinal sac

Products	Differences in % DE	p value	Test
All Ref	No significant differences	$p > 0.05$	ANOVA
All API	No significant differences	$p > 0.05$	ANOVA
All SEDDS	Significant differences	$p < 0.001$	ANOVA
All SD	Significant differences	$p < 0.001$	ANOVA

The study was repeated for all samples through rabbit intestinal sac for comparison. The finding was similar with the previous study which implies that membrane permeability of drugs is not species-dependant, since the composition of plasma membrane of intestinal epithelial cells is similar across the species. In case of rabbit intestinal sac less time is required as thickness of rabbit intestinal sac was much less than the chicken intestinal sac.

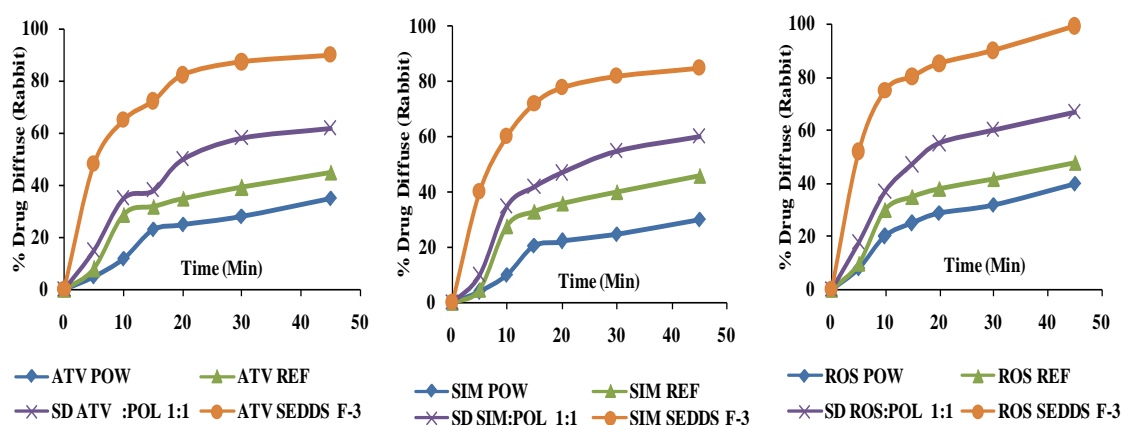


Figure 3.48: Permeability profiles of atorvastatin, simvastatin and rosuvastatin through rabbit intestinal sacs

3.17 Effect of SEDDS and SD on Plasma Lipid Profiles of Healthy Albino Rats

In-vivo performance of rosuvastatin was evaluated by using its pharmacodynamic effects (Ambike *et al.*, 2005). Hypolipidemic activity of rosuvastatin causes reduction in elevated total cholesterol (CH), low-density lipoprotein (LDL-CH) and triglycerides (TG) levels in blood. At the same time, it causes elevation of plasma high-density lipoprotein (HDL-CH) level, which promotes the removal of CH from peripheral cells and facilitates its delivery back to the liver. This pharmacodynamic effect is reported to be dose dependent (Vogel and Vogel, 1995) and hence was used as a basis for the comparison of *in-vivo* performance of SEDDS, SD and REF TAB. Administration of excess coconut oil, which is a rich source of saturated fatty acids, promotes biosynthesis of cholesterol in liver and leads to hypercholesterolemia (Elson., 1992).

The effect of SD ROS:POL 1:1 and ROS SEDDS F-3 formulations on plasma lipid profiles was determined by comparison with reference tablet (aqueous suspension of crushed powder containing 1 mg equivalent rosuvastatin/ml and 2% (*m/V*) gum acacia as a suspending agent). The preparations were administered in healthy albino rats of both sex and weighing between 150–180 g. Animals had free access to food and water. The animals were randomly divided into 5 treatment groups of 6 animals each, *viz.*, test treatment group for ROS SEDDS F-3 (SEDDS-TG), test treatment group for SD ROS: POL 1:1 (SD-TG) reference treatment group (R-TG), placebo treatment group (P-TG) and control treatment group (C-TG). The treatment was given for 21 days. Each treatment group received daily 1.5 ml of coconut oil orally in the morning throughout 21 days. SEDDS-TG, SD-TG, R-TG and P-TG additionally received ROS SEDDS F-3, SD ROS:POL 1:1 aqueous suspensions of ROS REF TAB and blank formulation respectively. The administered oral dose was 10 mg kg⁻¹ per day. Cholesterol level was measured by Blood Cholesterol Measuring Kit (**EasyMate**[®] I, Jhunan Township, Taiwan).

Statistical analysis for the differences in lipid profiles of treatment and control groups was done by the unpaired *t*-test and ANOVA (significance level *p* <0.05). The results were confirmed by Bonferroni's multiple comparison as a post-hoc test using a statistical package for social sciences (SPSS software, version 16.0; SPSS Inc., USA).

Initial CH level: The plasma lipid profiles of all the experimental groups at different time intervals are presented in Figure 3.49. No significant differences were in plasma CH was observed within or between five treatment groups on day zero (initial) due to random sampling of animals ($P > 0.055$).

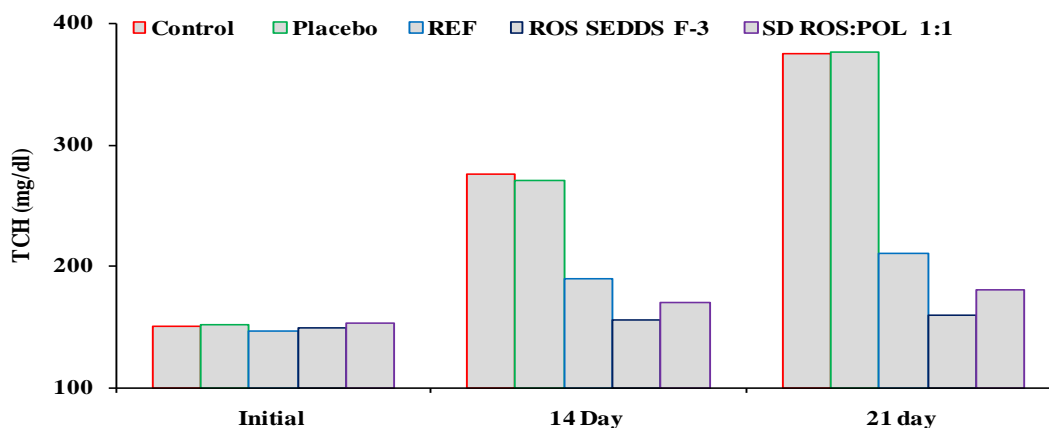


Figure 3.49: Plasma CH levels of rabbit for different treatments at different time intervals

CH level after 14 days: significant increases (1.7 to 1.9 fold) in plasma CH levels were observed ($p < 0.01$) in control treatment group (C-TG) and placebo treatment group (P-TG). After the same period, slight changes in plasma CH were also observed for ROS SEDDS F-3 (SEDDES-TG), SD ROS: OLP 1:1 (SD-TG) and ref treatment group (R-TG) ($p < 0.05$).

CH level after 21 days: after 21 days of treatment with coconut oil (1.5 ml per day, orally), P-TG and C-TG showed a marked increase in total CH (2.47-2.5-fold, respectively) ($p < 0.001$). After the same period, slight changes in plasma CH were also observed for ROS SEDDS F-3 SEDDES-TG), SD ROS: OLP 1:1 (SD-TG) and ref treatment group (RTG) ($p < 0.05$). On the contrary, as expected, in SEDDES-TG and SD-TG, the increase in total CH was much lower but significant ($p < 0.001$), while the R-TG showed marginal increase (2.1-fold and 2.12-fold, respectively) ($p < 0.001$) in plasma CH after 21 day treatment.

Effect of placebo: Lack of significant differences in plasma CH for C-TG and P-TG after the 21-day treatment inferred no appreciable effect of placebo components on the lipid profiles of experimental animals.

Effect of SEDDS, SD and REF: Comparison of plasma CH levels of C-TG against SEDDS-TG, SD-TG and R-TG revealed the lipid-lowering effect of SEDDS and SD of rosuvastatin. Plasma CH levels were significantly lower in the case of SEDDS-TG, SD-TG and R-TG in compared to C-TG (0.43, 0.48 and 0.56 fold respectively) ($p < 0.001$).

Comparison of SEDDS-TG and SD-TG against R-TG: after the 21-day treatment, plasma CH of SEDDS-TG and SD-TG were significantly lower (0.76-fold and 0.86-fold, respectively) ($p < 0.001$) in compared to R-TG. This clearly indicates the varying lipid-lowering effects of rosuvastatin obtained by administering SEDDS and SD of the drug. Thus, SEDDS-TG and SD-TG showed a significantly better *in-vivo* performance than REF TAB in terms of pharmacodynamic parameters. This observation is in accord with earlier reports (Kang *et al.*, 2005; Ambike *et al.*, 2005). Table 3.22 shows the summarized results of *in-vivo* performance study of SEDDS and SDs.

Table 3.22: Summary of ANOVA results for effect of SEDDS and SD on plasma lipid profiles of healthy albino rats

Day	Effect	p value
0	No significant differences in plasma CH within and between groups	$p > 0.055$
14	in case of P-TG and C-TG, significant increases (about 2 fold) in plasma CH	$p < 0.001$
21	in case of P-TG and C-TG, significant increases (about 2.5 fold) in plasma CH	$p < 0.001$
21	Lack of significant differences in plasma CH between C-TG and P-TG	$p > 0.05$
21	Plasma CH level of SEDDS-TG, SD-TG and R-TG were significantly lower than that of C-TG (0.43, 0.48 and 0.56 fold respectively)	$p < 0.001$
21	Plasma CH level of SEDDS-TG and SD-TG were significantly lower than that of R-TG (0.76 and 0.86 fold respectively)	$p < 0.001$

Enhanced pharmacodynamic performance of rosuvastatin formulated in SEDDS could be described as the combined effect of different mechanisms, like the presentation of drug in solubilized form, large interfacial area made available for absorption, enhanced dissolution in the presence of surfactants and increased cellular uptake of drug, probably due to inhibition of cellular efflux systems (Charman *et al.*, 1992; Shah *et al.*, 2011; Hunter and Hirst, 1997).

3.18 *In-Vivo* Bioavailability Study of Rosuvastatin SEDDS and SD

Oral bioavailability study in rabbits was performed by determining the concentration of rosuvastatin in blood samples following oral administration. Six healthy rabbits, 1.5-1.7 kg, fasted for 24 h before the experiment, were allocated to three groups at random. Rabbits were administered rosuvastatin SEDDS (ROS SEDDS F-3), SD ROS:POL 1:1 and ref tablet (ROS REF) within three periods of experiment. Washout interval among the administrations was kept at 7 days. The plasma profiles of rosuvastatin in rabbits following oral administration of reference tablet (ROS REF), ROS SEDDS F3 and SD ROS:POL 1:1 are represented in Figure 3.51. Pharmacokinetic parameters and the relative bioavailability (F_r) of rosuvastatin after oral administration to rabbit are shown in Table 3.23.

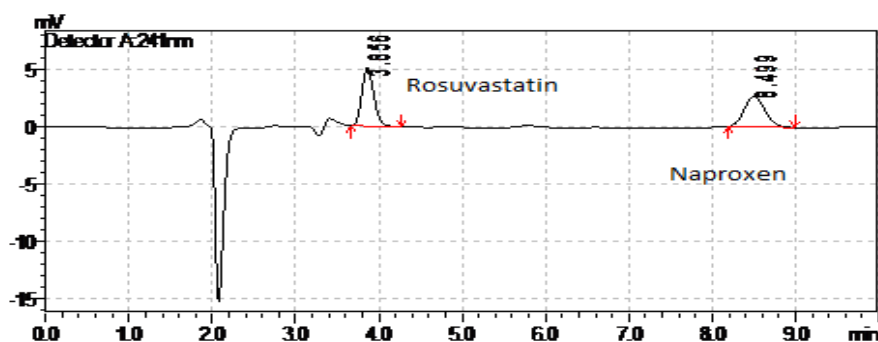


Figure 3.50: Typical HPLC chromatograms of rosuvastatin and naproxen

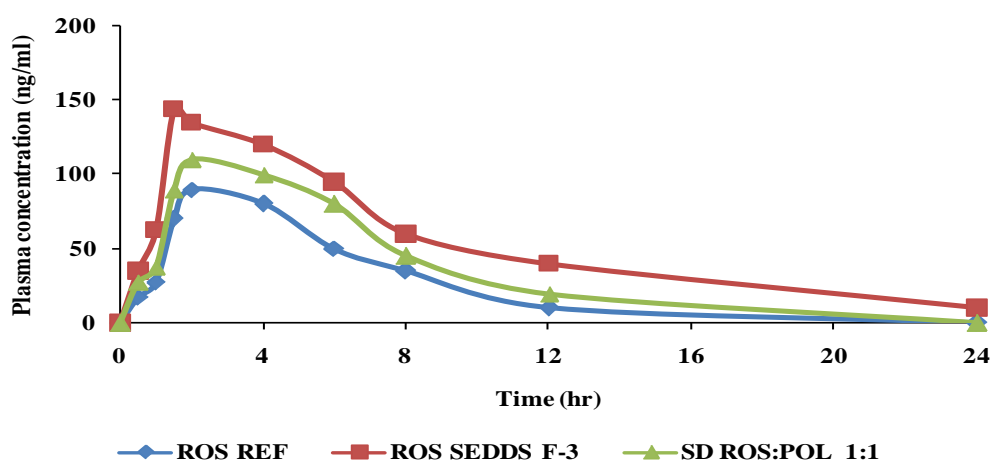


Figure 3.51: Plasma concentration profile of rosuvastatin after oral administration of SEDDS, SD and conventional tablet in rabbits ($n = 6$ and 6mg kg^{-1})

Statistical Analysis of Pharmacokinetic Data

Statistical analysis of the pharmacokinetic data was performed based on a non-compartmental model with *kinetica* (version 5.0). Data from the plasma concentration–time curve within 24 h after drug intake were used to obtain the peak plasma concentration (C_{\max} , ng/ml) and time of peak plasma concentration (T_{\max} , h) for reference tablet (ROS REF) and ROS SEDDS F-3 and SD ROS:POL 1:1. The area under the plasma concentration–time curve ($AUC_{0\rightarrow 24}$ h) was calculated using the linear trapezoidal method. The relative bioavailability (Fr) of the SEDDS to the conventional tablet with the same dose was calculated as: $Fr = [AUC_{\text{SEDDS } 0\rightarrow 24 \text{ h}} / AUC_{\text{REF } 0\rightarrow 24 \text{ h}}] \times 100\%$. The pharmacokinetic parameters were analyzed statistically by ANOVA test using SPSS software (version 16.0; SPSS Inc., USA).

The C_{\max} and $AUC_{0\rightarrow 24}$ h of the SEDDS were significantly higher than those of the tablet and SD. The T_{\max} of the SEDDS was less than that of the tablet and SD. The relative bioavailability of rosuvastatin in SEDDS F-3 and SD was 168.09 and 134.67 in compare to tablet. This might be due to solubilization and droplet-size reduction produced by SEDDS. So SEDDS increased the oral bioavailability of rosuvastatin. It might be a promising approach for rapid onset and effective absorption with oral administration of rosuvastatin.

Table 3.23: Pharmacokinetic parameters and bioavailability of rosuvastatin after oral administration of SEDDS and SD

Parameters	ROS MP	ROS SEDDS F-3	SD ROS:POL 1:1
T_{\max} (h)	2.1 ± 0.51	1.5 ± 0.43	2.0 ± 0.43
C_{\max} (ng/ml)	70.00 ± 2.31	144 ± 1.43	90 ± 1.43
AUC_{0-24h} (ng h/ml)	465.56 ± 0.51	781.45 ± 0.51	626.45 ± 0.51
Fr (%)	100.00	168.09	134.67

Although the effect of SEDDS on the absorption of drug has not been clarified, and *in-vitro* evaluating methods are still in its infancy, the progress of SEDDS has been greatly advanced by the achievements involved in models simulating the release and absorption in the gastrointestinal tract (Charman *et al.*, 1992; Constantinides, 1995; Gursoy and Benita, 2004). However, further discussion on the relationship between bioavailability and droplet size is needed.

Chapter Four: Conclusion

4.0 Conclusion

About 40% of new drug candidates are poorly water soluble and oral delivery of these drugs is difficult because of their low bioavailability, high intra- and inter-subject variability and a lack of dose proportionality. It is one of the major challenges to synthesize any new molecule, which is pharmacologically active for the researchers and pharmaceutical companies. *In-vitro* dissolution study of commercially available three statins (atorvastatin, simvastatin and rosuvastatin) implies that they are poorly soluble and they are considered as BCS II class drug as they but highly permeable. These three drugs (atorvastatin, simvastatin and rosuvastatin) are suitable candidate to increase dissolution rate for higher bioavailability. High intra- and inter-subject variability of marketed tablets also proved it. SEDDS and SD of these three drugs were prepared and evaluated by *in-vitro*, *in-vivo* and *ex-vivo* methods. SEDDS formulations consist of lipids, surfactants and cosurfactants, which are emulsified by aqueous medium under gentle digestive motility in the gastrointestinal tract. SD is the molecular dispersion of drugs in different carrier. Both SEDDS and SD could increase the dissolution and permeability of drugs by in different ways. SEDDS significantly decrease droplet size and restrain the secretion of drug efflux transporter P-gp. The low bioavailability of atorvastatin, simvastatin and rosuvastatin drugs is produced by the poor solubility and extensive first-pass metabolism in the gut wall and liver. The use of SEDDS for the delivery of these drugs could improve its solubility and permeability through mucous membranes significantly. Solubility of these three drugs in various excipients was analysed. Pseudo-ternary phase diagrams composed of lipid–cosurfactant–surfactant–water were mapped; the microemulsion region in each diagram was plotted and compared. The morphology and the droplet size/ distribution of SEDDS were observed by Malvern particle size analyzer. Droplet size and distribution, and long-term stability were investigated in detail. Optimal formulations can become microemulsions when dispersed with medium. The average droplet size of the optimal formulation is within 120 nm and shows Gaussian distribution. The rate and amount of the release of drug from SEDDS capsules were more than those from the conventional tablets in 0.1M HCl. *In-vitro* assessment of SEDDS and SDs prove the higher dissolution rate; an oral bioavailability study in rabbits was also performed for only rosuvastatin SEDDS and

SD. Diffusion through cellulose tubing and permeability through chicken and rabbit intestinal sacs proved the higher transmission rate through these membrane. *In-vivo* performance of rosuvastatin was evaluated by using its pharmacodynamic effects (hypolipidemic activity of rosuvastatin). Rapid reduction in elevated total CH, levels in blood of SEDDS proves the effectiveness of SEDDS over conventional tablets. We found that SEDDS might have the potential to advance the oral bioavailability of poorly water statins. After oral administration of 6 mg kg⁻¹ rosuvastatin to 6 rabbits, the oral bioavailability of SEDDS and SDs was increased by 1.68 fold for SEDDS and 1.34 fold for SD when compared with that of the conventional tablets. SEDDS was found more effect than SDs. This study indicates that the potential use of SEDDS and SDs for the oral delivery of these statins can be an alternative to improve its systemic availability. The development of SEDDS and SDs is promising for improving the oral bioavailability of poorly soluble drugs.

5.0 References

- Alam AA, Masum MAA, Islam RB, Sharmin F, Islam SMA. Formulation of solid dispersion and surface solid dispersion of nifedipine: A comparative study. *African Journal of Pharmacy and Pharmacology*, 2013; 7(25); 1707-1718.
- Allam AN, Gamal SSE, Naggar VF. Bioavailability: A Pharmaceutical Review. *Journal of Noble Drug Delivery Technology*, 2011; 1(1): 80-96.
- Allen LV, Popovich NG, Ansel HC. *Ansel's pharmaceutical dosage forms and drug delivery systems*, 8th Edition, Lippincott Williams and Wilkins, Philadelphia, 2004; P 236.
- Ambike AA, Mahadik KR, Paradkar A. Spray dried amorphous solid dispersions of simvastatin; a low T_g drug: *In-vitro* and *in-vivo* evaluations. *Pharmaceutical Research*, 2005; 22: 990–998.
- Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutical drug classification: the correlation of *in-vitro* drug product dissolution and *in-vivo* bioavailability. *Pharmaceutical Research*, 1995; 12: 413-420.
- Amrutkar C, Salunkhe K, Sanjay Chaudhari S. Study on self nano emulsifying drug delivery system of poorly water soluble drug rosuvastatin calcium. *World Journal of Pharmaceutical Research*, 2014; 3(4):21-37.
- Anderson NH, Bauer M, Boussac N, Khan-Malek R, Munden P, Sardaro M. An evaluation of fit factors and dissolution efficiency for the comparison of *in-vitro* dissolution profiles. *Journal of Pharmaceutical and Biomedical Analysis*, 1998; 17(4-5): 811-822.
- Arambepola C, Farmer AJ, Perera R, Neil HA. Statin treatment for children and adolescents with heterozygous familial hypercholesterolaemia: A systematic review and meta-analysis. *Atherosclerosis*, 2007; 195(2): 339-347.
- Ashok R, Pradeep RV. Preparation and *in-vivo* evaluation of SMEDDS (Self-Microemulsifying Drug Delivery System) containing fenofibrate. *AAPS PharmSciTech*, 2007; 9: 44–52.
- Atef E, Belmonte A. Formulation and *in-vitro* and *in-vivo* characterization of phenytoin SEDDS. *European Journal of Pharmaceutical Sciences*, 2008; 35: 257-263.

References

- Bachhav YG, Patravale VB. SMEDDS of glyburide: formulation, *in-vitro* evaluation, and stability studies. *AAPS PharmSciTech*, 2009; 10(2): 482-487.
- Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C *et al.* Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomized trials of statins. *Lancet*, 2005; 366(9493):1267-1278.
- Blatter F, Adriaan P, Szelaglewlez M. Crystalline forms of rosuvastatin calcium salt. US Patent Application Publication (No. US 7,932,387 B2) Apr. 26, 2011, 1-7.
- Bobe KR, Subrahmanya CR, Suresh S, Gaikwad DT, Patil MD, Khade TS, Gavitre BB, Kulkarni VS, Gaikwadb UT. Formulation and evaluation of solid dispersion of atorvastatin with various carriers. *International Journal of Comprehensive Pharmacy*, 2011; 1 (02): 1-6.
- Boddupalli BM, Banda K, Ramani R, Anisetti RN, Kamal ZN, Goje *et al.* Preparation, characterization, and tableting of a solid dispersion of simvastatin with super disintegrants. *Journal of Pharmacy Research*, 2010; 3(11):2568-2570.
- Box GEP, Wilson KB. On the experimental attainment of optimum multifactorial conditions. *Journal of the Royal Statistical Society Series*. 1951; 13: 1–12.
- Boza A, De la Cruz Y, Jordan G, Jauregui-Haza U, Aleman A, Caraballo I. Statistical optimization of a sustained-release matrix tablet of lobenzarit disodium. *Drug Development and Industrial Pharmacy*, 2000; 26: 1303–1307.
- BP, British Pharmacopoeia, The Pharmaceutical Press, Her Majesty's Office, London, Vol. 1, 2014:45 -1296.
- Breitenbach J, Schrof W, Neuman J. Confocal raman-spectroscopy: analytical approach to solid dispersions and mapping of drugs. *Pharmaceutical Research*, 1999; 16(7): 1109-1113.
- Broman E, Khoo C, Taylor LS. A comparison of alternative polymer excipients and processing methods for making solid dispersions of a poorly water soluble drug. *International Journal of Pharmaceutics*, 2001; 222(1): 139-151.

References

- Bugay DE. Characterization of the solid-state: spectroscopic techniques. *Advanced Drug Delivery Reviews*, 2001; 48(1): 43-65.
- Cade D, Madit N. Liquid filling in hard gelatin capsules-preliminary steps. *Bulletin Technology, Gattefosse*. 1996; 15-20.
- Cannon JB, Long MA. Emulsions, microemulsions, and lipid-based drug delivery systems for drug solubilization and delivery-Part II: Oral applications, in Liu R., (ed) *water-insoluble drug formulation*. 2nd ed., CRC Press, Boca Raton, FL, 2008; pp. 227-253.
- Cao F, Guo j, Ping Q. The physicochemical characteristics of freeze-dried scutellarin-cyclodextrin tetracomponent complexes. *Drug Development and Industrial Pharmacy*, 2005; 31(8): 747–756.
- Charman SA, Charman WN, Rogge MC, Wilson TD, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutical evaluation of an investigational lipophilic compound, *Pharmaceutical Research*, 1992; 9: 83–87.
- Charman WN, Stella VJ. Estimating the maximal potential for intestinal lymphatic transport of lipophilic drugs molecules. *International Journal of Pharmaceutics*.1986; 34: 175-178.
- Chaturvedi V, Bhargava B. Health Care Delivery for Coronary Heart Disease in India- Where are we headed? *American Heart Hospital Journal*, 2007; 5: 32-37.
- Chauhan B. Preparation and evaluation of gliben clamidepolyglycolized glycerides solid dispersions with silicon dioxide by spray drying technique. *European Journal of Pharmaceutical Science*, 2005; 26: 219–230.
- Chaulang G, Patil K, Ghodke D, Khan S, Yeole P. Preparation and characterization of solid dispersion tablet of furosemide with crosspovidone. *Research Journal of Pharmacy and Technology*, 2008; 1(4): 386-389.
- Cherson R. Bioavailability, Bioequivalence and Drug Selection. *Basic Pharmacokinetics*, 1997; 8: 1-117.
- Chiba Y, Kohri N, Iseki K, Miyazaki K. Improvement of dissolution and bioavailability for mebendazole, an agent for human echinococcosis, by preparing solid dispersion with polyethylene glycol, *Chemical and Pharmaceutical Bulletin*, 1991; 39: 2158– 2160.

References

- Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion systems. *Journal of Pharmaceutical Science*, 1971; 60: 1281–1302.
- Choudharya A, Ranaa AC, Aggarwalb G, Kumara V, Zakira F. Development and characterization of an atorvastatin solid dispersion formulation using skimmed milk for improved oral bioavailability. *Acta Pharmaceutica Sinica B*, 2012; 2(4): 421–428.
- Cilurzo F, Minghetti P, Casiraghi A, Montanari L. Characterization of nifedipine solid dispersions. *International Journal of Pharmaceutics*, 2002; 242(1-2): 313-317.
- Cirri M, Mura P, Mora PC. Liquid spray formulations of xibornol by using self-microemulsifying drug delivery systems. *International Journal of Pharmaceutics*, 2007; 340(1-2): 84-91.
- Cole ET. Liquid-filled hard-gelatin capsules. *Pharmaceutical Technology*, 1989; 12: 124-140
- Collnot EM, Baldes C, Wempe MF, Kappl R, Huttermann J, Hayatt JA, *et al.* Mechanism of inhibition of p-glycoprotein mediated efflux by vitamin E TPGS: influence on ATPase activity and membrane fluidity. *Molecular pharmaceutics*, 2007; 4: 456-474.
- Constantinides PP. Lipid microemulsion for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharmaceutical Research*, 1995; 12: 1561–1572.
- Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates *in-vitro* and *in-vivo*, *International Journal of Pharmaceutics*, 2004; 278:119–131.
- Craig DQM. The mechanisms of drug release from solid dispersions in water-soluble polymers. *International Journal of Pharmaceutics*, 2002; 231: 131–144.
- Crison JR, Amidon GL. Method and formulation for increasing the bioavailability of poorly water-soluble drugs. US Patent No. 5,993,858, issued November 30, 1999.
- Date AA, Nagarsenker MS. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. *International Journal of Pharmaceutics*, 2007; 329(1-2): 166-72.

References

- De MP, Rahier H, Van MB. The use of modulated temperature differential scanning calorimetry for the characterisation of food systems. *International Journal of Pharmaceutics*, 1999; 192(1): 77-84.
- Dhirendra K, Lewis S, Udupa N, Atin K. Solid dispersions: a review. *Pakistan Journal Pharmaceutical Science*, 2009; 22(2): 234-246.
- Dias R, Sakhare S, Mali K. *In-vitro* Absorption Studies of Mucoadhesive Tablets of Acyclovir. *Indian Journal of Pharmacy Education and Research*, 2010; 44(2): 183-188.
- Drooge JV. Characterization of the Mode of Incorporation of Lipophilic Compounds in Solid Dispersions at the Nanoscale Using Fluorescence Resonance Energy Transfer (FRET). *Journal of Macromolecular Rapid Communication*, 2006; 27: 1149–1155.
- Elson CE, Tropical oils: nutritional and scientific issues, *Critical Reviews in Food Science and Nutrition*. 31 (1992) 79–102.
- EMEA. European Agency for the Evaluation of Medicinal Products: Notes for Guidance on the Investigation of Bioavailability and Bioequivalence. 2001; Available at <http://www.emea.europa.eu/pdfs/human./ewp/140198en.pdf>.
- Enas EA, Chacko V, Pazhoor SG, Chennikkara H, Devarapalli HP. Dyslipidemia in South Asian patients. *Current Atherosclerosis Reports*, 2007; 9(5): 367-374.
- Fatima S, Usman S, Muhammad IN. Statistical evaluation of *in-vitro* dissolution profiles of different brands of simvastatin 20 mg tablets available in local market of karachi. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 5(3): 622-626.
- Fattah MA, Bhargava HN. Preparation and *in-vitro* evaluation of solid dispersions of halofantrine. *International Journal of Pharmaceutics*, 2002; 235(1-2): 17–33.
- FDA Food and Drug Administration, Center for Drug Evaluation and Research Guidance for industry: Dissolution testing of immediate release solid oral dosage forms, 1997.
- FDA, Food and Drug Administration, Guidance for industry, Waiver of *In-vivo* Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms based on a Biopharmaceutics Classification System, 2000.

References

- FDA, Food and Drug Administration. Guidance for industry, Bioavailability and bioequivalence studies for orally administered drug products - General considerations, 2003.
- Ferrec E, Christophe C, Artussen P, Brayden D, Fabre G, Gires P, *et al.* *In-vitro* models of the intestinal barriers. *ATLA* 1999; 29: 649.
- Forster A, Hempenstall J, Rades T. Characterization of glass solutions of poorly water-soluble drugs produced by melt extrusion with hydrophilic amorphous polymers. *Journal of Pharmacy and Pharmacology*, 2001; 53(3): 303-315.
- Garcia-Zubiri IX. Thermal stability of solid dispersions of naphthalene derivatives with [beta]-cyclodextrin and [beta]-cyclodextrin polymers. *Thermal Analysis of Pharmaceutical*, 2006; 444: 57–64.
- Geethalakshmi A, Divya Mahalingan VK, Rao N, Krishna KH. Enhancement of solubility and dissolution rate of atorvastatin calcium by co-solvent evaporation. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 2(5): 3790-3806.
- Ginski MG, Polli JE. Prediction of dissolution–absorption relationship from a dissolution/Caco-2 system. *International Journal of Pharmaceutics*, 1999;177: 117.
- Giri TK, Alexander A, Tripathi DK. Physicochemical classification and formulation development of solid dispersion of poorly water soluble drugs. *International Journal of Pharmaceutical and Biological Archives*, 2010; 1(4): 309-324.
- Goldberg AH, Galbaldi M, Kaning KL. Increasing in dissolution rates and gastrointestinal absorption via solid solution and eutectic mixture experimental evaluation of griseofulvin-succinic acid solution. *Journal of Pharmaceutical Sciences*, 1966; 55: 4870-492.
- Goldberg AH, Gibaldi M, Kanig JL. Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures I. Theoretical considerations and discussion of the literature. *Journal of Pharmaceutical Science*, 1965; 54: 1145-1148.
- Griffin BT, O’Driscoll CM. A comparison of intestinal lymphatic transport and systemic bioavailability of saquinavir from three lipid based formulations in the anaesthetized rat model. *Journal of Pharmacy and Pharmacology*, 2006; 58: 917-925

References

- Gubbi SR, Jarag R. Formulation and characterization of atorvastatin calcium liquisolid compacts. *Asian Journal of Pharmaceutical Sciences*, 2010; 5(2): 50-60.
- Gupta P, Kakumanu VK, Bansal AK. Stability and solubility of celecoxib-PVP amorphous dispersions: a molecular perspective. *Pharmaceutical Research*, 2004; 21(10): 1762–1769.
- Gursoy RN, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine and Pharmacotherapy*. 2004; 58: 173–182.
- Hasegawa S. Effects of water content in physical mixture and heating temperature on crystallinity of troglitazone-PVP K30 solid dispersions prepared by closed melting method. *International Journal of Pharmaceutics*, 2005; 302: 103–112.
- Hauss DJ, Fogal SE, Ficorilli JV, Price CA, Roy T, Jayaraj AA *et al.* Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water soluble LTB4 inhibitor. *Journal of Pharmaceutical Sciences*, 1998; 87: 164-169.
- Higuchi T: Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of Pharmaceutical Sciences*, 1961; 50: 847-875.
- Hixon AW and Crowell JH: Dependence of reaction velocity upon surface and agitation. *Industrial and Engineering Chemistry Research*, 1931; 23: 923-931.
- Hörter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced Drug Delivery Reviews*, 1997; 25:3–14.
- Hunter J, Hirst BH. Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral absorption, *Advanced Drug Delivery Review*. 1997; 25: 129–157.
- Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Slick HE, Grove J. MDCK cells: a tool for membrane permeability screening. *Journal of Pharmaceutical Science*. 1999; 88: 28.
- Islam S, Dey LR, Shahriar M, Dewan I, Islam SMA. Enhancement of dissolution rate of gliclazide using solid dispersions i: characterization and dissolution rate comparison. *Bangladesh Pharmaceutical Journal*, 16(1): 45-52, 2013.

References

- Jagadeesan R, Radhakrishnan M. Novel approaches in the preparation of solid dispersion on solubility: a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 5(3): 1000-1004.
- Jatwani S, Rana AC, Singh G, Aggarwa G. Solubility and Dissolution enhancement of simvastatin using synergistic effect of hydrophilic carriers *Der Pharmacia Lettre*, 2011; 3(6): 280-293.
- Joshi M, Pathak S, Sharma S, Patravale V. Solid microemulsion preconcentrate (Nanosorb) of artemether for effective treatment of malaria. *International Journal of Pharmaceutics*, 2008; 362(1-2): 172-8.
- Kadu PJ, Kushare SS, Thacker DD, Gattani SG. Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS, *Pharmaceutical Development and Technology*, 2011; 16(1): 65-74.
- Kale V, Kasliwal RH, Avari JG, Attempt to design continuous dissolution–absorption system using everted intestine segment for *in-vitro* absorption studies of slow drug release formulations. *Dissolution Technology*. 2007; 5:31-36.
- Kalia A, Poddar M. Solid Dispersions: an approach towards enhancing dissolution rate. *International Journal of Pharmacy and Pharmaceutical Science*, 2011; 3: 9-19.
- Kalyanwat R, Patel S. Solid dispersion: a method for enhancing drug dissolution. *International Journal of Drug Formulation Research*, 2010; 1(3):1-14.
- Kamble PM, Shaikh KS, Chaudhari PD. Application of liquisolid technology for enhancing solubility and dissolution of rosuvastatin. *Advanced Pharmaceutical Bulletin*, 2014; 4(2):197-204.
- Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, *et al.* Development of self-microemulsifying drug delivery system for oral availability enhancement of simvastatin in beagle dogs, *International Journal of Pharmaceutics*, 2005;. 274:65–73.
- Kaplan SA. Biopharmaceutical considerations in drug formulation design and evaluation. *Drug Metabolism Reviews*, 1972; 1:15–34.
- Kapoor B, Kaur R, Kour S, Behl H, Kour S. Solid Dispersion: an evolutionary approach for solubility enhancement of poorly water soluble drugs. *International Journal of Recent Advances in Pharmaceutical Research*, 2012; 2: 1-16.

References

- Kapure VJ, Pande VV, Deshmukh PK. Dissolution enhancement of rosuvastatin calcium by liquisolid compact technique. *Journal of Pharmaceutics*, 2013; 2013:1-9.
- Karanth H, Shenoy VS, Murthy RR. Industrially feasible alternative approaches in the manufacture in the solid dispersion: a technical report. *AAPS Sci Tech*, 2006; 7(4): E1-E8.
- Karavas E. Application of PVP/HPMC miscible blends with enhanced mucoadhesive properties for adjusting drug release in predictable pulsatile chronotherapeutics. *European Journal of Biopharmaceutics*, 2006; 64: 115–126.
- Karim A, Gokhale R, Cole M, Sherman J, Yeramian P, Bryant M *et al.* HIV protease inhibitor SC-52151: a novel method of optimizing bioavailability profile via a microemulsion drug delivery system. *Pharmaceutical Research*, 1994; 11: S368.
- Kasim NA, Whitehouse M, Amachandran C, Bermejo M, Lennernas H, Hussain AS, *et al.* Molecular properties of WHO essential drugs and provisional biopharmaceutical classification, *Molecular Pharmaceutics*, 2004; 1(1): 85-96.
- Katteboina S, Chandrasekhar P, Balaji S. Approaches for the development of solid self emulsifying drug delivery systems and dosage forms. *Asian Journal of Pharmaceutical Sciences*, 2009; 4(4): 240-253.
- Kaur J, Aggarwal G, Singh G, Rana AC. Improvement of drug solubility using solid dispersion. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2012; 4(2): 50.
- Kaushal AM, Guptam P, Bansal AK. Amorphous drug delivery systems: molecular aspects, design, and performance. *Critical Reviews in Therapeutic Drug Carrier System*, 2004; 21(3): 133-193.
- Kerc J, Srcic S. Thermal analysis of glassy pharmaceuticals. *Thermochimica Acta*, 1995; 248: 81-95.
- Khaled KA. Formulation and evaluation of hydrochlorothiazide liquisolid tablets. *Saudi Pharmaceutical Journal*, 1998; 6(1): 39-46.
- Kim HJ, Yoon KA, Hahn M, Park ES, Chi SC. Preparation and *in-vitro* evaluation of self-microemulsifying drug delivery systems containing idebenone. *Drug Development and Industrial Pharmacy*, 2000; 26: 523-529.

References

- Kimura M, Shizuki M, Miyoshi K, Sakai T, Hidaka H, Takamura H *et al.* Relationship between the molecular structures and emulsification properties of edible oils. *Journal of Plant Biochemistry and Biotechnology*, 1994; 58: 1258-1261.
- Koga k, Kusawake Y, Sugioka YLN, Takada K. Enhancing mechanism of labrasol on intestinal membrane permeability of the hydrophilic drug gentamicin sulfate. *European Journal of Pharmaceutics and Biopharmaceutics*. 2006; 64: 82-91.
- Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q₁₀: formulation development and bioavailability assessment. *International Journal of Pharmaceutics*, 2001; 212: 233-46.
- Korsmeyers RW, Gummy R, Doelker EM, Buri P and Peppas NA: Mechanism of solute release from porous hydrophilic polymers, *International Journal of Pharmaceutics*, 1983; 15: 25-35.
- Kulkarni NS, Ranpise NS, Govind Mohan G. Development and evaluation of solid self nano-emulsifying formulation of rosuvastatin calcium for improved bioavailability. *Tropical Journal of Pharmaceutical Research*, 2015; 14 (4): 575-582.
- Kumar A, Sharma S, Kamble R. Self-emulsifying drug delivery system (SEDDS): Future Aspects. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010; 2: 7-13.
- Kumar RS. Design, development and evaluation of fenofibrate and rosuvastatin nanodroplets. *European Scientific Journal* December, 2013;4:606-617.
- Kumar SK, Elango K, Deattu N, Akilandeshwari A. Enhancement of dissolution rate and formulation development of rosuvastatin calcium solid dispersion tablets employing starch phosphate as carrier *International Journal of Pharmacy Research & Science*, 2014, 02(2), 164-170.
- Kyatanwar AU, Gajbhiye ND, Jadhav KR, Kadam VJ. Solid self-emulsifying drug delivery systems: Review. *Journal of Pharmacy Research*, 2010; 3(4): 877-882.
- Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery system. *Advanced Drug Delivery Reviews*, 2000; 45: 89-121.
- Leppert PS, Fix JA. Use of averted intestinal rings for *in-vitro* examination of oral absorption potential. *Journal of Pharmaceutical Sciences*, 1994; 83(7): 976-81.

References

- Leuner C, Dressman JB. Improving drug solubility for oral delivery using solid dispersions, *European Journal of Pharmaceutical Sciences*. 50 (2000) 47–60.
- Levet-Trafit B, Gruyer MS, Marjanovic M, Chou RC. Estimation of oral drug absorption in man based on intestine permeability in rats. *Life Sciences*, 1996; 58(24): PL359-63
- Li J, Guo Y, Zografi G. The solid-state stability of amorphous quinapril in the presence of beta-cyclodextrins. *Journal of Pharmaceutical Science*, 2002; 91(1): 229-243.
- Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 2004; 58: 265-78.
- Lloyd GR. A calorimetric investigation into the interaction between paracetamol and polyethylene glycol 4000 in physical mixes and solid dispersions. *European Journal of Biopharmaceutics*, 1999; 48: 59–65.
- Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J *et al.* Diagnosis, prevention, and management of statin adverse effects and intolerance: proceedings of a Canadian Working Group Consensus Conference. *Canadian Journal of Cardiology*, 2011; 27(5): 635-662.
- Masum MAA, Sharmin F, Islam RB, Islam MA, Islam SMA. Application of solid dispersion technique in improving solubility of ibuprofen by poloxamers. *Journal of Pharmacy Research*, 2013;1(5); 498-505.
- Mei X, Etzler FM, Wang Z. Use of texture analysis to study hydrophilic solvent effects on the mechanical properties of hard gelatin capsules. *International Journal of Pharmaceutics*. 2006; 324: 128–135.
- Nainwal P, Sinha P, Singh A, Nanda D, Jain DA. A comparative solubility enhancement study of rosuvastatin using solubilization techniques *International Journal of Applied Biology and Pharmaceutical Technology* , 2011; 2(4): 1-14.
- Narasaiah VL, Jimidi B, Goli V. Enhancement of dissolution rate of atorvastatin calcium using solid dispersions by dropping method. *International Journal of PharmTech Research*, 2011; 3(2): 652-659.

References

- Narasaiah VL, Reddy KB, Kishore K, Kumar MR, Rao PS. Reddy VB. Improved dissolution rate of atorvastatin calcium using solid dispersions with PEG-4000. *Journal of Chemical and Pharmaceutical Research*, 2010b; 2(3):304-311.
- Narasaiah VL, Reddy KB, Kishore K, Kumar MR, Rao PS. ReddyVB. Enhanced Dissolution Rate of Atorvastatin Calcium using Solid Dispersion with PEG 6000 by Dropping Method. *Journal of Pharmaceutical Science & Research*, 2010a;2 (8): 484-491.
- Nazzal S, Khan MA. Controlled release of a self-emulsifying formulation from a tablet dosage form: Stability assessment and optimization of some processing parameters. *International Journal of Pharmaceutics*, 2006; 315: 110–121.
- Nipun TS, Islam SMA. SEDDS of gliclazide: Preparation and characterization by *in-vitro*, *ex-vivo* and *in-vivo* techniques *Saudi Pharmaceutical Journal* 2014; 22: 343–348.
- Nokhodchi A, Talari R, Valizadeh H, Jalali MB. An investigation on the solid dispersions of chlordiazepoxide. *International Journal of Biomedical and Science*, 2007; 3(3): 210-216.
- O'Driscoll CM. Mini review: lipid-based formulations for intestinal lymphatic delivery. *European Journal of Pharmaceutical Sciences*. 2002; 15: 405-415.
- Oishi TS, Nimmi I, Islam SMA. Comparative *in-vitro* bioequivalence analysis of some generic tablets of atorvastatin, a bcs class II compound. *Bangladesh Pharmaceutical Journal*, 2011; 14 (1): 61-66.
- Parmar N, Bagda A , Patel M, Patel S. Formulation strategy for dissolution enhancement of simvastatin, *International Journal of Pharmaceutical Science and Research*, 2012; 3(10): 3817-3822.
- Patel D, Sawant KK. Oral bioavailability enhancement of acyclovir by self-microemulsifying drug delivery systems (SMEDDS). *Drug development and industrial pharmacy*, 2007; 33(12): 1318-26.
- Patel PA, Chaulang GM, Akolkotkar A, Mutha SS, Hardikar SR, Bhosale AV. Self emulsifying drug delivery system: A Review. *Research Journal of Pharmacy and Technology*, 2008; 1(4): 313-323.

References

- Patel S, Jani G, Patel M. Development of self-emulsifying formulation of ionizable water insoluble BCS class-II drug: rosuvastatin calcium. *Inventi Rapid: Pharm Tech* Vol. 2013, Issue 2, 1-6.
- Patil P, Joshi P, Paradkar A. Effect of formulation variables on preparation & evaluation of Gelled SEDDS of Ketoprofen. *AAPS Pharmaceutical Science and Technology*, 2004; 5(3): 34-42.
- Patil P, Patil V, Paradkar A . Formulation of a self-emulsifying system for oral delivery of simvastatin: In vitro and in vivo evaluation. *Acta Pharm*, 2007; 57:111–122.
- Paul PK, Dey LR, Shahriar M, Dewan I, Islam SMA, Comparative *in-vitro* dissolution study of spironolactone in binary and tertiary solid dispersion: Model dependant and independent approaches. *Journal of Drug Delivery & Therapeutics*, 2012; 2(4): 73-80.
- Peppas NA: Analysis of Fickian and non-Fickian drug release from polymers. *Pharmaceutica Acta Helvetiae*, 1985; 60:110 -111.
- Pikal MJ, Lukes AL, Lang JE, Gaines K. Quantitative crystallinity determinations for beta-lactum antibiotics by solution calorimetry: correlations with stability. *Journal of pharmaceutical Science*, 1978; 67(6): 767-73.
- Pokharkar VB. Development, characterization and stabilization of amorphous form of a low T_g drug. *Journal of Powder Technology*, 2006; 167: 20–25.
- Popy FA, Dewan I, Parvin MS, Islam SMA. Evaluation of *in-Vitro* equivalence for tablets containing poorly water soluble compound atorvastatin, *Dissolution Technology*, 2012; 19(4): 30-33.
- Porter CJ, Charman WN. *In-vitro* assessment of oral lipid based formulations. *Advanced Drug Delivery Review*, 2001; 50(1): 127-47.
- Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Advanced Drug Delivery Reviews*, 2008; 60(6): 625-37.
- Pouton CW. Lipid formulations for oral administration of drugs: Non-emulsifying, self emulsifying and self micro-emulsifying drug delivery system. *European Journal of Pharmaceutical Sciences*, 2000; 11(2): 593-598.

References

- Pouton CW. Self-emulsifying drug delivery systems: Assessment of the efficiency of emulsification. *International Journal of Pharmaceutics*, 1985; 27(2-3): 335-348.
- Rane Y, Mashru R, Sankalia M, Sankalia J. Effect of hydrophilic swellable polymers on dissolution enhancement of carbamazepine solid dispersions studied using response surface methodology. *AAPS PharmSciTech*, 2007; 8(2): E1-E11.
- Rao M, Mandage Y, Thanki K, Bhise S, Dissolution improvement of simvastatin by surface solid dispersion. *Technology Dissolution*, 2010; 17(2): 27-34.
- Rawat M, Saraf S, Saraf S. Influence of selected formulation variables on the preparation of enzyme-entrapped eudragit S100 microspheres. *AAPS PharmSciTech*: 2007; 8: E116. <http://dx.doi.org/10.1208/pt0804116>
- Rege BD, Kao JP, Polli JE. Effects of nonionic surfactants on membrane transport in caco-2 cell monolayers, *European Journal of Pharmaceutical Sciences*, 2002; 16: 237-246.
- Revathi S, Raju D. Self emulsifying drug delivery system: A Review. *World journal of Pharmacy and Pharmaceutical Sciences*, 2012; 2(1): 89-107.
- Rogers TL, Hu JH, Brown J, Young T, Johnston KP, Williams RO. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray freezing into liquid. *European Journal of Pharmaceutics and Biopharmaceutics*, 2002; 54(3): 271-280.
- Rokad V, Nagda C, Nagda D. Design and evaluation of solid self-emulsifying drug delivery system of rosuvastatin calcium. *Journal of Young Pharmacists*, 2014; 6(3): 37.
- Rupal J, Kausha J, Mallikarjuna SC, Dipti P. Preparation and evaluation of solid dispersions of aceclofenac. *International Journal of Pharmaceutical Science and Drug Research*, 2009;1:32-35.
- Sánchez-Lafuente C, Furlanetto S, Fernández-Arévalo M, Alvarez-Fuentes J, Rabasco AM, Faucci MT, Pinzauti S, Mura P. Didanosine extended-release matrix tablets: optimization of formulation variables using statistical experimental design. *International Journal of Pharmaceutics*. 2002; 237: 107–118.

References

- Sapra K, Sapra A, Singha SK, Kakkar S. Self Emulsifying Drug Delivery System: A Tool in Solubility Enhancement of Poorly Soluble Drugs. *Indo Global Journal of Pharmaceutical Sciences*. 2012; 2(3): 313-332.
- Sayyad A, Sawant SD. Techniques of solubility enhancement of poorly soluble drug with special emphasis on solid dispersion. *Journal of Pharmacy Research*, 2010; 3: 2494-2501.
- Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundamental and Clinical Pharmacology*, 2004; 19: 117-125.
- Sebhatu T, Angberg M, Ahlneck C. Assessment of the degree of disorder in crystalline solids by isothermal microcalorimetry. *International Journal of Pharmaceutics*, 1994; 104: 135-144.
- Sekiguchi K, Obi N. Studies on absorption of eutectic mixture I. A comparison of sulfathiazole and that of ordinary sulfathiazole in man. *Chemical and Pharmaceutical Bulletin*, 1961; 9: 866-872.
- Sekiguchi K, Obi N. Studies on absorption of eutectic mixture II. Absorption of Eutectic Mixture II. Absorption of fused conglomerates of chloramphenicol and urea in rabbits. *Chemical and Pharmaceutical Bulletin*, 1964; 12: 134-144
- Serajuddin AT, Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. *Journal of Pharmaceutical Sciences*, 1999; 88: 1058–1066.
- Serajuddin ATM, Sheen PC. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water soluble drug from solid dispersions. *Journal of Pharmaceutical Sciences*, 1988; 77: 414-417.
- Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A *et al.* Formulation development and optimization using nanoemulsion technique: A Technical Note. *AAPS Pharmaceutical Science and Technology*, 2007; 8(2): E1-E6.
- Shah NH, Carvajal MT, Patel CI, Infeld NH, Malick AW, Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving *in-vitro* dissolution and oral absorption of lipophilic drugs, *International Journal of Pharmaceutics*, 1994; 106:15–23.

References

- Shah Y, Iqbal Z, Ahmad L, Khan A, Khan, MI. Shabnam Nazir, Fazli Nasir. Simultaneous determination of rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detection: Method development, validation and optimization of various experimental parameters. *Journal of Chromatography B*, 2011; 879: 557–563.
- Sharma B, Saini V, Sharma A. Preparation, Characterization and *In-Vitro* Evaluation of Atorvastatin Calcium Solid Dispersions with Various Hydrophilic Polymers and Its FDT Formulation. *Current Pharma Research*, 2012. 2(4), 620-630.
- Sharma DK, Joshi SB. Solubility enhancement strategies for poorly water soluble drugs in solid dispersion. *Asian Journal of Pharmaceutics*, 2007; 1: 7-8.
- Sharma M, Garg R, Gupta GD. Formulation and evaluation of solid dispersion of atorvastatin calcium. *Journal of Pharmaceutical and Scientific Innovation*, 2013; 2(4): 73-81.
- Shen H, Zhong M. Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin. *Journal of Pharmacy and Pharmacology*. 2006; 58:1183–1191.
- Sheth NS, Mistry RB. A Review: Self Emulsifying Drug Delivery System. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 3(2): 23-28.
- Shin SC, Cho CW. Physicochemical characterizations of piroxicam-poloxamer solid dispersion. *Pharmaceutical Development and Technology*, 1997; 2(4): 403-407.
- Shukla JB, Koli AR, Ranch KM, Parikh RK. Self microemulsifying drug delivery system. *Pharma Science Monitor*, 2010; 1(2):13-33.
- Simonelli AP, Mehta SC, Higuchi WI. Dissolution rates of high energy polyvinylpyrrolidone (PVP)-sulfathiazole co precipitates. *Journal of Pharmaceutical Sciences*, 1969; 58(5): 538-549.
- Singh B, Kapil R, Nandi M, Ahuja N. Developing Oral Delivery Systems using Formulation by Design (FbD): Vital Precepts, Retrospect and Prospects. *Expert Opinion on Drug Delivery*, 2011; 8: 1341–1360.
- Singh SK, Dodge J, Durrani MJ, Khan MA. Optimization and characterization of controlled release pellets coated with experimental latex: I. Anionic drug. *International Journal of Pharmaceutics*, 1995; 125: 243–255.

References

- Sinha S, Ali M, Baboota S, Ahuja A, Kumar A, Ali J. Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir. *American Association of Pharmaceutical Scientist Pharm Sci Tech*, 2010; 11(2): 518–527.
- Subramanian N, Ray S, Ghosal SK, Bhadra R, Moulik SP. Formulation Design of Self- microemulsifying Drug Delivery Systems for Improved Oral Bioavailability of Celecoxib. *Biological and Pharmaceutical Bulletin*, 2004; 27: 1993–1999.
- Sukanya M, Kishore VS Design and development of solid dispersions of simvastatin for enhancing the solubility. *American Journal of PharmTech Research*, 2012; 2(4): 733-740.
- Sunita SS, Manisha VP, Fatima IM, Amol SS. Durgacharan AB. D’Souza JI. Physicochemical characterization and solubility enhancement of simvastatin using solid dispersion technology *World Journal of Pharmaceutical Research*, 2012; 1(1): 297-304.
- Swathi T, Krishna MV, Kumar DS, Krishnavevi J. Enhancement of solubility and dissolution rate of rosuvastatin by solid dispersion technique. *Journal of Pharmaceutical and Scientific Innovation*, 2013; 2(6): 36-40.
- Tanaka N, Imai K, Okimoto K, Jeda SR, Ibuki YT, Higaki K, *et al.*,. Developments of novel sustain –release system disintegration controlled matrix tablet (DCMT) with solid dispersion granules of nifedipine (II): *In- vivo* evaluation. *Journal of Controlled Release*, 2006; 122:51-56.
- Tanaka N. Development of novel sustained release system, disintegration-controlled matrix tablet (DCMT) with solid dispersion granules of nifedipine. *Journal of Control Release*, 2005; 108: 386–395.
- Tiwari R, Tiwari G, Srivastava B, Rai AK. Solid dispersions: an overview to modify bioavailability of poorly water soluble drugs. *International Journal of PharmTech Research*, 2009; 1(4): 1338-1349.
- Tukker JJ, *In-vitro* methods for the assessment of permeability. In: Dressman, J.B., Lennernas, H. (Eds.), *Oral Drug Absorption*. Marcel Dekker, New York, 2000; p. 51.
- Urbanetz NA. Stabilization of solid dispersions of nimodipine and polyethylene glycol 2000. *European Journal Pharmaceutical Science*, 2006; 28: 67–76.

References

- USP. US Pharmacopoeia National Formulary, USP 35/NF 30, United States Pharmacopoeia Convention Inc., Rockville, MD, 2012: 182-1883.
- Van D. Characterization of the molecular distribution of drugs in glassy solid dispersions at the nano-meter scale, using differential scanning calorimetry and gravimetric water vapour sorption techniques. *International Journal of Pharmaceutical*, 2006; 310: 220–229.
- Vanden MG. Evaluation of Inutec SP1 as a new carrier in the formulation of solid dispersions for poorly soluble drugs. *International Journal of Pharmaceutics*, 2006; 3(16): 1–6.
- Vasanthavada M, Tong WQ, Joshi Y, Kislalioglu. Phase behavior of amorphous molecular dispersions I: determination of the degree and mechanism of solid solid solubility. *Pharmaceutical Research*, 2004; 21(9): 1598-1606.
- Vilas PC, Nayan GA, Bhushan RR, Sunil PP. A Review on self micro-emulsifying drug delivery system. *International Journal of Pharmaceutical Sciences*, 2013; 4(1): 3628-3648.
- Vilhelmsen T. Effect of a melt agglomeration process on agglomerates containing solid dispersions. *International Journal of Pharmaceutical*, 2005; 303: 132–142.
- Vogel HG, Vogel WH, *Drug Discovery and Evaluation: Pharmacological Assays*, Springer, Verlag, Berlin 1997, pp. 604–611.
- Wagner JG: Interpretation of present dissolved-time plots derived from *in-vitro* testing of conventional tablets and capsules. *Journal Pharmaceutical Science*, 1969; 58: 1253 – 1257.
- Wankhade V, Tapar K, Pande S, Bobade N. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for Gliclazide. *Der Pharmacia Lettre*, 2010; 2(4): 132-143
- WHO Expert Committee on Specifications for Pharmaceutical Preparations. WHO Technical Report Series, No. 937, Annex 8. Proposal to waive *in-vivo* bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms; World Health Organization: Geneva, Switzerland, 2006.
- WHO List of Essential Drugs, 12th ed., revised April 2002. (<http://www.who.int/medicines/organization/par/edl/eml2002core.pdf>).

References

- Wierzbicki, Anthony S. New lipid-lowering agents. *Expert Opinion on Emerging Drugs*, 2003; 8(2): 365-376.
- Won DH. Improved physicochemical characteristics of felodipine solid dispersion particles by supercritical anti-solvent precipitation process. *International Journal of Pharmaceutical*, 2005; 301: 199–208.
- Yoshihashi Y. Estimation of physical stability of amorphous solid dispersion using differential scanning calorimetry. *Journal of Thermal Analysis Calorimetric*, 2006; 85: 689–692.