IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

TO FORMULATE SILVER NANOPARTICLES OF DICLOFENAC SODIUM ALONGWITH POLYHERBALS *PLUCHEA LANCEOLATA*, *VITEX NIGUNDO* AND CURCUMIN AND EVALUATE ANTI-INFLAMMATORY ACTIVITY BY TOPICAL APPLICATION.

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ABSTRACT

Current review on In situ gelling systems includes polymeric formulations that are in solution forms before entering the body, but change to gel forms under the physiological conditions. Eye is the most sensitive organ of the body. Designing of ocular drug delivery system is the most challenging field for pharmaceutical scientists. Topical administration of ophthalmic drugs is used to alleviate the symptoms and signs caused by ocular surface inflammatory disorders, to treat infections, for glaucoma or intraocular inflammation. The objective of ourwork is to formulate an ocular delivery system of Ciprofloxacin. Due to its elastic properties, in-situ gels resist the ocular drainage of drug leading to longer contact times with ocular surface. The solution-gel transition depends on one or combination of different stimuli, like ph change, temperature modulation, solvent exchange, ultra violet irradiation and the presence of specific ions or molecules. Drug delivery systems having such properties can be widely used for sustained vehicle preparations of the bioactive molecules. Mainly *In situ gel*s are administered by oral, ocular, rectal, vaginal, injectable, and intraperitoneal routes. The *In situ gel* forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems.

Keywords: Gels, in-situ gel, ophthalmic drugs, glaucoma, bioactive molecules, sustained release, polymers, and drug delivery systems.

INTRODUCTION

The oral route is most preferred for chronic drug therapy in human beings. Probably at least 90% of all drugs are administered by oral route. Oral delivery of 50% of the drug compounds is facing problems because of the high lipophilicity (Low hydrophilicity) and low permeability of the drug itself.

The solubility issues complicating the delivery of new drugs, and also affects the delivery of many existing drugs. Relative to highly soluble compounds, low drug solubility often manifests itself in a host of in vivo consequences, including decreased bioavailability, increased chance of food effect, more frequent incomplete release from the dosage form and higher inter-patient variability.

Poorly soluble compounds also present many in vitro formulation obstacles, such as severely limited choices of delivery technologies and increasingly complex dissolution testing with limited or poor correlation to the in vivo absorption.

Poor oral bioavailability has the consequence of more variability and poorly controlled plasma concentration and drug effects to the patients. In recent years, much attention has been focused on lipid based formulations for delivering,

1.2 Biopharmaceutics Classification System (BCS) class II and Biopharmaceutics Classification System (BCS) class IV drug candidates which suffer limited oral bioavailability, high intra- and intersubject variability and lack of dose proportionality (Gursoy and Benita, 2004).

Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution (Amidon et al., 1995). The prospects in delivering poorly soluble drugs will grow in significance in the coming years as innovator companies rely upon NCEs for a larger share of the revenue within the pharmaceutical market.

Poorly soluble compounds also present poor correlation fo years as innovator companies rely upon NCEs for a larger share of the revenue within the pharmaceutical market. Poorly soluble compounds also present poor correlation for in-vitro studies and the in-vivo absorption.

These in vivo and in vitro characteristics and the difficulties in achieving predictable and reproducible in vivo / in vitro correlations are often sufficiently formidable to halt development on many newly synthesized compounds due to solubility issues.

Of equal challenges which the development candidates; despite possessing ideal pharmacological characteristics are to be left aside due to insufficient oral bioavailability. Poor oral bioavailability has the consequence of more variable and poorly controlled plasma concentration and drug effects.

Biopharmaceutical Classification System For the effective formulation and to sort out the drug related problem, one should have to be very familiar with the Biopharmaceutical Classification System (BCS). The introduction of the Biopharmaceutical Classification System (BCS) in FDA guidelines represents a major step forward in the regulation of oral drug products.

The guidelines tried to classify drug substances into four categories according to their solubility and permeability properties given in

Table 1: Biopharmaceutical Classification System of Drugs

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

Bioavailability Enhancement Poor bioavailability by the per-oral route can be due to poor solubility, degradation in GI lumen, poor membrane permeation, premucosal clearance and presystemic elimination.

Any of the approaches, which can alter these characteristics, should help in improving the bioavailability of the drugs.

1.3 Generally, four major approaches are followed for overcoming the poor bioavailability.

The Formulations approach It involves the modification of the formulation, manufacturing process or the physiochemical properties of the drug without changing the chemical structure. The dissolution rate, solubility and/or permeability are generally modified by this method.

The Chemical approach It involves the modification of chemical substituents groups, the physiochemical properties or prodrug approaches, which leads to increase drug solubility and permeability

The Pharmacokinetic approach It involves the modification of chemical structure, drug combinations, dose and regiments to alter the pharmacokinetic behaviour of the drug.

The Biologic approach It involves the change in the route and rate of administration of the drug to the human tissue. This can be achieved by many ways, one of them is the simultaneous administration of the two or more drug which alter the human physiology by many ways, like Basic Metabolic Rate (BMR) changing, enzymatic induction or inhibition etc which ultimately change the pharmacokinetics of the desired administered drug.

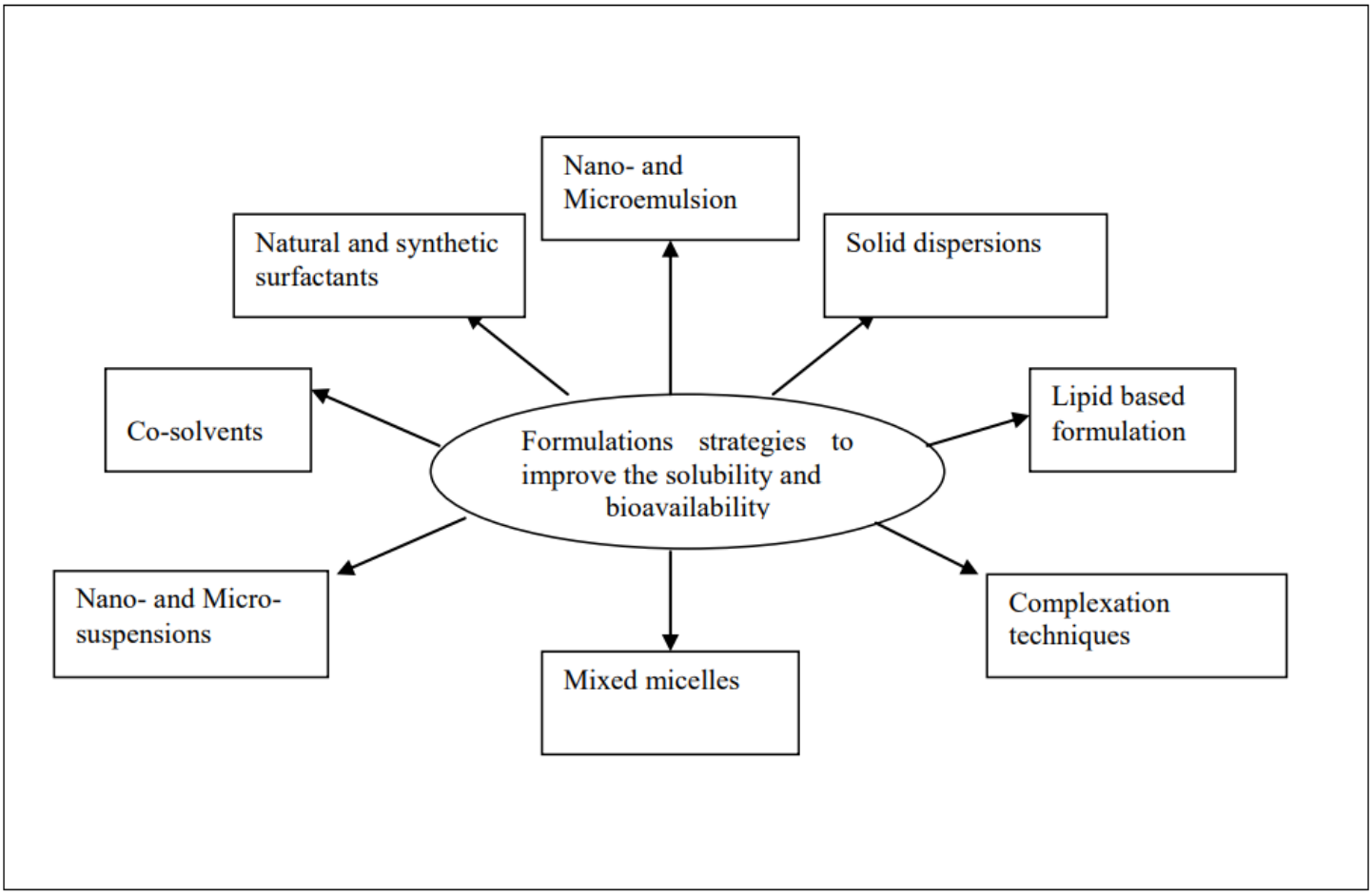

Figure 1: Formulation strategies to improve solubility and bioavailability of the drug substances

1.4 Microemulsion One of the promising technologies is lipid based nanoemulsion or microemulsion drug delivery system, which is being applied to enhance the oral bioavailability of the poorly soluble drugs. It was Hoar and Schulman who generated a clear single-phase solution by titrating a milky emulsion with medium chain alcohol such as hexanol andtherefore introduced the microemulsion concept as early as 1940s.

Schulman and coworkers subsequently in 1959 coined the term microemulsion as the droplet sizes (100-600 nm) were much smaller than those of milky ordinary emulsions (Schulman et al., 1959) and has then been defined and redefined on many occasions.

The term microemulsion implies a close relationship to ordinary emulsions (macroemulsions). This is misleading because the microemulsion state embraces a number of different microstructures, most of which has little in common with classical two-phase emulsions. Microemulsions are readily distinguished from normal emulsions by their transparency, low viscosity, and more fundamentally by their thermodynamic stability.

1.5 Nanoemulsion

The nanoemulsions can thus be defined as thermodynamically stable, transparent (or translucent) dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size less than 100 nm. The observed transparency of these systems is due the fact that the maximum size of nanoemulsion

droplets is less than the one-fourth of the wavelength of visible light (approximately 150 nm). Droplet size in thermodynamically stable nanoemulsions is usually 10-100 nm (Sinto and Shaprio, 2004).

Bouchemal et al., 2004, has defined nanoemulsions as kinetically stable system but approaching thermodynamic stability due to its long term physical stability (with no flocculation or coalescence) covering the size range between 100 - 600 nm. The homogeneous systems that can be prepared over a wide range of surfactant concentrations and oil to water ratios (20-80%) are all fluids of low viscosity.

Nanoemulsion provides ultra low interfacial tensions and large o/w interfacial areas. Nanoemulsions being colloidal nanodispersions of oil in water (or water in oil), thermodynamically stabilized by an interfacial film of surfactant(s) and co-surfactant(s) have revealed tremendous potential in nanoengineering of various inorganic materials (Date and Patravale, 2004).

1.5 Self -nanoemulsifying drug delivery system (SNEDDS)

SNEDDS is defined as isotropic mixtures of oil and surfactant and cosurfactants that form o/w nanoemulsion upon mild agitation followed by dilution in aqueous media, such as GI tract. Self-microemulsifying formulations spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification (Charman et al., 1992; Shah et al., 1994; Constantinides et al., 1995; Wakerly et al., 1986; Craig et al., 1993).

When SNEDDS are administered orally, the droplet size might be smaller than those in vitro, because bile salts would be incorporated into the surfactant layers of the emulsion droplets. Thus SNEDDS are related to nanoemulsions except that they are concentrate of oil and surfactant, and form nanoemulsion when diluted by GI fluids in vivo. Thus, for lipophillic drug compounds that exhibit dissolution rate limited absorption, these systems may offer an improvement in the rate and extent of absorption.

1.6 Theories of Nanoemulsification

Many approaches have been used to explore the mechanisms of nanoemulsion formation and its stability. Some emphasize the formation of an interfacial film and the production of ultra low interfacial tension (mixed film theories); others emphasize the monophasic nature of many nanoemulsions (solubilization theories). Thermodynamic theories consider the free energy of formation of the nanoemulsions and the bending elasticity of the film.

1.7 Advantages of nanoemulsion

1. They are thermodynamically stable.
2. They act as supersolvents (acts as a solvents for both the hydrophobic and hydrophilic drugs), improving the solubility and thermodynamic activity of the drug.
3. The small particle size of the microemulsion as well as both the hydrophilic and lipophillic domains of microemulsion enhances the oral and percutaneous uptake of the drugs.
4. They act as potential reservoir of the drugs through which pseudo-zero order kinetics can be obtained.
5. The small size of the droplets give large interfacial area from which drug can be quickly released,

improving the oral absorption of poorly water soluble drugs

6. Ease of preparation with no significant energy contribution.

1.8 Disadvantages of nano emulsion

1. Limitation of the lipid excipients.
2. Stability solely dependent on the lipid stability.
3. For the formulation stability, one should have to select only synthetic oils.

CHAPTER 2

2.1 LITERATURE REVIEW

Patil et al (2002)had formulated a gelled self-emulsifying drug delivery system (SEDDS) containing ketoprofen as an intermediate in the development of sustained release solid dosage form. Captex 200 (an oil), Tween 80 (a surfactant), and Capmul MCM (a cosurfactant) were used to formulate SEDDS.

Breitenbach(2003) in his article, reviewed the process technology with regard to the set up and specific elements of the extruder as well as its application. Melt extrusion processes are currently applied in the pharmaceutical field for the manufacture of a variety of dosage forms and formulations such as granules, pellets, tablets, suppositories, implants, stents, transdermal systems and ophthalmic inserts.

Kang et al (2004) prepared the self-microemulsifying drug delivery system (SMEDDS) for oral bioavailability enhancement of a poorly water soluble drug, simvastatin. Solubility of simvastatin was determined in various vehicles. SMEDDS is mixture of oils, surfactants, and cosurfactants, which are emulsified in aqueous media under conditions of gentle agitation and digestive motility that would be encountered in the gastrointestinal (GI) tract.

Chambin et al(2005)studied the influence of cryogenic grinding on properties of a selfemulsifying formulation. Recently, self-emulsifying drug delivery systems (SEDDS) have been developed as a method to deliver lipophilic drugs. Gelucire® 44/14 is an excipient, from the lauroyl macrogolglycerides family, producing a fine oil-in-water emulsion when introduced into an aqueous phase under gentle agitation as SEDDS, improving thereby solubility of poorly water-soluble drugs and their bioavailability.

You et al (2006) in the study of novel formulation design about water insoluble oily drug found that preparation of zedoary turmeric oil microspheres with self-emulsifying ability and evaluation in rabbits. To enhance in vivo absorption of zedoary turmeric oil (ZTO) and develop new formulations of a water insoluble oily drug, novel ZTO microspheres with self-emulsifying ability, called self-emulsifying microspheres here, were prepared in a liquid system by the quasiemulsion solvent diffusion method.

Franceschinis et al(2007) studied on self-emulsifying pellets prepared by wet granulation in high-shear mixer and the influence of formulation variables and preliminary study on the in vitro absorption. A method of producing self-emulsifying pellets by wet granulation of powder mixture composed of microcrystalline

cellulose, lactose and nimesulide as model drug with a mixture containing mono- and di-glycerides, polisorbate 80 and water, in a 10l high shear mixer was investigated.

Nazzal and Mansoor (2008) prepared the controlled release of a self-emulsifying formulation from a tablet dosage form and the stability assessment and optimization of some processing parameters. The objective of this study was to evaluate the effect of some processing parameters on the release of lipid formulation from a tablet dosage form.

Grove et al.(2010) identified two self-microemulsifying drug delivery systems (SMEDDS) containing either medium chain triglycerides (MC-SMEDDS) or long chain triglycerides (LCSMEDDS), with the same ratio between lipid, surfactant and co-surfactant. The SMEDDS ended up having a composition of 25% lipid, 48% surfactant and 27% co-surfactant, MC-SMEDDS.

Serratoni et al (2012) was to study and formulate a controlled release system, which could be used for the oral administration of highly water-insoluble drugs. Pellets have been prepared by extrusion/spheronization containing two model drugs methyl and propyl parabens.

Abdalla and Mader (2013) was to investigate the feasibility of producing solid self-emulsifying pellets using the extrusion/spheronization technique. Pellets were made from a mixture of C18 partial glycerides, Solutol (R) HS15 and microcrystalline cellulose.

Nielsen et al(2014) describes the evaluation and characterization of a self-nanoemulsifying drug deliverysystem (SNEDDS) consisting of a nonionic surfactant (Cremophor RH40), a mixture of long chain mono-, di-, and triacylglycerides (Maisine 35-1 and Sesame oil) and ethanol.

Shafiq et al (2015) was to design a thermodynamically stable and dilutable nanoemulsion formulation of Ramipril, with minimum surfactant concentration that could improve its solubility, stability and oral bioavailability.

Date and Nagarsenker, (2016) Self-nanoemulsifying drug delivery systems (SNEDDS) were developed with the objective to overcome problems associated with the delivery of cefpodoxime proxetil (CFP), a poorly bioavailable high dose antibiotic having pH dependant solubility.

Rhee et al (2017) was to develop an aqueous parenteral formulation containing itraconazole (ITZ) using an o/w microemulsion system. A mixture of benzyl alcohol and medium chain triglyceride (3/1) was chosen as the oil phase. Pseudoternary phase diagrams of the microemulsion formations were constructed in order to determine the optimum ratio of oils, the concentration range of surfactant and cosurfactant and the optimum ratio between them.

Park et al.2018 A semisolid self-emulsifying system (SES) of itraconazole consisting of oleic acid, polysorbate 80 and coajuvant (citric acid) was prepared by a hot-melt technique and then compared with hydroxypropylmethylcellulose (HPMC) solid dispersion (SD) coated onto inert sugar spheres as a reference formulation for in vitro and in vivo disposition in rats.

Cuine et al., (2019). The study was to investigate the impact of a change in the proportions of lipid, surfactant and co-solvent on the solubilisation capacity of self-emulsifying formulations of danazol during in vitro dispersion and digestion studies and correlation with in vivo bioavailability in beagle dogs.

Woo et al.,(2021) Silymarin has been used to treat hepatobiliary diseases. However, it has a low bioavailability after being administered orally on account of its low solubility in water. In order to improve the dissolution rate, silymarin was formulated in the form of a self-microemulsifying drug delivery system (SMEDDS).

Wang et al., (2023). A self-microemulsifying drug delivery system (SMEDDS) for enhancement of oral absorption of a poor water-soluble drug, alpha-Asarone (ARE), is reported. Solubility of ARE was determined in various vehicles. SMEDDS consisted of a mixture of oils, surfactants, and cosurfactants.

CHAPTER 3

3.1 AIM & OBJECTIVES

Atorvastatin is a synthetic cholesterol-lowering agent called HMG-CoA (3 hydroxy-3- methylglutaryl-coenzyme A) reductase inhibitor.This enzyme is involved in cholesterol biosynthesis by catalyzing the conversion reaction of HMG-CoA to mevalonate.

After oral administration alone, atorvastatin is rapidly absorbed; maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to atorvastatin dose.

The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability is attributed to low solubility, presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism

So to eleviate these problems there are three basic appeoaches 1. Reduce the drug dose 2. Increase the drug bioavailability and 3. To reduce the presystemic drug metabolism/ clearence Such above mentioned approaches can be opted by many formulation strategies; one of them is self-emulsifying drug delivery systems (SEDDS).

Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil, surfactant, surfactant and co-surfactant that form fine oil-in-water or water-in-oil emulsion when introduced into aqueous medium under gentle agitation

Nanoemulsion/ microemulsion have a higher solubilization capacity than simple micellar solutions and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions, because they can be manufactured with little energy input (heat or mixing) and has a long shelf life. The nanosized droplets leading to enormous interfacial area associated with nanoemulsion would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery.

The specific objectives of this study are:

1) To enhance the solubility and bioavailability of water insoluble drug atorvastatin by using self-nanoemulsifying drug delivery system (SNEDDS).

2) Development and characterization of solid (Pellets) self-nanoemulsifying drug delivery system (SSNEDDS) of Atorvastatin.

3) To perform in-vitro studies of the formulation.

CHAPTER 4

4.1 MATERIAL & METHOD

4.2 DRUG PROFILE

ATORVASTATIN CALCIUM

The atorvastatin calcium component of is chemically described as [R- (R*,R*)]-2-(4- fluorophenyl)-ß,a-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino) carb -onyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Its empirical formula is (C33H34 FN2O5) 2Ca•3H2O. The Atorvastatin was selected for the development of solid self-nanoemulsifying approaches. Atorvastatin a hypolipidemic agent and is official in USP. The profile of drug is described as follows:

MOLECULAR STRUCTURE

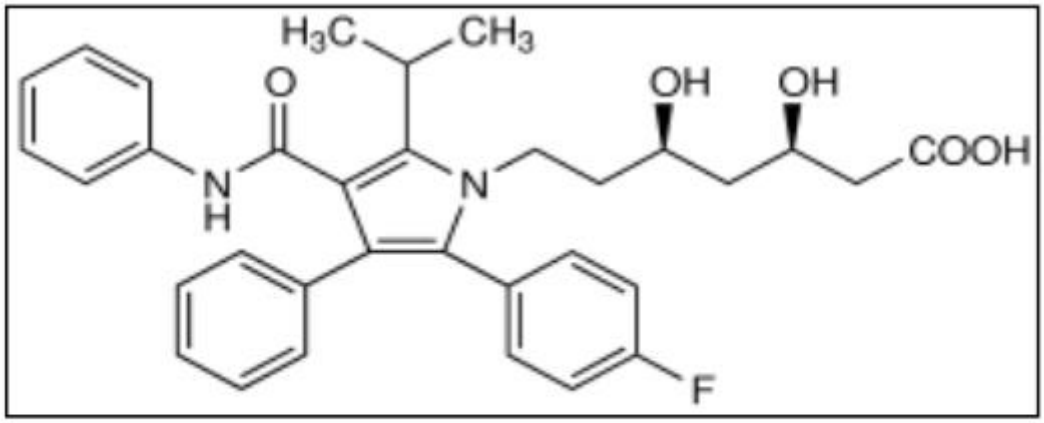

Fig 3.1: Chemical structure of Atorvastatin

Figure 2: Chemical structure of Atorvastatin

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

Molecular Formula: [C33H35FN2O5] 2 Ca .3H2O.

Generic Name: Atorvastatin calcium

Molecular Weight: 1209.4 g/mol

Category: Cardiovascular Agents 3.1.7.

Sub-category: HMG-CoA Reductase Inhibitor 3.1.8

Percentage Purity: 98.0% - 101.0% 3.1.9.

Calcium percentage: 3.3-3.6%

Physical Properties

Appearance: White to off white amorphous powder.

Solubility: Freely soluble in methanol and soluble in dimethylsulphoxide (DMSO) and dimethyl formamide (DMF); insoluble in aqueous solution with pH less than 4.0. It is very slightly soluble in distilled water,Phosphate buffer (7.4) and acetonitrile;slightly soluble in ethanol. 20.4 ug/mL (pH 2.1), 1.23 mg/mL (pH 6.0).

Stability: Stable under ordinary conditions 3.2.3. Pka : 4.46 3.2.4. Log P: 6.36 (Octanol/Water) 3.2.5. Melting point: 159.2-160.7°C

PHARMACOLOGY OF ATORVASTATIN (USP, CLARKE'S ANALYSIS AND MARTINDALE)

Atorvastatin, a synthetic cholesterol-lowering agent, is a medicine called HMG-CoA (3 hydroxy3-methylglutaryl-coenzyme A) reductase inhibitor. This enzyme is involved icholesterol biosynthesis by catalyzing the conversion reaction of HMG-CoA to mevalonate. The function of lowering the amount of cholesterol leads to the result in clearing the LDP (low-density lipoprotein) cholesterol in the blood by increased LDL receptors. The calcium salt of atorvastatin is used in the treatment of primary hypercholesterolema and dyslipidemia

Mechanism of Action

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL; atorvastatin also reduces LDL production and the number of LDL particles.

4.3 PREFORMULATION STUDIES

- UV spectral analysis
- FT-IR spectral analysis
- Characterization of the drug sample
- Physical properties
- Organoleptic characterization

- FT-IR spectral analysis
- Partition co-efficient determination
- Differential scanning calorimetric analysis (DSC)

4.4 ANALYTICAL METHODOLOGY

- Preparation of calibration curve in different solvent for in-vitro studies
- By spectrophotometric method
- In methanol i) Determination of λ max. ii) Preparation of calibration curve
- In distilled water containing 5% methanol i) Determination of λ max. ii) Preparation of calibration curve
- In distilled water containing self-emulsifying mixtures i) Determination of λ max. ii) Preparation of calibration curve.

4.5 Component Selection for The Nanoemulsifying Drug Delivery System.

4.6 Formulation Development

4.7 Characterization of Optimized Nanoemulsion

- Refractive index measurement
- Viscosity measurements
- Droplet size distribution
- Transmission electron microscopy (TEM) study.
- Optimization of formulations on the basis of in-vitro drug release performance.

4.8. Methods Employed for The Preparation Of Self-Nanoemulsifying Pellets

4.9 In vitro characterization of the optimized pellets

Bulk density, tapped density and Hausner factor ,Carr's flowability index ,% Compressibility ,Angle of repose ,Friability

4.10 Particle size analysis

4.11 Method to study in vitro release rate

4.12 Dissolution studies at different pH

CHAPTER 5

RESULT & DISCUSSION

5.1 Preformulation Studies

5.2 Authentication of the drug sample

By UV spectral analysis

Procedure

10 mg of Atorvastatin calcium (AT Calcium) was dissolved in methanol and volume was made upto 100ml with the same. 2ml of the above solution was diluted upto 10ml with methanol. The solution was scanned on Shimadzu Double Beam Spectrophotometer (UV1601) between 200nm to 400nm and showed absorption (Figure 5.1).

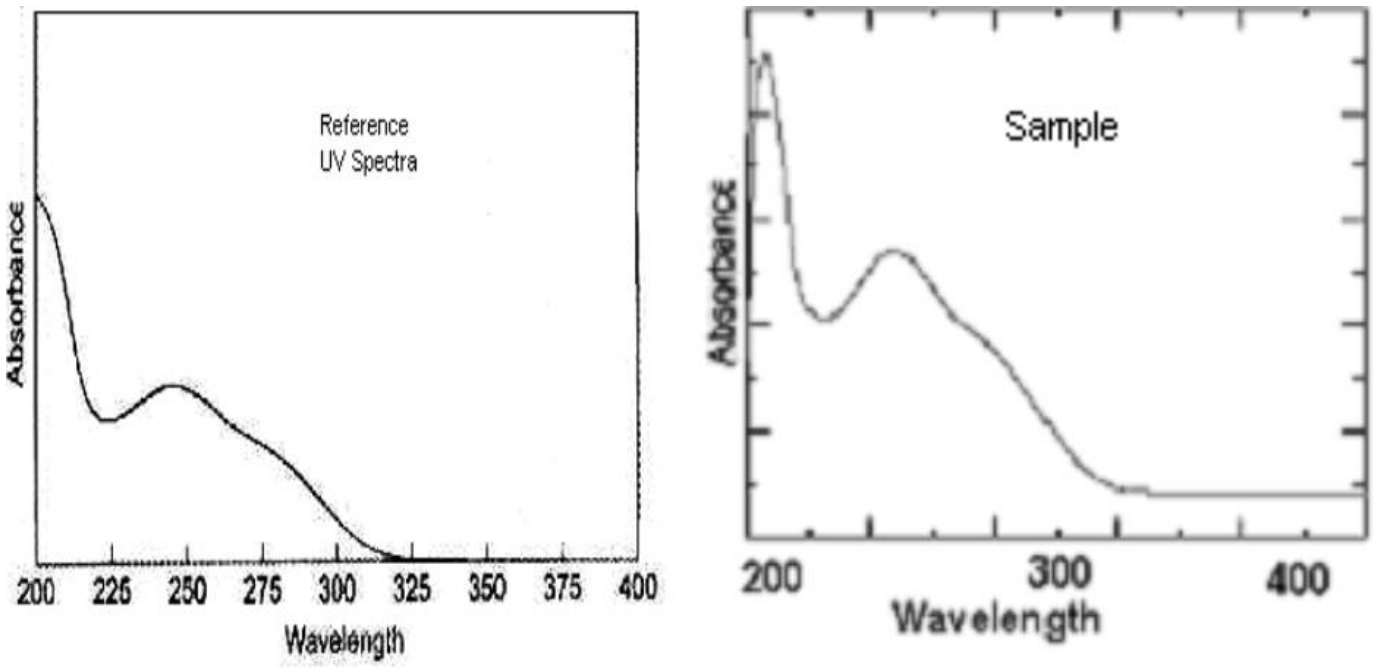

Figure 5.1: Comparison of UV-spectra of atorvastatin in methanol (λ_{max} – 247. 5nm) With referenc **(Clark's analysis, 2006)**

Figure 3: UV-Spectra of Atorvastatin in methanol

The λ max was found to be 247.5 nm and was found to be identical with reference spectra.

FT-IR spectral analysis

Potassium bromide (KBr) was mixed with the AT Calcium powder in the ratio of (100:1) and using high-pressure hydraulic machine made pellets. FTIR of the pellet was recorded on FTIR spectrometer 1520 (ARBRO Pharmaceutical Limited, Delhi) and compared with that of the standard (Figure 5.2)

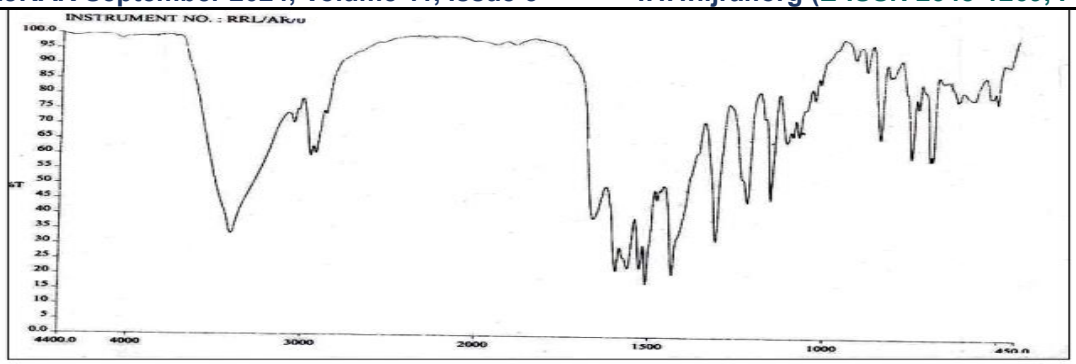

Figure: A

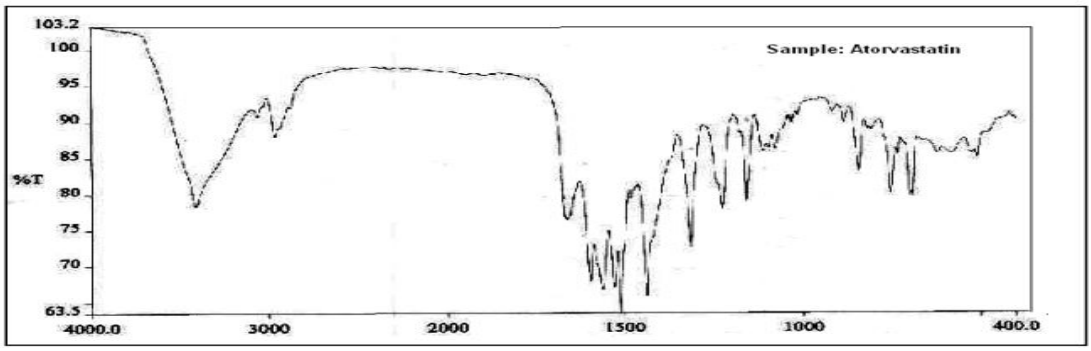

Figure: B

Figure 5.2: FTIR-spectra of AT Calcium reference (A) and sample (B)

Figure 4: FTIR-Spectra of Atorvastatin calcium

The FT-IR absorption spectra of atorvastatin was obtained using KBr pellet technique and the spectra was found to exhibit characteristics absorption bands at 3240, 1627, 1620 1180,1100,3600, 828 cm-1 , showing N-H, C=O, C=C, C-O, C-N, O-H and aromatic substitution bands respectively of AT Calcium.

Characterization of the drug sample

Physical properties AT Calcium that was received as a gift sample from (Lupin research laboratory, Pune, Maharashtra) was characterized for various physical properties like color, nature, taste, melting point, loss on drying (Table 5.1).

Table 2 : Physical properties of the AT Calcium drug sample

S. No	Parameters	Inferences
1.	Appearance	White amorphous powder
2.	Melting Point	159.0-160^{0}C
3.	Solubility	Freely soluble in methanol & soluble in DMSO & DMF, very slightly soluble in water.
4.	PKa	4.46
5.	Log P	6.36 (octanol : water)

The melting point was determined by the capillary tube method using a melting point apparatus (Scientific apparatus, India) and by differential scanning calorimeter (DSC), Loss on drying was done by the IR-LOD instruments. The melting point of the drug sample was determined by capillary method was found to be 159-162 0 C. The reported value was 159.2-160.7°C. It shows the sample was authentic

Differential scanning calorimetric analysis (DSC)

Endothermic peaks were obtained for the given amorphous drug AT Calcium using aluminium pan in triplicate. The thermogram was shown in the Figure 5.4.

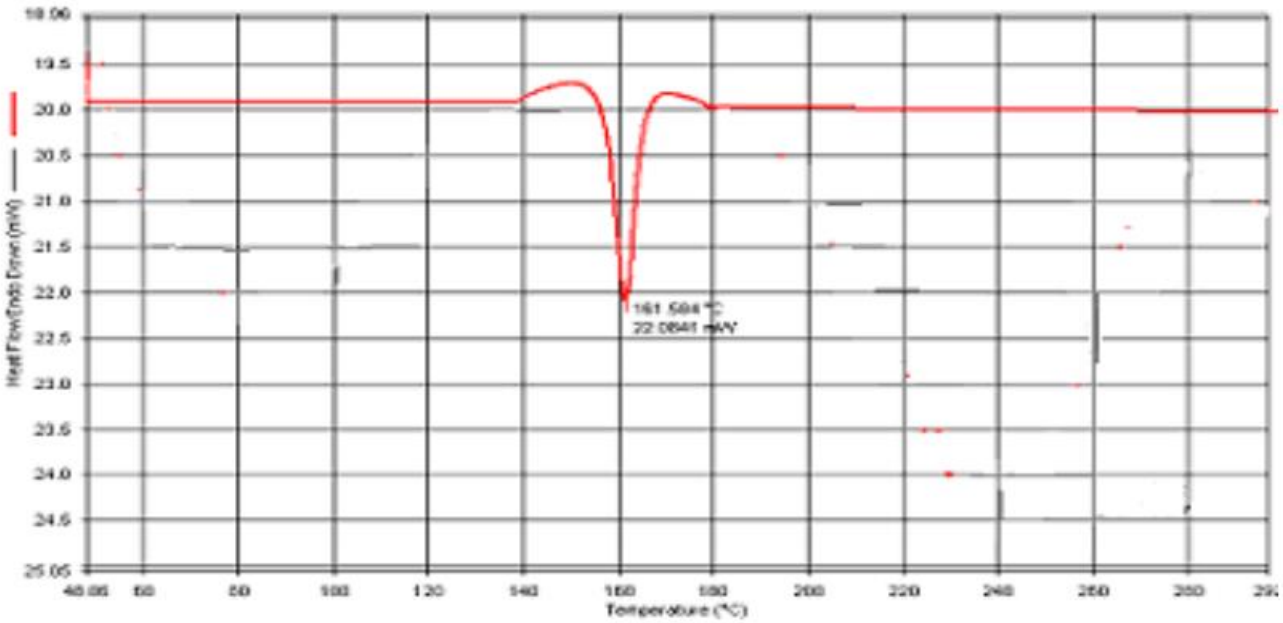

Figure 5.4: Differential scanning calorimetric -spectra of atorvastatin reference sample

Figure 5: DSC of Atorvastatin

5.3 Analytical Methodology

10 mg of AT Calcium was dissolved in methanol and volume was made up to 100ml with methanol. From the stock (100µg/ml) different dilutions from 2 to 20 µg/ml were prepared with the same diluting medium to prepare calibration curve (Figure 5.6 and Table 5.2)

Table 3 : Calibration curve of atorvastatin in methanol at 247.5nm

S.No	Concentration (µg/ml)	Absorbance±SD (n=3)
1	0	0
2	2	0.0569 ± 0.02
3	4	0.1203 ± 0.08
4	6	0.1837 ± 0.15
5	8	0.2406 ± 0.17
6	10	0.3105 ± 0.22
7	12	0.3674 ± 0.15
8	14	0.4373 ± 0.28
9	16	0.4812 ± 0.14
10	18	0.5614 ± 0.05
11	20	0.6210 ± 0.31

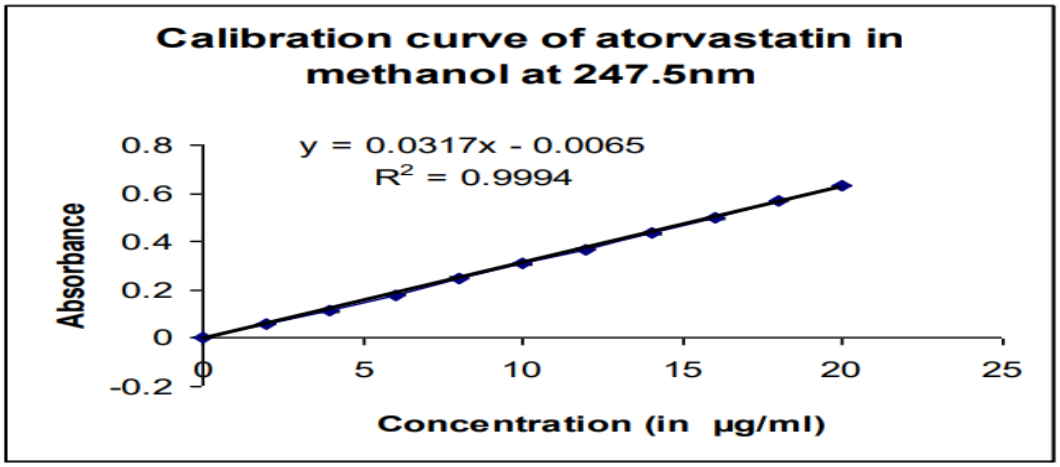

Figure 5.6: Calibration curve of atorvastatin in methanol at 247.5nm

Figure 6: Calibration curve of Atorvastatin

In distilled water containing 5% methanol 10 mg of AT Calcium was dissolved in 5 ml methanol and volume was made up to 100ml with distilled water. From the stock (100µg/ml) different dilutions from 2 to 20 (µg/ml) were prepared with the same diluting medium to prepare calibration curve

Table 4: Calibration curve of atorvastatin in distilled water containing 5% methanol

S.No.	Concentration (µg/ml)	Absorbance± SD (n=3)
1	0	0
2	2	0.0554
3	4	0.1172
4	6	0.1790
5	8	0.2408
6	10	0.3026
7	12	0.3644
8	14	0.4262
9	16	0.4880
10	18	0.5499
11	20	0.6116
12	22	0.6734
13	24	0.7352
14	26	0.7970
15	28	0.8588
16	30	0.9206

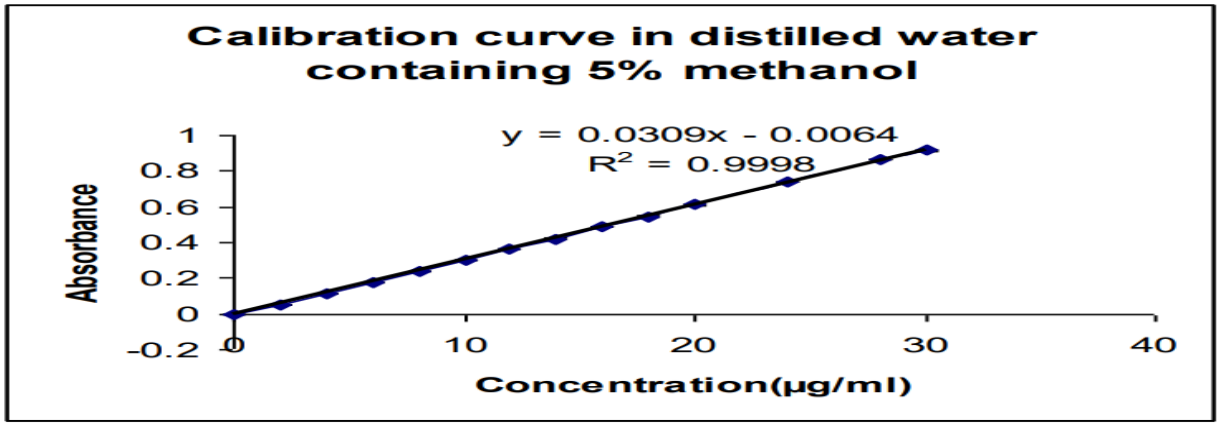

Figure 5.8: Calibration curve of atorvastatin in distilled water containing 5% methanol

Figure 7: Calibration curve of Atorvastatin in distilled water in 5 % methanol

The prepared samples were scanned on Shimadzu Double Beam Spectrophotometer (UV 1601) between 200nm to 400nm. The equation of regression was found to be Y = 0.0309 x –0.0064 with correlation of co-efficient as 0.9998.

5.4 Formulation Development

Component Selection For The Nanoemulsifying Drug Delivery System

Components

Both long and medium chain triglyceride oils with different degrees of saturation have been tried for the design of nanoemulsion formulations. Edible oils, which could represent the logical and preferred lipid excipients choice for the development of nanoemulsion, are not frequently screened due to their poor ability to dissolve large amounts of lipophillic drugs. 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 (Kimura et al., 1996; Hauss et al., 1998; Constantinides, 1995).

The surfactant chosen must be able to lower interfacial tension to a very small value to aid dispersion process during the preparation of the nanoemulsion. Provide a flexible film that can readily deform around droplets and be of the appropriate lipophillic character to provide the correct curvature at the interfacial region for the desired nanoemulsion type. Safety is a major determining factor in choosing a surfactant as large amounts of surfactants may cause GI irritation. Non-ionic surfactants are less toxic than ionic surfactants. Nonionic surfactants typically have lower CMCs than their ionic counterparts.

Solubility determination in the various oils, surfactants and co-surfactants For formulating solid self-nanoemulsifying drug delivery system (SSNEDDS) the solubility of the drug in different oils is an essential step for the nanoemulsion formulation. So before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of nanoemulsion drug delivery system.

Procedure 2 ml of different oils was taken in small vials separately and excess amount of the drug was added to each vial. The vials were tightly stopperd and were continuously stirred for 72 hrs in mechanical shaker at 250C and after that, oils were centrifuged. The supernatant was separated and dissolved in methanol and solubility was quantified by HPLC method at 247nm after appropriate dilution with methanol.

Table 5 : Solubilty of Atorvastatin

Table 6.1: Solubility of AT Calcium in different oils, surfactants and co surfactants

S.No	Oils	Solubility (mg/ml)
1	Oleic acid	52.01±1.67
2	Isoproylmyristate (IPM)	16.22±0.25
3	Olive oil	08.25±0.45
4	Triacetin	02.81±1.69
5	Jojoba oil	06.57±0.96
6	Castor oil	08.35±0.84
7	Groundnut oil	05.25±1.52
8	Liquid paraffin	04.19±1.68
9	Labrafac	11.30±0.58

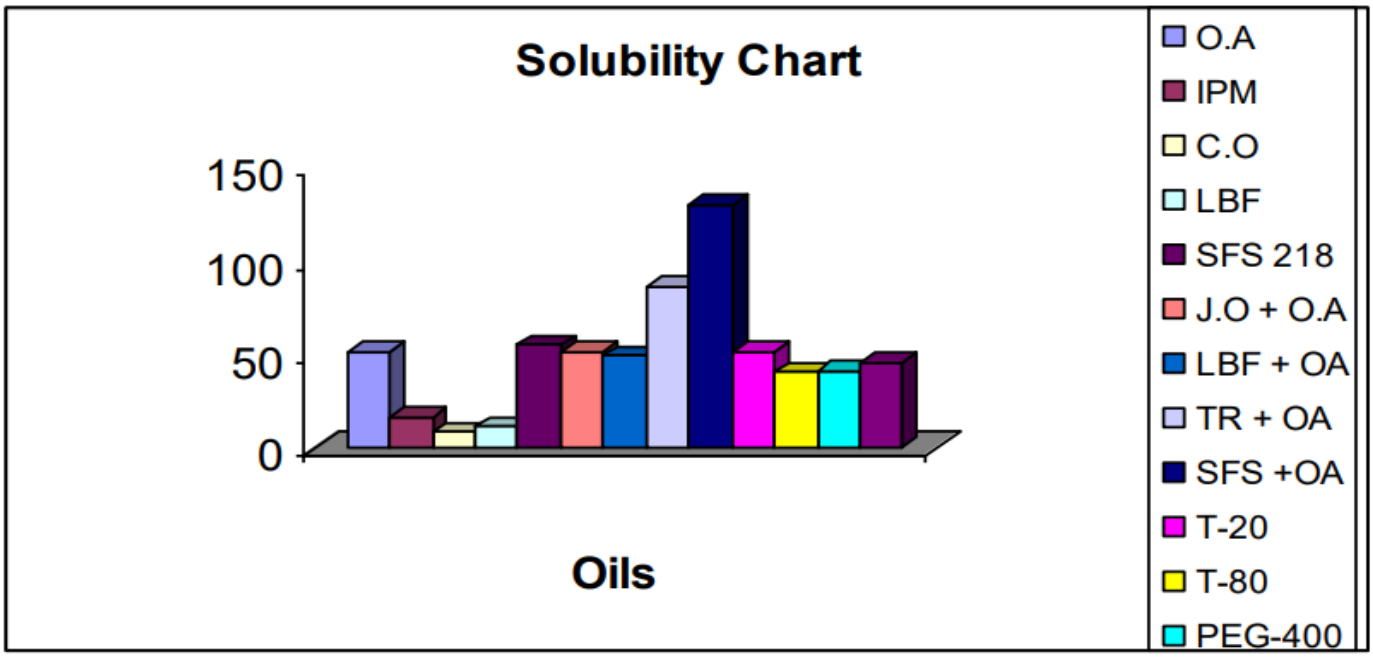

Figure 6.1: Statistical solubility chart of AT Calcium in different oils

Figure 8: Statistical solubility of Atorvastatin calcium

Abbreviation used: O.A = Oleic acid, IPM = Isopropyl myristate, C.O = Castor oil, LBF =Labrafac, SFS = Sefsol oil, J.O =Jojoba oil, TR = Triacetin, T-20 = Tween 20, T-80 = Tween 80, PEG-400 = Polyethlene glycol-400

On the basis of above study it was concluded that the solubility in the combination of oils like Triacetin + Oleic acid and Sefsol + Oleic acid was found to be favourable (Table 6.1 and Figure 6.1) for the microemulsion preparation of AT Calcium. Among them the oil mixture Triacetin and Oleic acid itself was not completely miscible with each other and solubility of AT Calcium was also less and did not meet the dose criteria of AT Calcium. The maximumsolubility of AT Calcium i.e. 130.06 ± 2.68 mg/ml was obtained in mixture of Sefsol and Oleic acid and was selected as oil phase for further formulations developments.

Construction of pseudo-ternary phase diagrams

Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1) and the stock of 100 mL of each groups was prepared (Table 6.2). These Smix ratios were chosen in increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams for the nanoemulsions formation.

Table 6: Different volumes of Surfactant and Cosurfactant taken to make a stock Smix ratio

S.No	Volume of Surfactant (mL)	Volume of Cosurfactant (mL)	Ratio of Smix
1	100	0	1:0
3	50	50	1:1
2	33.3	66.7	0.5:1 or 1:2
6	25	75	1:3
4	66.7	33.3	2:1 or 1:0.5
5	75	25	3:1
7	80	20	4:1

Procedure For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratios from 1:9 to 9:1 in different small glass test tubes. Sixteen different combinations of oil and each Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

For the determination of existence zone of microemulsion, pseudoternary phase diagrams were constructed using water titration method (Shafiq et al., 2007). To construct pseudoternary phase diagrams, the oil phase (oleic acid: Sefsol, 1:1) was mixed with different ratio of surfactant and cosurfactant (Tween 20 and Carbitol® respectively) and mixture was titrated with distilled water until it turned turbid.

Examine each and every point I detailed and note it down. Pseudo ternary phase diagrams were drawn by using data obtained in aqueous titration method as shown in Figure. 6.2 (A-G). The amount of water added to give water concentration in the range of 5-95%of total volume at 5% intervals.

After every 5% addition of the water to the oil and Smix mixture, visual observation were made as shown in Table 6.8. The ratio of surfactant and co surfactant (Tween 20 and Carbitol®) were used for the titration are, 1:0,1:1,1:2,1:3,2:1,3:1 and 4:1 respectively.

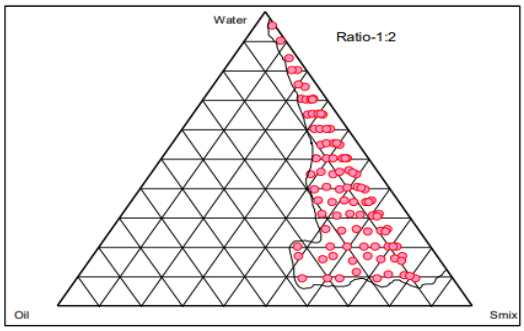

Figure 6.2 C Phase diagram of Smix 1:2

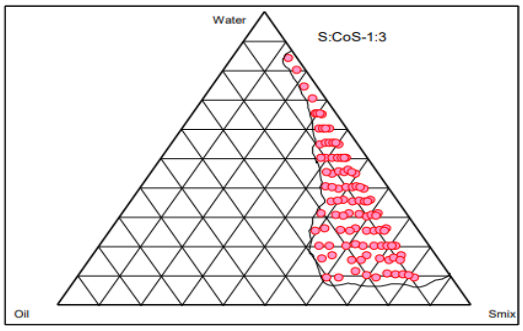

Figure 6.2 D Phase diagram of Smix 1: 3

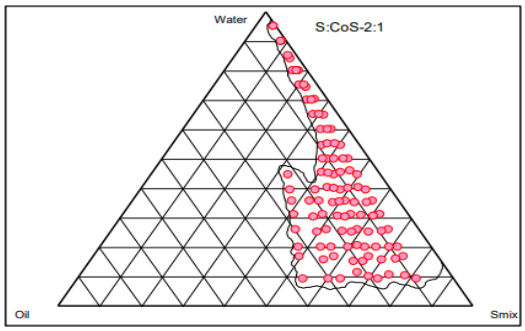

Figure 6.2 E Phase diagram of Smix 2:1

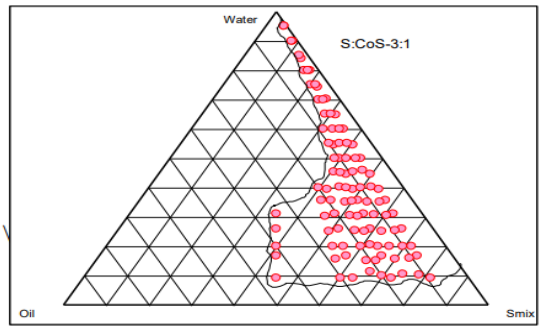

Figure 6.2 F Phase diagram of Smix 3:1

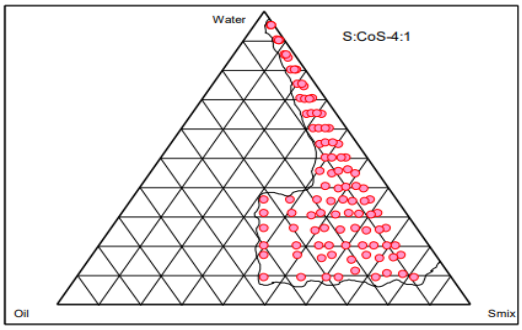

Figure 6.2 G Phase diagram of Smix 4:1

Figure 6.2 (A-G): Pseudoternary phase diagram consisting of oil, sefsol 218 and oleic acid, surfactant, Tween 20 and co-surfactant, carbitol.

Figure 9: Pseudoternanry phase diagrams

Phase diagram is one of the primary steps and make a backbone for the nanoemulsion durg delivery system, particularly when the aim is to accurately delineate a phase boundary (Lawrence and Rees, 2000). Observations are made carefully to separate metastable systems from phase boundary, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous (Craig et al., 1995).

The relationship between the phase behaviour of a mixture and its composition can be selected with the aid of a phase diagram (Lawrence and Rees, 2000). Sefsol 218 (oil) and oleic acid (oil), Tween 20, Tween 80 (surfactants) and Carbitol (co surfactants), were used to study the phase diagrams in detail. The systems were observed for visual clarity and flowability characteristics referring to Table 6.8. Those which did not show a change in the miniscus after tilting to an angle of 90° were classified as nano gels a metastable system were not selected .

After taking observation, pseudo ternary phase diagrams were constructed based on the observations marked during titration. Phase diagrams were constructed separately for each ratio of Smix prepared in Table 6.2, so that o/w nanoemulsion regions could be identified. In the phase diagrams (Figure. 6.1, A-G) only o/w nanoemulsion region is shown. After building the backbone of the nanoemulsion delivery system, different formulations were selected at different point from the phase diagram justifying the drug dose.

Selection of the formulations from phase diagram

From each phase diagram constructed, different formulations were selected from nanoemulsion region so that drug could be incorporated into the oil phase; therefore, following criteria were made for the selection of different formulation from phase diagrams:

The dose of AT Calcium varies between 5 mg to 20 mg. But most frequently used dose is 10 mg.

The oil concentration should be such that it dissolves the drug (10 mg) easily.

From each phase diagram different concentration of oil, which solubilized 10 mg of AT Calcium, was selected at a difference of 5% (10, 15 and 20%)

For each percentage of oil selected, that formula was taken from the phase diagram, which used minimum concentration of Smix for its nanoemulsion formation

The emphasis for the selection of formulations was given on the minimum concentration of the Smix, from the phase diagram.

As per saturation solubility studies of AT Calcium in oily mixture (Sefsol 218 : Oleic acid ::1:1), around 130± 2.68 mg of drug was solubilized per ml of mixtures.

The concentration, 10% (100 µL) of oil in 1 mL of formulation is just able to solubilize 10 mg of AT Calcium. Therefore 10% was selected as the least oil concentration to be taken for one mL formulation from the phase diagram.

The drug stock solutions in oil mixture were prepared in such a way that 10 mg dose is present in each formulation complying the oil percentage for each formulas as shown in the Table 6.

Table 7: Formulations from pseudoternary phase diagrams of smix ratio

Table 6.3: Selected formulations from pseudoternary phase diagram of Smix ratio 1:1, 1:2, 1:3

Smix Ratio (S:CoS)	Code	Percentage v/v of different components in formulation		
		Oil	Smix	Aque
1:1 (Figure 6.2 B)	1	10	30	60
	2	10	38	55
	3	15	38	47
	4	15	45	45
	5	20	40	45
	6	20	40	35
	7	25	45	35
	8	25	31	30
1:2 (Figure 6.2C)	9	10	40	59
	10	10	40	50
	11	15	45	45
	12	15	38	40
	13	20	40	47
	14	20	36	45
	15	25	40	49
	16	25	37	35
1:3 (Figure 6.2 D)	17	10	40	53
	18	10	44	50
	19	15	50	41
	20	15	48	35
	21	20	48	32
	22	20	50	30

Vigorous studies were done for the phase diagram construction, phase boundry were plotted on the ternary phase diagram.

After building the backbone of the nanoemulsion delivery system, different formulations were selected at different point from the pseudophase diagram which justified the drug dose considering the drug solubility in the oils phase.

As per saturation solubility studies of AT Calcium in oily mixture, Sefsol 218:Oleic acid (1:1), around 130 mg of drug can be solubilized per mL.

The concentration 10% (100 µL) of oil in 1 mL formulation is just able to solubilize 10 mg of AT Calcium. Therefore 10% was selected as the least oil concentration to be taken for one mL formulation from the phase diagram.

The selected formulations shown in Table 6.6 and 6.7 were screened on the basis of the thermodynamic stability studies.

5.4 Development of drug containing nanoemulsion formulation

The drug stock solutions in oil mixture were prepared in such a way that 10 mg dose is present in each formulation complying the oil percentage for each formulae as shown selected from the phase diagram.

This was prepared by dissolving the 1000 mg of drug individually in the 10, 15, 20 and 25 mL of oily mixture, which complies the 10%, 15%, 20% and 25% oil compositions respectively in the formulae.

The drug stock table is shown in the Table 6.4.

Table 8: Preparation of drug stock for each formulae selected in phase diagram

S.NO	Oil percentage in formulations	Amount of drug (mg)	Volume of oil (mL)	Final concentration (mg/µL)
1	**10%**	**1000**	**10**	**10 mg/100 µL**
2	**15%**	**1000**	**15**	**10 mg/150 µL**
3	**20%**	**1000**	**20**	**10 mg/200 µL**
4	**25%**	**1000**	**25**	**10 mg/250 µL**

Screening of formulations on the basis of thermodynamic stability studies

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant:co-surfactant mixture and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates micro emulsion from emulsions that have kinetic stability and will eventually phase separate (Lawrence and Rees., 2000). The thermodynamic stability studies was performed on the basis of following tests.

Table 8: Thermodynamic stability tests of different selected formulations from Smix ratio 1:1, 1:2, 1:3

Oil used: Sefsol 218+Oleic acid (1:1) , **Surfactant used**: Tween 20, **Cosurfactant used**: Carbitol, **External phase:** Distilled water								
Smix Ratio (S:CoS)	**S.N**	**Percentage v/v of different components in formulations**			**Observations based on the thermodynamic stability studies**			**Inference**
		Oil	**Smix**	**Water**	**H/C**	**Cent**	**Freez**	
1:1 (Figure 6.1 B)	1	10	30	60	×	√	×	Failed
	2	10	38	55	√	√	√	Passed
	3	15	38	47	√	√	√	Passed
	4	15	45	45	√	√	√	Passed
	5	20	40	45	√	√	√	Passed
	6	20	40	35	√	√	√	Passed
	7	25	45	35	√		√	Passed
	8	25	31	30	√	√	√	Passed
1:2 (Figure 6.1C)	9	10	40	59	×	√	√	Failed
	10	10	40	50	√	√	√	Passed
	11	15	45	45	√	√	√	Passed
	12	15	38	40	√	√	√	Passed
	13	20	40	47	√	√	√	Passed
	14	20	36	45	√	√	√	Passed
	15	25	40	49	-	×	√	Failed
	16	25	37	35	-	×	√	Failed
1:3 (Figure 6.1D)	17	10	40	53	√	√	√	Passed
	18	10	44	50	√	√	√	Passed
	19	15	50	41	√	√	√	Passed
	20	15	48	35	√	√	√	Passed
	21	20	48	32	√	√	√	Passed
	22	20	50	30	×	√	√	Failed

Table 9: Thermodynamic stability tests of different selected formulations from Smix ratio 2:1, 3:1 & 4:1.

Oil used: Sefsol 218+Oleic acid (1:1) , **Surfactant used**: Tween 20, **Cosurfactant used**: Carbitol, **External phase:** Distilled water								
Smix Ratio (S:CoS)	**S.N**	**Percentage v/v of different components in formulations**			**Observatios based on the thermodyanamic stability studies**			**Inference**
		Oil	**Smix**	**Water**	**H/C**	**Cent**	**Freez**	
2:1 (Figure 6.1E)	23	25	35	55	√	√	√	Passed
	24	25	40	50	√	√	√	Passed
	25	10	42	43	-	×	-	Failed
	26	10	45	40	√	√	√	Passed
	27	15	40	40	√	√	√	Passed
	28	15	45	35	√	√	√	Passed
	29	20	38	37	√	√	√	Passed
	30	20	40	50	√	√	√	Passed
3:1 (Figure 6.1F)	31	25	40	45	-	×	-	Failed
	32	25	42	42	-	×	-	Failed
	33	10	43	40	-	×	-	Failed
	34	10	45	40	√	√	√	Passed
	35	15	40	35	-	√	×	Passed
	36	15	45	35		√	√	Passed
	37	20	40	38	-	×	-	Failed
	38	20	38	35	√	√	√	Passed
4:1 (Figure 6.1G)	39	25	40	50	√	√	√	Passed
	40	25	45	45	-	-	-	Failed
	41	10	45	40	√	√	√	Passed
	42	10	50	35	-	×	-	Failed
	43	15	45	35	-	×	-	Failed
	44	15	50	30	-	×	-	Failed
	45	20	40	35	√	√	√	Passed
	46	20	45	30	√	√	√	Passed

5.6 Results and discussion

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano or micro emulsion from emulsions that have kinetic stability and will eventually phase separate (Lawrence and Rees, 2000). Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for dispersibility test. Those

formulations, which survived thermodynamic stress tests, were taken for dispersibility test to see the visual clarity after infinite dilution (Table 6.6 and 6.7).

Dispersibility tests

The efficiency of self-emulsification of oral nanoemulsion was assessed using a standard USP XXII dissolution apparatus 2 (Pouton, C.W., 1997). One ml of each formulation was added to 500 mL of distilled water and in 0.1N HCl respectively at 37 ± 0.5 oC. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The in vitro performance of the formulations was visually assessed using the following grading system (Table 6.5). Those formulations that passed the thermodynamic stability and also dispersibility test in Grade A were taken for the further studies. Further from each Smix Group one formulation is selected, having the least Smix concentration irrespective of Smix ratio used, but passing dispersibility test in Grade A in distilled water as well as in 0.1N HCl (Table 6.9 and 6.10).

Table 10: Observation table of Dispersibility study

S.No.	Observation	Grade
1	Rapidly forming (within 1 min) nanoemulsion, having a clear or slight bluish	A
2	Rapidly forming, slightly less clear microemulsion, in bluish colour	B
3	Fine milky emulsion that formed within 2 minutes	C
4	Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).	D
5	Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.	E

Table 11: Dispersibility tests of selected formulations from Smix ratio 1:1, 1:2 and 1:3

Smix Ratio (S:CoS)	S.N	Percentage v/v of different components in formulation			Observations based on the Dispersibility tests in distilled water and 0.1 N HCl.		Inference
		Oil	Smix	Aque	Distilled water	0.1 N HCl	
1:1 (Figure 6.1B)	1	10	30	60	-	-	-
	2	10	38	55	Grade A	Grade A	Passed
	3	15	38	47	Grade A	Grade A	Passed
	4	15	45	45	Grade A	Grade C	Failed
	5	20	40	45	Grade A	Grade C	Failed
	6	20	40	35	Grade B	Grade C	Failed
	7	25	45	35	Grade A	Grade C	Failed
	8	25	31	30	Grade B	Grade C	Failed
1:2 (Figure 6.1C)	9	10	40	59	-	-	-
	10	10	40	50	Grade A	Grade A	Passed
	11	15	45	45	Grade A	Grade A	Passed
	12	15	38	40	Grade A	Grade A	Passed
	13	20	40	47	Grade A	Grade C	Failed
	14	20	36	45	Grade B	Grade B	Failed
	15	25	40	49	-	-	-
	16	25	37	35	-	-	-
1:3 (Figure 6.1D)	17	10	40	53	Grade A	Grade A	Passed
	18	10	44	50	Grade A	Grade A	Passed
	19	15	50	41	Grade A	Grade C	Failed
	20	15	48	35	Grade A	Grade A	Passed
	21	20	48	32	Grade B	Grade C	Failed
	22	20	50	30	-	-	-

Table 12: Dispersibility tests of selected formulations from Smix ratio 2:1, 3:1 and 4:1

Smix Ratio (S:CoS)	S.N	Percentage v/v of different components in formulation			Observations based on the Dispersibility tests in distilled water and 0.1 N HCl.		Inference
		Oil	Smix	Aque	Distilled water	0.1 N HCl	
2:1 (Figure 6.1E)	23	25	35	55	Grade A	Grade B	Failed
	24	25	40	50	Grade A	Grade A	Passed
	25	10	42	43	-	-	-
	26	10	45	40	Grade A	Grade C	Failed
	27	15	40	40	Grade A	Grade C	Failed
	28	15	45	35	Grade A	Grade C	Failed
	29	20	38	37	Grade A	Grade C	Failed
	30	20	40	50	Grade B	Grade C	Failed
3:1 (Figure 6.1F)	31	25	40	45	-	-	-
	32	25	42	42	-	-	-
	33	10	43	40	-	-	-
	34	10	45	40	Grade A	Grade C	Failed
	35	15	40	35	Grade A	Grade C	Failed
	36	15	45	35	Grade A	Grade B	Failed

	37	20	40	38	-	-	-
	38	20	38	35	Grade A	Grade C	Failed
4:1 (Figure 6.1G)	39	25	40	50	Grade A	Grade A	Passed
	40	25	45	45	-	-	-
	41	10	45	40	Grade A	Grade B	Failed
	42	10	50	35	-	-	-
	43	15	45	35	-	-	-
	44	15	50	30	-	-	-
	45	20	40	35	Grade A	Grade C	Failed
	46	20	45	30	Grade A	Grade B	Failed

Results and discussion

A number of formulations passed the thermodyanamic stability (Accelerated stability) tests (Tables 6.6 and 6.7). So it was not possible to go for the further study by selected all the formulations. Finally five formulations were selected on the basis of the dispersibility test in distilled water as well as in 0.1N HCl considering the minimum surfactant concentrations from all the phase diagram (Table 6.9 and 6.10).

Characterization Of Selected Formulations

Refractive Index

Refractive index of selected formulations was determined using an Abbe type refractrometer. It was standardized using castor oil (Table 6.12).

Table 13: Refractive index of pure components and selected nanoemulsion formulations

Pure Component	R. I ± SD (n=3)	Formulation	R. I ± SD (n=3)
Sefsol 218	1.399±0.006	GM1	1.404±0.011
Tween 20	1.401±0.020	GM2	1.403±0.022

Results and Discussion

Refractive index of selected formulations was determined using an Abbe type refractrometer. It was standardized using castor oil. It is also used as a parameter in the determination of droplet size distribution of nanoemulsion as the droplet size measurement is done by light scattering observed at 90° angle. The observation table shows that as the concentration of the of the oils increases in the formulation, the RI increases as obsevered in GM4 and GM5. Formulation GM3 shows that as the amount of the co-surfactants increases, the rigidity of the structure decreases and so ultimately the RI decreases. RI also affected by the size of the oils globules, as the globules size increases the RI incease as reported in the formulation GM1 and GM2 shown in Table 6.12.

Viscosity

The viscosity of the prepared nanoemulsion formulations were determined as such without dilution by Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) using spindle # CPE40 at 25 ±0.5°C. The software used for the viscosity calculations was Rheocalc V2.6. The parameters, which were set after optimizing the procedure, were listed in the Table 6.13.

Table 14: Specification for the viscosity determination

Parameters	Optimized specifications
Sample (g)	0.5
Speed (rpm)	6
Data Interval (min)	10
Loop Start	CP-41
Wait time (min)	5
Temperature (°C)	25±0.5
Share rate (1/sec)	7.5(N)

Table 15: Observation table of viscosity measurements

	Mean viscosity ± SD (cps)	Mean viscosity ± SD (cps)
GM1	27.18±0.22	27.51±1.01
GM2	43.01±0.99	43.42±0.99
GM3	10.01±0.12	10.03±0.91
Gm4	30.15±0.09	30.37±1.21
GM5	35.92±0.12	35.73 ± 1.18

Results and discussion

The viscosities of the optimized formulations were determined. The values are shown in Table 6.14 and the process parameters were shown in the Table 6.13. It was observed that the viscosity of all the formulations is ranges between 10-43 cps. Formulation GM3, has the minimum viscosity (10 ± 0.91 cps), which shows that the amount of co-surfactants is directly proportional to the film flexibility. The viscosity of finally selected formulations, GM1 was found to be 27.51 ± 1.01 cps

Droplet size analysis (Particle size distribution)

Droplet size of the prepared nanoemulsion was determined by using photon correlation spectroscopy, which analyzes the fluctuations in light scattering due to Brownian movement of the particles (Attwood et al., 1992). The formulation (0.1 mL) was dispersed in 50 mL (500 dilution) of distilled water in a volumetric flask and gently mixed by inverting the flask and measurement done using a Zetasizer (Nano ZS-90, UK). Light scattering was monitored at 25°C at a 90° angle.

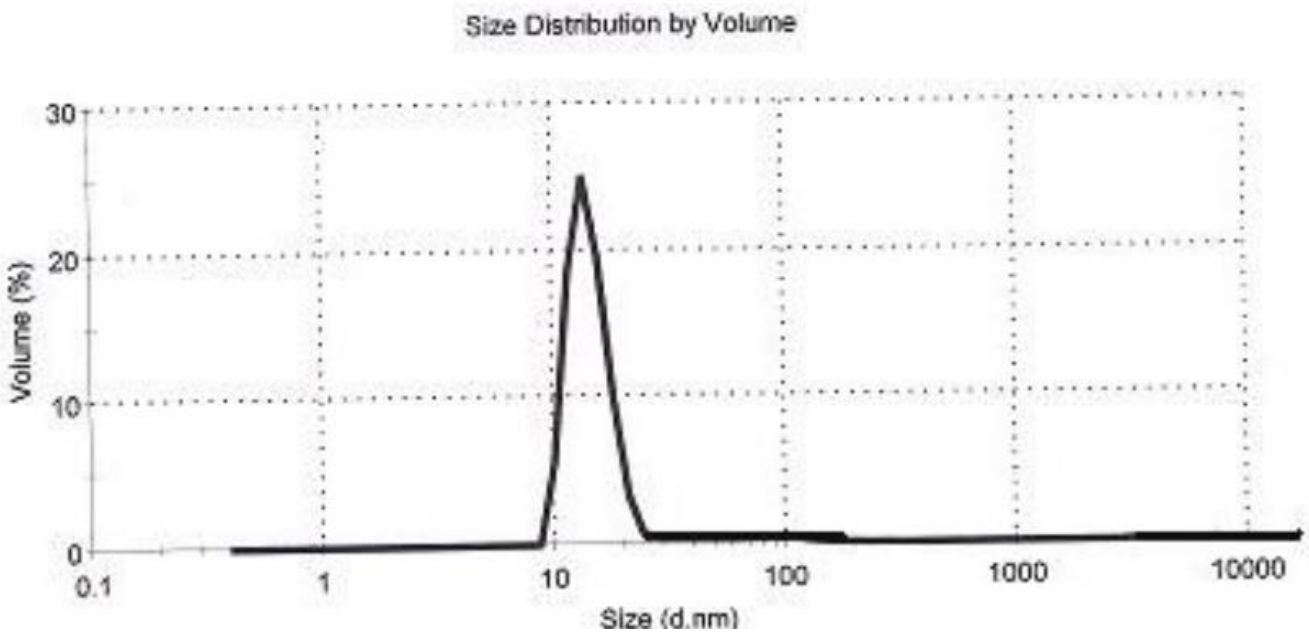

Figure 10: Droplet size distribution of formulation (GM 1)

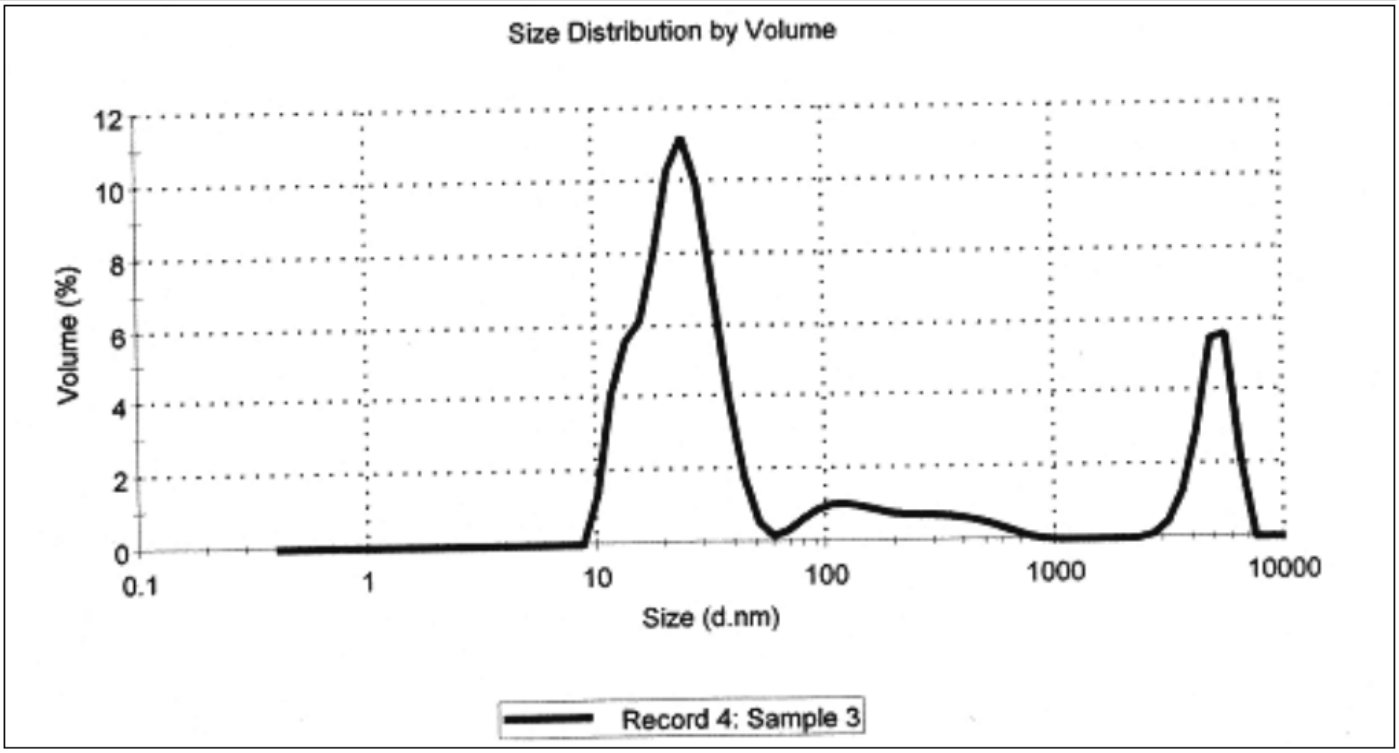

Figure 11: Droplet size distribution of formulation (GM 2)

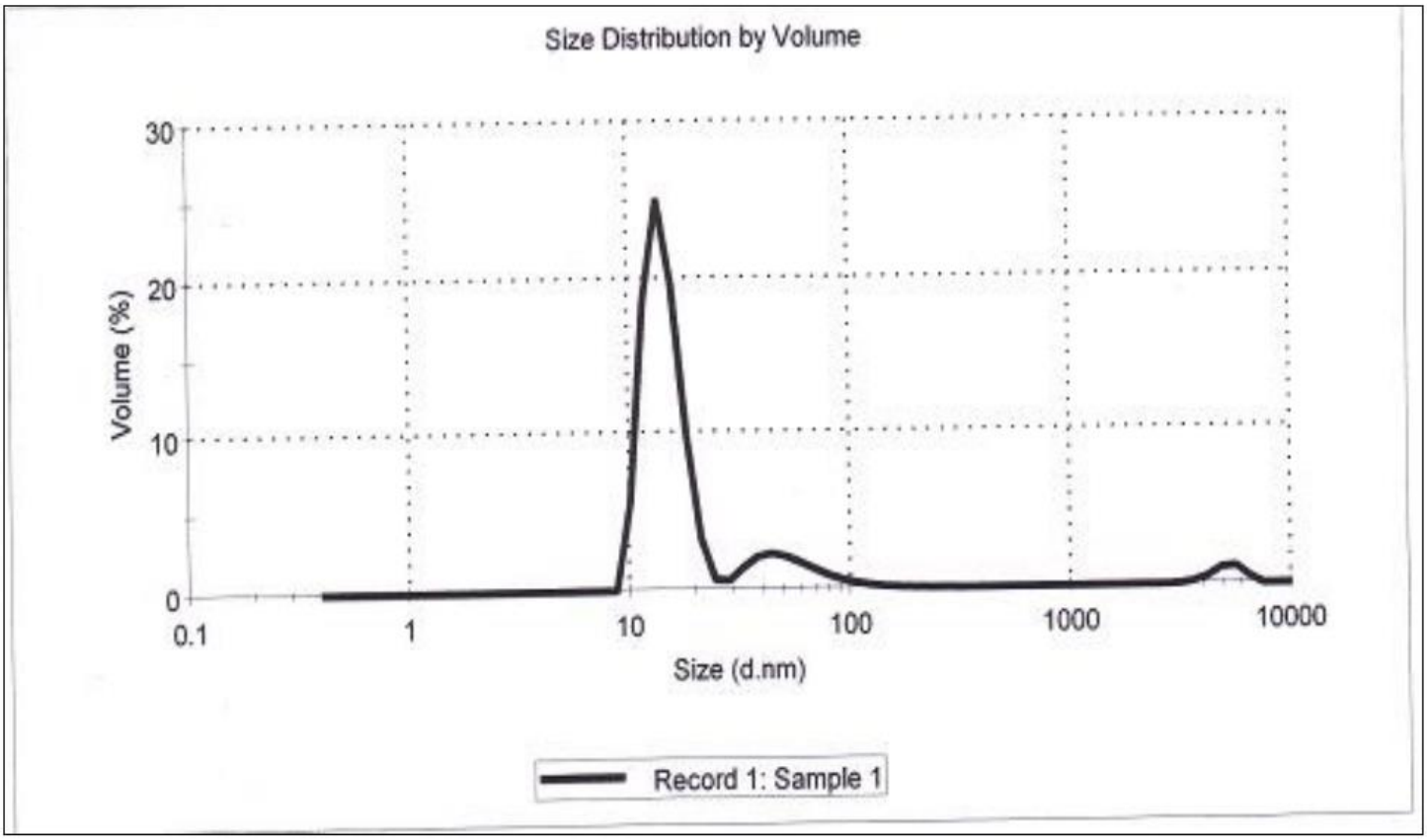

Figure 12: Droplet size distribution of formulation (GM 3)

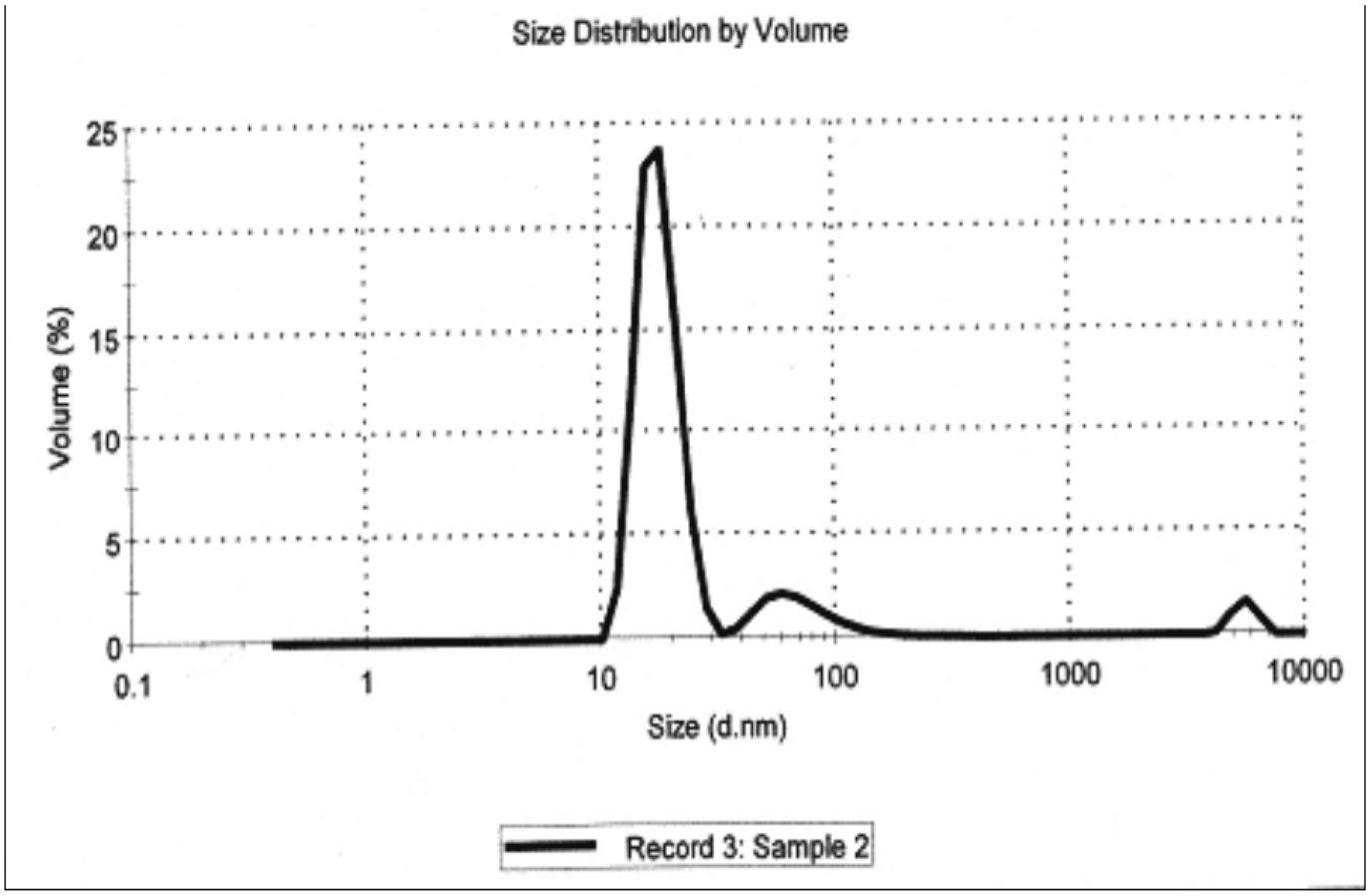

Figure 13: Droplet size distribution of formulation (GM 4)

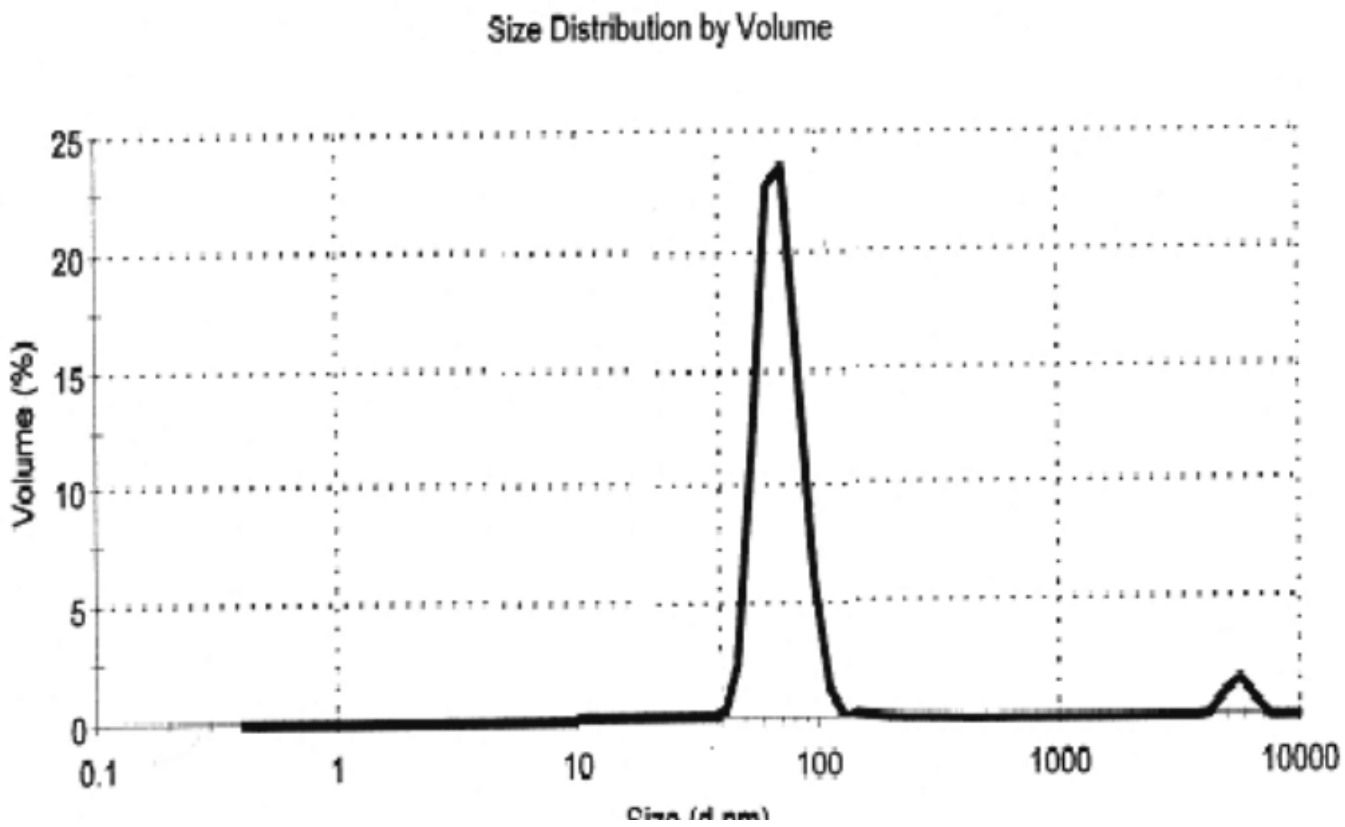

Figure 14: Droplet size distribution of formulation (GM 5)

Results and discussion

The size and size distribution analysis was performed on selected formulatios by using Malvern Zetasizer (Nano ZS-90 U.K).Droplet size analyses of the selected formulations showed that the size increased with the increase in concentration of oil in the formulations GM1, GM2 (Table 6.15 and 6.16) and increase in the concentration of co-surfactants in formulation GM3 (Table 6.17).

This may be due to the increase in the oil concentration form 10% to 15% v/v although having the Smix concentration same which shows enability to disperse the increased amount of oils. The statistical distribution of droplet size is shown in the Figure 6.4, 6.6, 6.8, and 6.10.

The mean droplet size of the formulation GM1, GM2, GM3 and GM4 were 42.8 nm, 195 nm, 107 nm and 81.6 nm respectively. In GM1 formulation 82.5% droplets were found to be 14.4 nm range .The droplet size in the case of GM2 and GM3 were found to be i.e. 195 and 107 respectively, as compared to GM1, this attribute to the fact that formulation GM2 and GM3containing higher concentrations of oil and co-surfactant respectively.

The result also reveals that co-surfactant (Carbitol ®) doesn't play a vital role as compared to the surfactant (Tween 20®) for the nanoemulsifications of oily mixture (sefsol: oleic acid). The polydispersity was found to be minimum in the case of GM1 (0.237) and GM4 (0.397) as compared to GM2 (0.478) and GM3 (0.407).

Transmission electron microscopy

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM) TOPCON 002B operating at 200 KV and of a 0.18 nm capable of point to point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. In order to perform the TEM an observation, the nanoemulsion formulation was diluted with distilled water (1/100). A drop of the diluted nanoemulsion was directly deposited on the Copper holey film grid and observed after putting fixing agent and drying it in the filtered air. The photographs are shown in the Figure 6.11 (A- D).

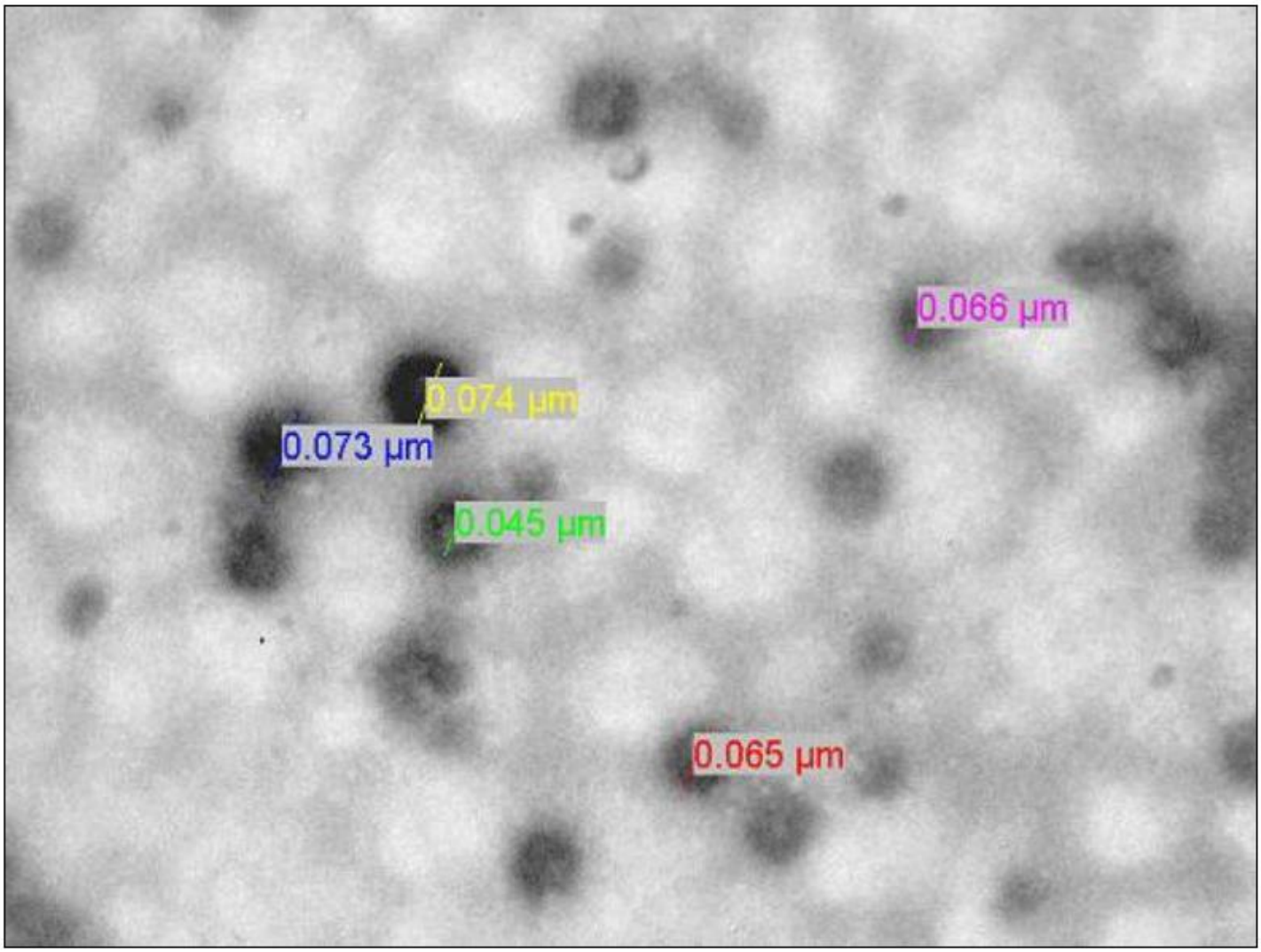

Figure 15: TEM of formulation GM1

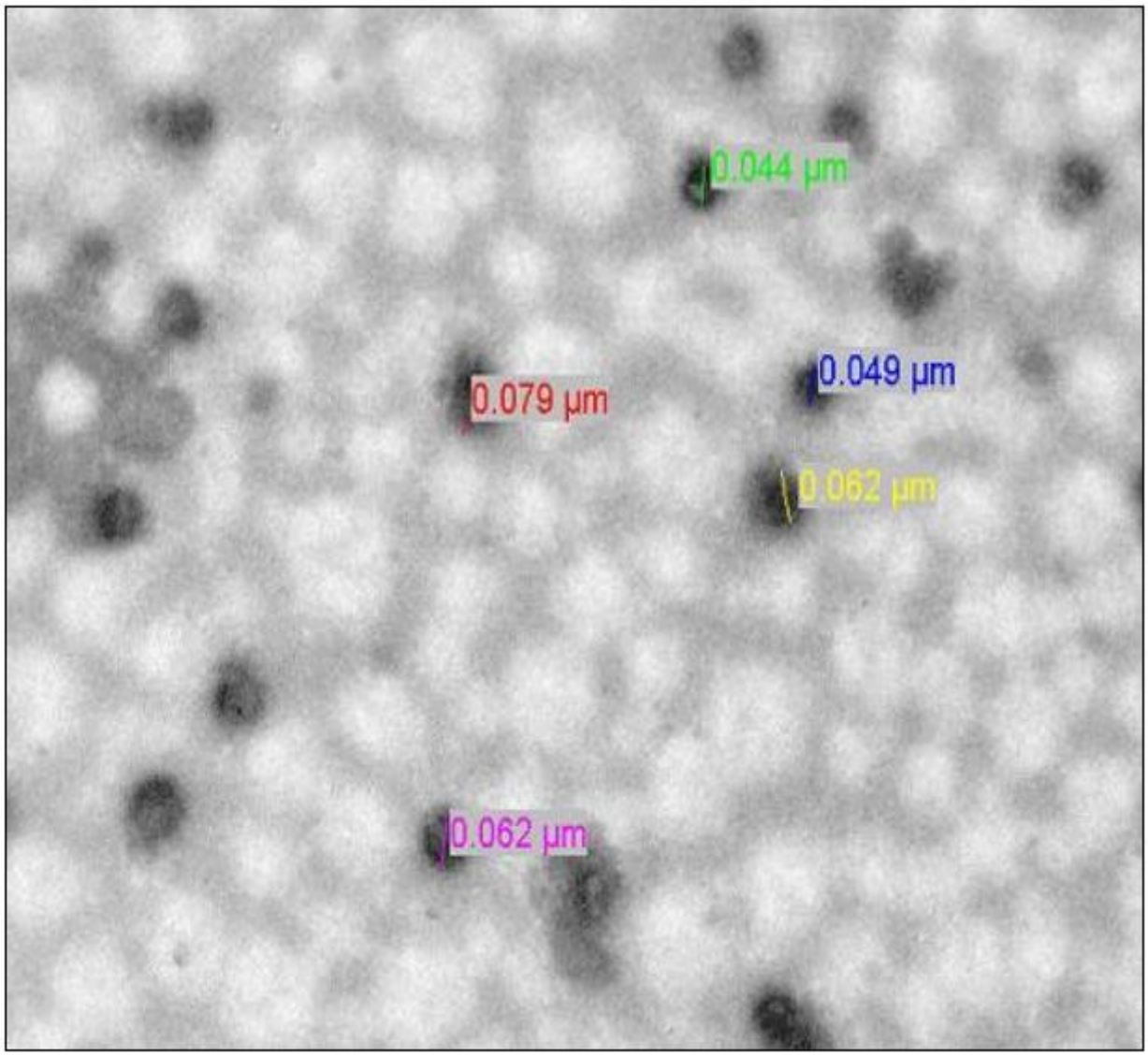

Figure 16: TEM of formulation GM2

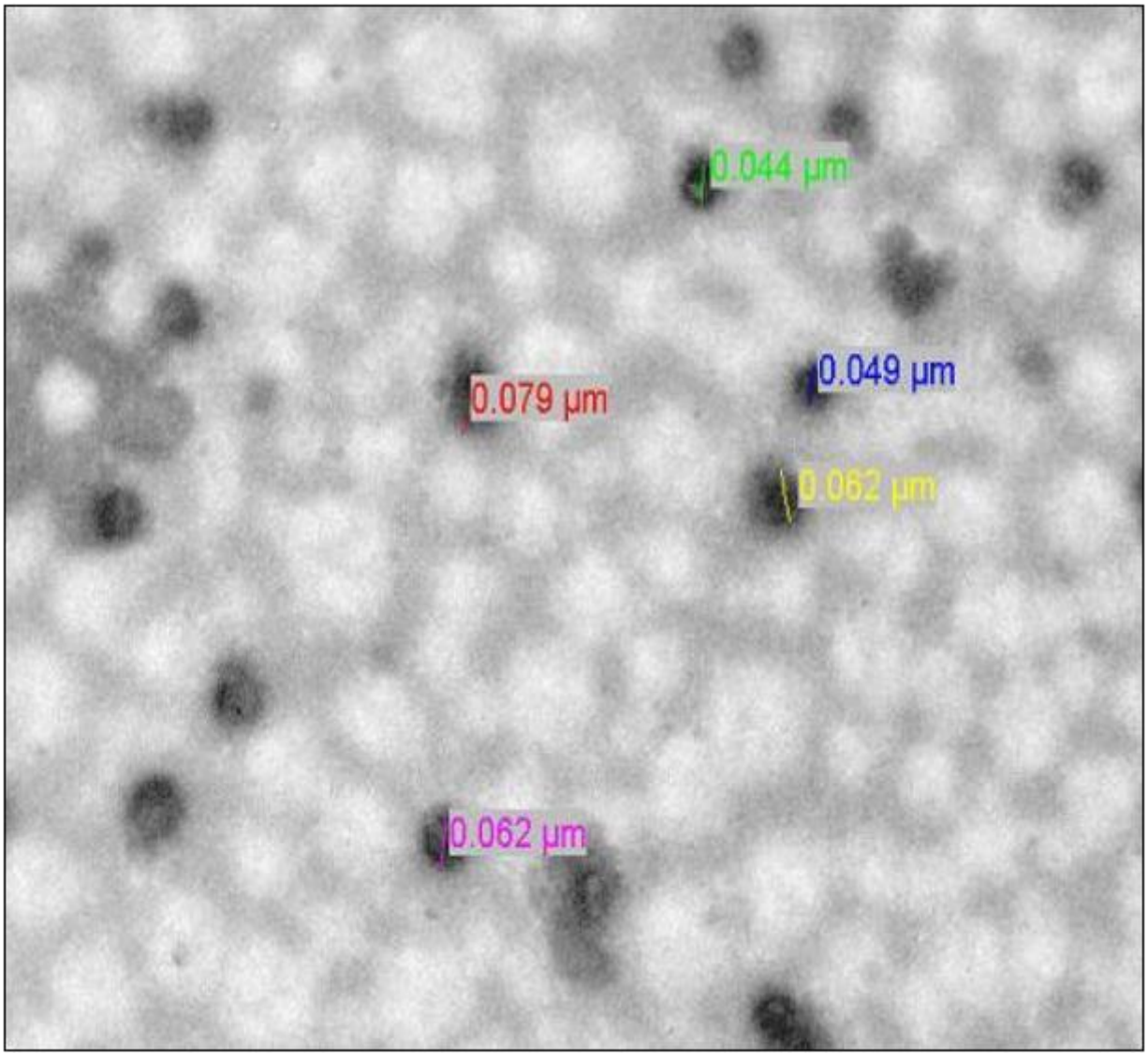

Figure 17: TEM of formulation GM3

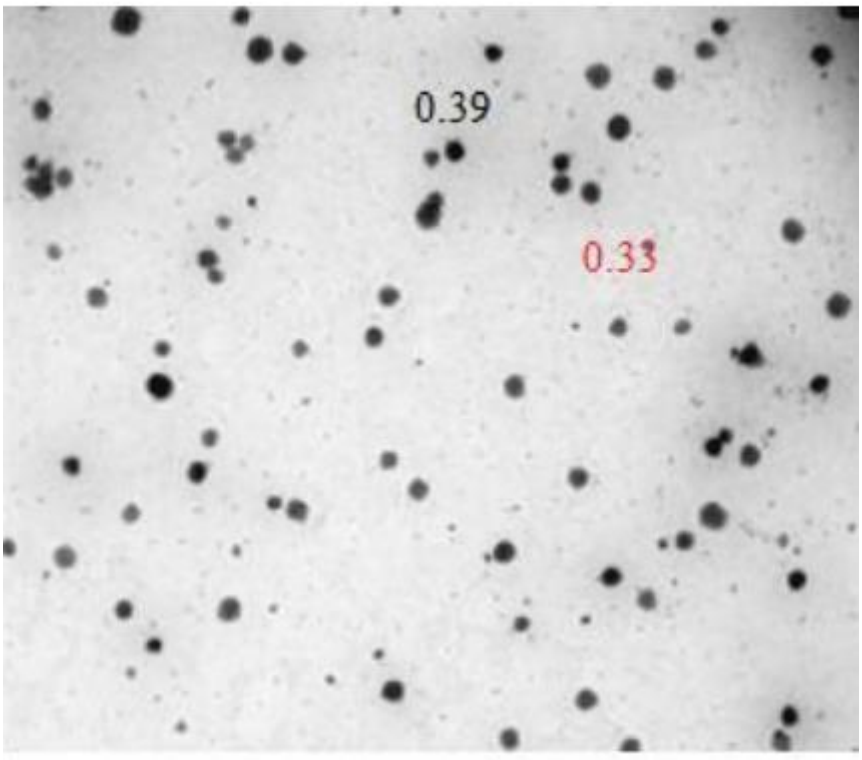

Figure 18: TEM of formulation GM4

Results and discussion

The nanoemulsion appears dark and the surroundings was bright in TEM as it was clear from the image (Figure. 6.11[A-G]), a "positive" image is seen using TEM. Some equally distributed droplet sizes are measured using TEM, as it is capable of point-to-point resolution. The droplet size is in agreement with the results obtained from droplet size analysis. The droplet of all selected formulations was found to be in the range of 19 nm to 79 nm.

In-vitro drug release performance

The study was performed by using dialysis bag method (Shafiq et al., 2007) 6.2.5.1.

Dialysis membrane specification

The dialysis membrane used in the study was Cellulose membrane (Sigma, USA). Tubing as such without treatment is stored at room temperature. Its Capacity was 60 mL/feet; average flat width was 2.5 mm, diameter, and 16 mm. Its molecular weigh cut off was 12000 g/mole.

Treatment of dialysis bag

a) Removed the glycerin by washing in running water for 3-4 hours.

b) Removed sulphur compounds by treating it with 0.3% w/v sodium sulphide solution in water at 800 C for 1 minute.

c) Washed with hot water at 600 C for 2 minutes.

d) Acidified the procured dialysis bag with 0.2% v/v H2SO4 in distilled water

e) Rinsed it with hot water to remove acid.

f) Stored the dialysis bag in the dissolution medium in refrigerator in which you want to carry the dissolution experiments so that the pores remains open

In vitro drug release study

In vitro release test was performed in 250 ml of distilled water, which was based on USP XXIV method (Dissolution apparatus I.P. 2, at 100 rpm and 37 ± 0.5 0C). 1 ml of nanoemulsion formulation (Single dose containing 10 mg of AT Calcium) was placed in treated dialysis bag (MWCO 12,000 g/mole; Sigma, USA) and 1 mL samples was withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2,2.5,3,3.5, 4,4.5,5.5, 6,6.5,7, 8,9, 10, 12, 22 and 24 h) and same amount of distilled water was replaced (Kang et al., 2004).

The withdrawn 1 ml samples were diluted with 3 ml methanol and analyzed for the drug content by using developed RP-HPLC at 247 nm. The same method was used for the suspension containing 10 mgof AT Calcium in 1 ml distilled water.

The release of drug from different selected nanoemulsion formulations was compared with drug suspension and finally selected formulation was used for the further study i.e. for preparation of solid self-nanoemulsifying drug delivery system (SSNEDDS).

Table 16: IN-VITRO dissolution performance of optimized formulations

Time (Hrs)	Name of different formulations					
	GM1	GM2	GM3	GM4	GM5	Drug suspension
	%CR	%CR	%CR	%CR	%CR	%CR
0	0	0	0	0	0	0
0.5	31.43±45.9	23.67±0.91	32.56±1.91	31.78±1.94	32.78±2.89	07.77±0.31
1	37.98±6.79	39.87±1.78	37.67±3.89	34.89±1.90	35.89±1.56	08.07±0.43
1.5	51.54±3.43	54.90±1.21	52.92±1.89	39.90±1.98	37.78±1.12	8.61±0.18
2	59.13±0.87	58.89±0.89	57.89±1.89	41.89±0.15	39.69±1.89	09.61±0.11
2.5	63.43±0.89	61.45±1.26	61.45±0.78	43.97±2.87	41.76±0.18	10.77±0.31
3	71.67±1.56	65.56±0.89	67.82±1.23	48.65±1.98	43.67±1.09	10.87±0.43
3.5	79.68±0.89	68.41±1.89	71.72±3.45	52.67±1.78	47.98±2.12	10.99±0.08
4	85.12±1.32	71.67±5.41	77.89±2.43	58.89±1.72	49.45±4.79	11.19±0.18
4.5	87.89±3.32	74.89±1.43	81.56±4.53	60.12±0.71	51.52±3.72	11.57±0.31
5	89.69±1.21	78.65±0.45	85.87±5.31	64.12±1.71	53.64±5.71	11.97±0.43
5.5	91.69±0.98	80.98±0.92	89.08±0.78	69.89±0.71	58.76±8.90	12.01±0.08
6	98.05±0.90	82.03±1.12	94.97±0.89	71.69±0.71	60.01±1.00	12.91±1.98
6.5	99.65±1.29	82.36±2.12	93.97±0.56	72.69±0.81	61.06±1.31	13.77±0.98
7	99.55±1.29	82.16±2.12	93.37±0.56	73.69±0.81	60.06±1.44	13.07±0.91
8	99.06±1.21	82.26±2.32	92.97±0.56	71.69±0.81	61.06±0.38	12.77±0.90
9	99.65±1.29	82.36±2.12	93.97±0.56	72.69±0.81	61.06±1.41	13.07±0.91
10	99.05±1.22	81.16±2.12	92.91±0.36	70.49±0.51	60.06±0.21	12.01±0.08
12	91.69±0.98	80.98±0.92	89.08±0.78	69.89±0.71	58.76±8.90	11.11±0.18
22	85.12±1.32	71.67±5.41	77.89±2.43	58.89±1.72	49.45±4.79	11.17±0.61
24	87.89±3.32	74.89±1.43	81.56±4.53	60.12±0.71	51.52±3.72	11.90±0.13

%CR =Percentage cumulative release

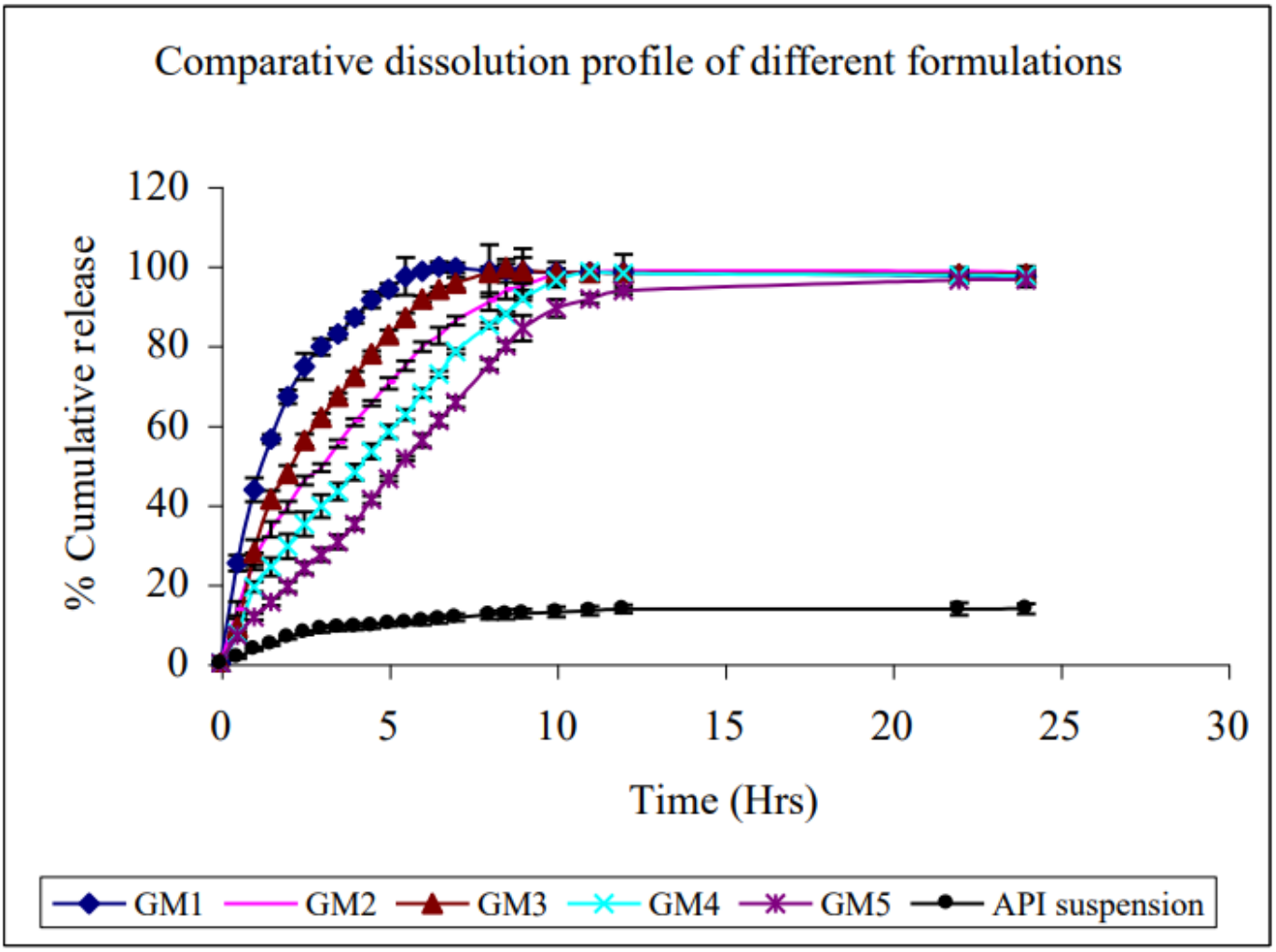

Figure 19: In-vitro optimization of AT nanoemulsion by dialysis bag method in distilled water

Results and discussions

Dissolution study was performed by dialysis bag method in distilled water for the final selection of formulation for further development of solid self-nanoemulsifying dosage forms. The dissolution study was performed for 24 hrs. Drug dissolution from formulation GM1 was very fast as 99.65±1.29% of drug released in 6.5 hrs; while formulations GM2, GM3, GM4 andGM5 showed comparatively slow release i.e. 82.36±2.12, 93.97±0.56, 72.69±0.81 and 61.06±1.31 in 6.5 hrs.

In contrast to this drug released from API suspension was found to be very low i.e. 13.77±0.98% in 6.4 hrs. This result was attributed to the fact that formulation GM1 is having comparatively smaller size (42.8 nm) of oil droplets and hence the larger surface area for dissolution as justified by droplet size distribution and by TEM photograph. The another possible reason was the oil concentration in formulation GM1 (10%) as compared to the GM2 (15%), although having the same concentrations of the Smix, which was not sufficient to emulsify the increased amount of oil in GM2. On the basis of above discussion the formulation GM1 was selected for development of pellets (SSNEDDS).

Methods employed for the preparation of Self-nanoemulsifying pellets

SES is normally prepared as liquid dosage forms that can be administrated in soft gelatin capsules or hard gelatin capsules for ease of administration, which have some disadvantages especially in the manufacturing process (in-process controls), with consequent high production costs.

Other than this, it also posses certain problems such as leaking, leaching of components from the capsule shell, and interaction of SES with capsule shell components are often observed for such liquid-filled capsules. Solidification of liquid systems has been a challenge that has attracted wide attention due to handling difficulties and machinability and stability problems that are often encountered with liquids.

An alternative method, which is currently investigated by several authors, is the incorporation of liquid selfemulsifying ingredients (oil/surfactant/water mixture) into a powder in order to create a solid dosage form (pellets, tablets, capsules). Various attempts have been reported in literature to transform liquids into solids self-emulsifying systems.

Formulation of self-nanoemulsifying pellets (SSNEDDS)

Pellets were prepared by extrusion-spheronization method. The dry excipients (MCC, Starch) and drug containing self-nanoemulsifying formulation were mixed to homogeneity (10 min). Distilled water was used as the wetting agent and a dough of suitable consistency for extrusion was formed. The dough was extruded through a 1.0-mm mesh screen in an extruder at 16 rpm. [Caleva Ltd, UK]. The extrudates were spheronized for 5 min at 500 rpm (Caleva Ltd, U.K). The obtained pellets were dried in an oven at 50o C.

Optimization of self-nanoemulsifying pellets (SSNEDDS)

The preparation of pellets by the method outlined above involved various process variables due to the fact that involved two steps i.e. extrusion and spheronisation. The following process variables were optimized on the basis of physical appearance, strength and sphericity of pellets.

Concentration of microcrystalline cellulose

Different amounts of the microcrystalline cellulose (Avicel 102) were tried for the formulation of self-nanoemulsifying pellets. The optimum amount was selected on the basis of components leaching behaviour. Fixed quantity of formulation GM1 (460 mg) containing 10 mg of AT Calcium and different amount of microcrystalline cellulose (Avicel PH102) were mixed separately and wet mass was prepared by taking water as a granulating agent. The granules were obtained by passing the wet mass through sieve no.18 and then spheronised

Table 17: Optimization of microcrystalline cellulose concentration

Formulation code	Formulation (GM1) (mg)	MCC (mg)	Appearance	Sphericity	Inference (Leaching of oil)
A1	460	340	Rods	-	Leaching
A2	460	365	Dumb-bell	-	Leaching
A3	460	390	Dumb-bell	-	Leaching
A4	460	415	Dumb-bell	-	Leaching
A5	460	440	Elliptical	-	Leaching
A6	460	465	Elliptical	-	Leaching
A7	460	490	Elliptical	-	Leaching
A8	460	515	Non-spherical	-	Leaching
A9	460	540	Non-spherical	-	Leaching
A10	460	565		+	Leaching
A11	460	590	Smooth	+	Less leaching agglomerated
A12	460	615	Smooth	+	Less Leaching
A*13	460	640	Smooth	+	No Leaching
A14	460	665	Bulky	+	Agglomerated
A15	460	690	Bulky	+	Agglomerated
A16	460	715	Bulky	+	Agglomerated

Results and discussion

Taking constant amount of the self-nanoemulsifying mixture of formulation GM1, the amount of microcrystalline cellulose (MCC) was gradually increased to get the granules free from leaching of components from the surface of pellets. In each step of optimization 25 mg of MCC was increased and the surface morphology as well as leaching potential was examined.

Finally 640 mg of MCC (58.2% of total formulation) was optimized and pellets were spherical and free from leaching possibilities. Formulation A 13 having 640 mg of MCC was considered optimized, as the concentration beyond it did not have any appreciable or better effects on the formulation. Morever further increase in MCC quantity would have added to the bulk of the final dose thusmaking its dispensing difficult. Therefore 640 mg was considered as a ceiling point in terms of concentration of MCC (Table 6.20).

Concentration of granulating fluids

Different granulating fluids like water, ethyl alcohol (EtOH), isopropyl alcohol (IPA) alone and in combinations and different concentration of starch paste was used as binder for preparation of pellets in order to see their strength. Results are shown in Table 6.21

Table 18: Optimization of granulating fluid

Formulation code	Granulating mediun	Friability
B1	Water	Passed
B2	Ethanol	Failed
B3	IPA	Failed
B4	Ethanol+IPA(1:1)	Failed
B5	Water+Ethanol	Failed
B6	Water + IPA (1:1)	Failed
B7	Starch paste 0.5%	Passed
B8	Starch paste 1%	Passed

B9	Starch Paste 1.5%	Passed
B10	Starch Paste 2%	Passed

* **SEM** = Self-emulsifying miture

* SEM concentration and MCC concentration were kept at optimum level

Results and discussion

Different binding fluids were tried to get the pellets of sufficient strength (satisfactory friability) (Table 6.21). In the present pelletization process, MCC was used as the pelletizing diluent, which also acts as a binder after swelling in the water. During optimization it was observed that friability of pellets was satisfactory when water alone was used as a granulating fluid. In contrast to this when alcohols (EtOH, IPA) and mixture of alcohol and water were used as a granulating fluid the strength of pellets was very less. The plausible explanation is that these organic solvents hinder the swelling potentials of MCC.

Another explanation is that the organic solvents act as solubilizing agents for SNEDDS composition, which can lead to destabilization of nanoemulsion structure, resulting in the failure of the system.

In order to obtain the selfnanoemulsifying pellets of the desired strength, starch paste of different concentration (0.5 – 2%) was also tried as a granulating agent. It was observed that the strength of pellets obtained after using starch paste as a granulating agent showed no drastic change as compared to the strength of pellets obtained after using water as a granulating agent.

The slight positive change in the strength was presumably attributed to the presence of MCC as well as starch paste, where both act synergistically as a binding agent.

It is also in conformity with earlier literature reports (Nazzal et al., 2002d). MCC itself acts as a release retardant thus restricting the release of the active moiety from the pellets. Therefore in order to alleviate the possibility of reduced release of the drug the use of binding agents like starch paste was avoided. On the basis of the aforementioned justification water was considered to be an optimized granulating agent for pelletization.

Optimization of spheronization speed

After optimizing the amount of MCC and binding fluids, the effect of the various process variables related to spheronization like speed and time using spheronizer (Caleva process solutions Ltd.) were optimized. The basis of sphericity of the obtained pellets was taken as a dependent variable. The other process variable was the spheronisation speed which was varied from 500 rpm to 1600 rpm (Tables 6.22 and 6.23).

Table 19: Optimization of spheronizer speed (rpm)

Spheronization speed (rpm)	Shape	Inference (Sphericity)
500	Rod/ cylindrical	No
600	Rod/ cylindrical	No
700	Dumb bell	Comparatively good
900	Dumb bell	Comparatively good
1200*	**Almost spherical**	**Excellent and uniform**
1500	No any effects	Uneven shape
1600	Almost spherical with fines	Aggregated

Optimization of spheronization time

After optimization of the spheronization speed, spheronization time was optimized taking the constant value of the spheronization speed (Table 6.23). The final formulation selected on satisfactory values of bulk density and appropriate pellets shape.

Table 20: Optimization of Spheronizer time (min)

Formuation code	Spheronization time	Bulk density (g/cc)	Shape of pellets
D1	2	0.442 ± 0.024	Rod/cylindrical
D2	4	0.432 ± 0.015	Rod/cylindrical
D3	5	0.430 ± 0.018	Rod
D4*	**6**	**0.429 ± 0.011**	**Spherical**
D5	7	0.431 ± 0.021	Irregular rod
D6	8	0.435 ± 0.017	Dumb bell

Results and Discussion

Obtained pellets were evaluated visually on the basis of shapes, spherisity and bulk density of pellets. It was well explained earlier that particulate approaches to sphericity will show minimum bulk density. Observation Table shows that formulation D4, measuring minimum bulk density (0.429 ± 0.011 g/cc) after spheronising for 6 min was selected for the final pelletization process at spheronization speed for 1200 rpm (Table 6.23).

In vitro characterization of the optimized pellets

As a matter of fact, in vitro characterization is well known to be the method of choice for the pharmaceutical industry to develop effective delivery system. Therefore various parameters as mentioned below were assessed for reaching at the optimized formulation

Bulk density, tapped density and Hausner factor Pellets (10g) were placed in a 25 ml volumetric cylinder and their volume was determined. The bulk density was calculated as g/cm3 . The cylinder was then tapped 1250 times and the volume was determined again afterward to calculate the tapped density (Steckel and Nogly., 2004).

On the basis of the tapped density and the bulk density, Hausner factor was determined by the following formulas

$$\text{Hausner's ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Carr's flowability index

The flow properties of the powdered lipid formulations were determined by the Carr's method. The following four tests were measured: (1) compressibility, (2) angle of repose, (3) angle of spatula, and (4) uniformity coefficient or cohesion. The flowability index (FI) was then calculated with the point scores as described (Carr, R.L, 1965). . The result is shown in Tables 6.26

% Compressibility

10 g of formulation was poured lightly into a 25ml graduated cylinder. It was tapped until no further change in volume was observed. Bulk density [ρb] (g/cm3), and tapped density [ρp] (g/cm3) were calculated as the weight of the formulations divided by its volume before and after tapping, respectively. Percentage compressibility was computed from the following equation: (Nazzal and Mansoor., 2006). The result is given in Tables 6.26

$$\%\text{Compressibility} = \rho p - \frac{\rho b}{\rho p} \times 100$$

ρp = Tapped density, ρb =Bulk density B.

Angle of repose Angle of repose was measured using a protractor for the heap of granules formed by passing 10 g of pellet sample through a funnel at a height of 8 cm from the horizontal surface (Woodruff and Nuessle.,1972; Hellen et al.,1993b). The angle of repose was calculated by using following formulae. The result is given in Tables 6.2

$$\tan\theta = \frac{\text{Vertical height of heap}}{\text{Radius of heap base}}$$

θ = Angle of repose

Friability

10 g of pellets together with 25 steel/glass spheres (6.35mm diameter) were rotated in a Roche friabilator for 10 min, the resulting material were placed on a 250 μm sieve and shaken for 5 min, the amount of material passed through the sieve was weighted and expressed as a percentage (Steckel and Nogly., 2004).

$$\%\ \text{Friability} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Where the initial weight of the pellets before friability testing and the final weight of pellets retained above the sieve with 0.355 mm aperture size after friability testing were determined (Tables 6.24).

Table 21: In vitro characterization of optimized self-nanoemulsifying pellets

S.No	Parameters	Results
1	Bulk density	0.429 ± 0.01
2	Tapped density	0.468 ± 0.013
3	Hausner's factor	1.09 ± 0.09
4	% Compressibility	8.3 ± 0.54
5	Angle of repose	31.6^0 ± 0.25
7	% Friability	0.957±0.102
8	Particle size distribution	840 to 1410 µm

Particle size analysis

Particle size distribution was determined by sieve analysis. Fifty grams of pellets were put on the top of the sieve with a series of openings ranging from 1700 µm (sieve no. 12), 1400 µm (sieve no 14) 1000 µm (sieve no. 18), 850 µm (sieve no. 20), 425 µm (sieve no 40) to 250 µm (sieve no. 60). The results are reported as percentage of weight retained on each sieve size (Umprayn et al., 1999). Particle size was determined by the method mentioned earlier and results are shown in Table 6.25.

Table 22: Particle size analysis self-nanoemulsifying pellets

Weight of pellet	Sieve no	Sieve sizes (µm)	Weight retained (gm)	%Weight retained
50 gm	12	1700 µm	0.2	0.4
	14	1400 µm	7.6	15.2
	18	1000 µm	9.2	18.4
	20	850 µm	21.0	42.0
	40	425 µm	10.5	21.0
	60	250 µm	1.5	3.0

Results and discussion

Bulk density (0.429 ± 0.01), tapped density (0.468 ± 0.013) and Hausner´s ratio (1.09 ± 0.09) were determined, indicating the good compressibility and filling properties in the capsules. Carr´s flowability index was also determined which shows good correlation between angle of repose (31.60 ± 0.25) and compressibility (8.3 ± 0.54) (Table 6.26).

The friability of the pellets were found to be satisfactory (0.957±0.102) indicating low strength. On the basis of sieve analysis shown in the Table 6.27 it was found that 75.6 % of total pellets were retained on the sieve 14/20. Therefore, it was concluded that most of the pellets were in the particle size range of 840 to 1410 µm. The statistical distributions of the pellets were shown in the Figure 6.13.

In vitro drug release rate

Comparative in-vitro dissolution studies of self-nanoemulsifying pellets as well as marketed tablets Atorlip® (Cipla) and Avas® (microlab) were carried out in USP dissolution apparatus II using 250 ml of three different dissolution media i.e. distilled water (Figure 6.14 and Table 6.26), simulated gastric fluid (pH 1.2) (Figure 6.15 and Table 6.27), and simulated intestinal fluid (pH 7.4) (Figure 6.16 and Table 6.28). Speed was adjusted at 50 rpm. The 1.0 ml sample was removed at each time interval and the same was replaced with the diluting fluid. The samples were filtered using millipore syringe filter and the drug content was estimated using developed RP-HPLC method at 247 nm

Table 23: In vitro dissolution study of self-nanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in distilled water

Time (min)	SNE pellet		Tablet-Microlab		Tablet-Cipla	
	CR µg/mL	%R	CR µg/mL	%R	CR µg/mL	%R
0	5	00	5	00	5	00
3	10	35.10	10	30.1	10	20.10
6	15	60.3	15	35.1	15	32.2
10	20	80.1	20	45.2	20	40.1
12	25	100.02	25	50.1	25	60.04
15	30	100.05	30	65.4	30	65.40
20	35	99.34	35	85.3	35	90.81
25	40	99.32	40	95.2	40	99.70
30	45	99.30	45	95.0	45	99.42
60	50	99.29	50	95.4	50	99.56

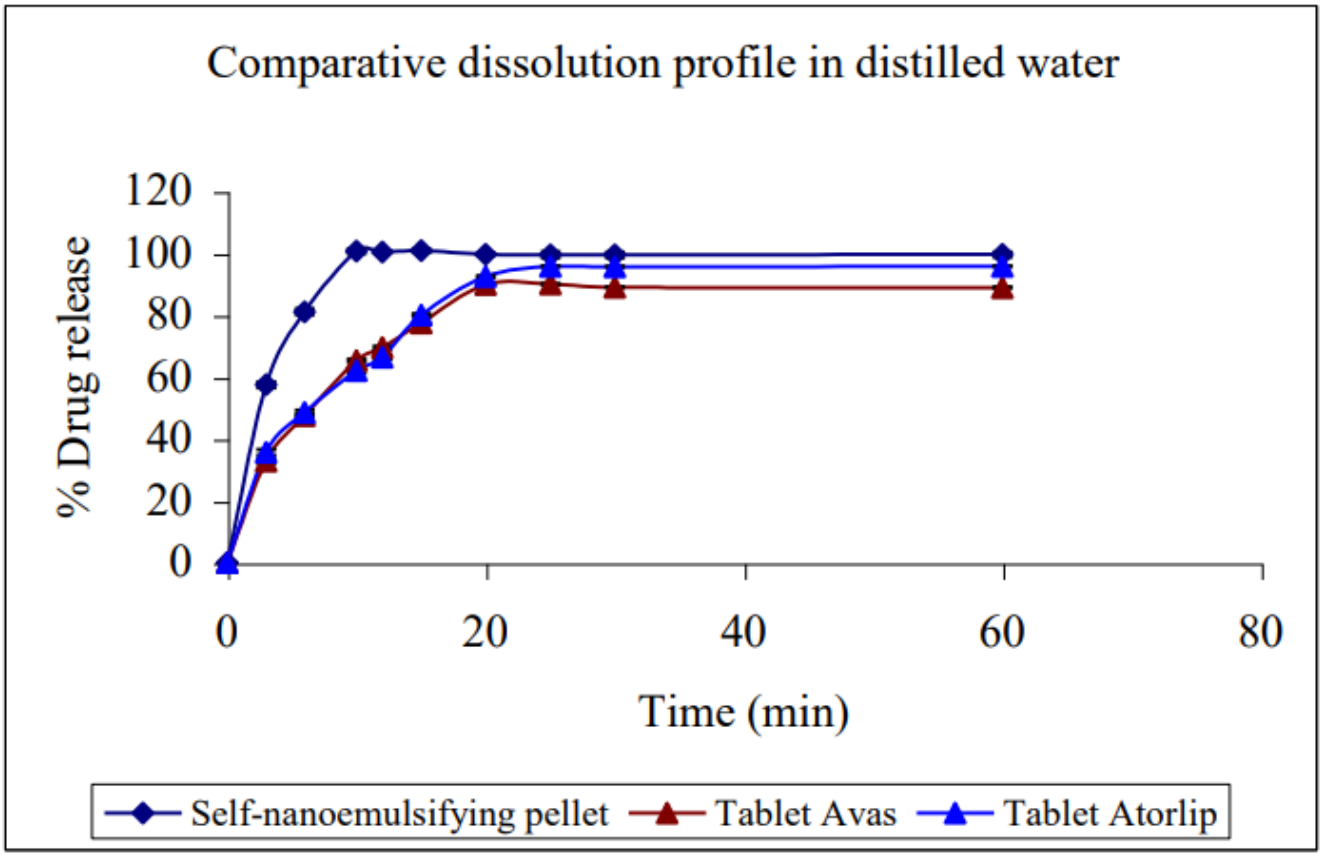

Figure 20: Comparative dissolution profile of % cumulative drug release from selfnanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in distilled water

Table 24: In vitro dissolution study of self-nanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in simulated gastric fluid (pH 1.2)

Time (min)	SNE pellet		Tablet-Microlab		Tablet-Cipla	
	CR µg/mL	%R	CR µg/mL	%R	CR µg/mL	%R
0	5	00	5	00	5	00
3	10	35.60	10	30.23	10	22.35
6	15	59.33	15	34.51	15	35.37
10	20	81.14	20	46.42	20	40.84
12	25	99.64	25	51.17	25	58.93
15	30	99.80	30	64.04	30	66.28
20	35	99.45	35	83.54	35	91.57
25	40	99.38	40	93.82	40	96.37
30	45	99.32	45	95.70	45	98.38
60	50	99.14	50	97.34	50	99.37

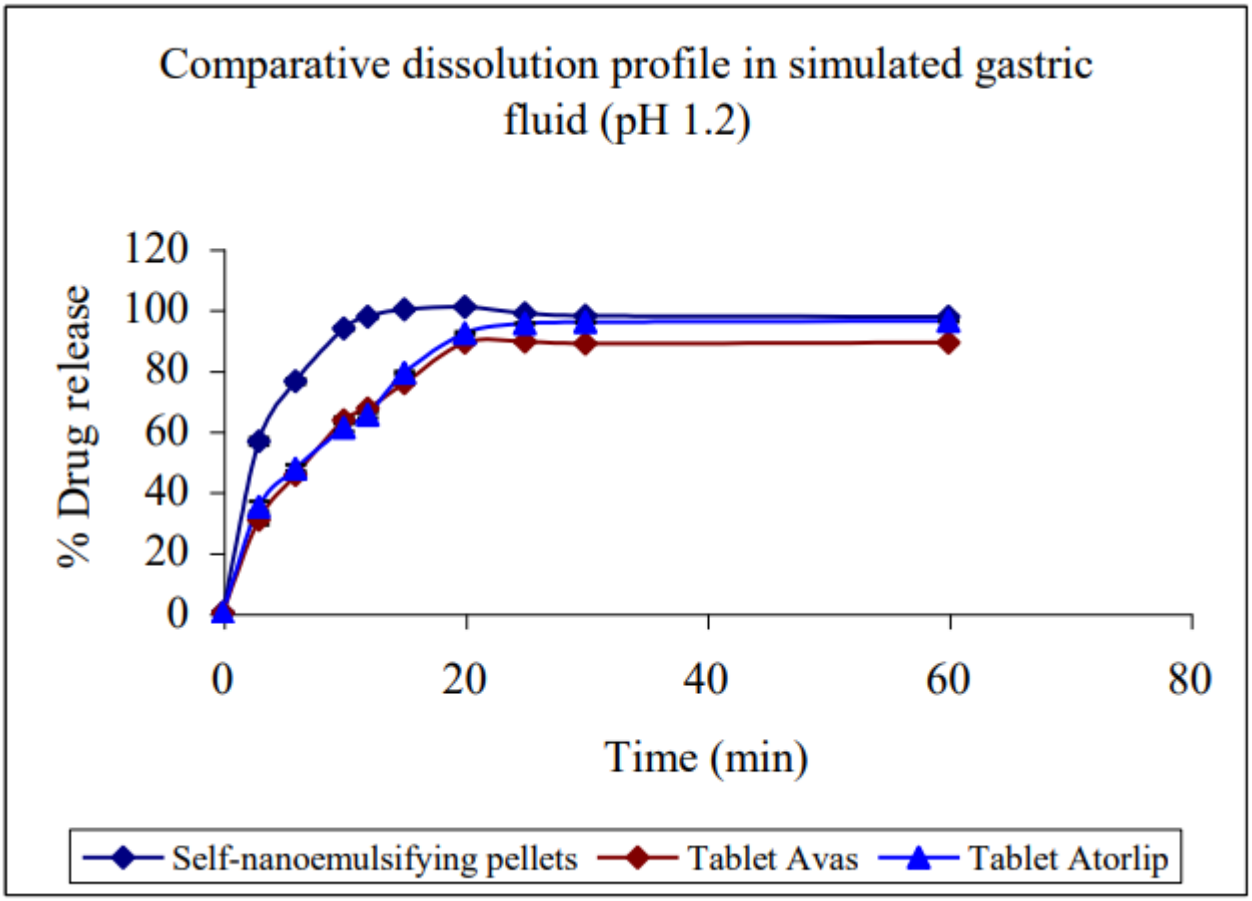

Figure 21: Comparative dissolution profile of % cumulative drug release from selfnanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in simulated gastric fluid (pH 1.2)

Table 25: In vitro comparative dissolution study of self-nanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in simulated intestinal fluid (pH 7.4)

Time (min)	SNE pellet		Tablet-Microlab		Tablet-Cipla	
	CR µg/mL	%R	CR µg/mL	%R	CR µg/mL	%R
0	5	00	5	00	5	00
3	10	36.16	10	31.36	10	19.35
6	15	60.53	15	35.05	15	38.47
10	20	85.68	20	45.46	20	43.85
12	25	96.47	25	52.37	25	55.19
15	30	96.38	30	67.74	30	66.78
20	35	97.75	35	84.24	35	93.97
25	40	98.58	40	97.46	40	96.87
30	45	99.27	45	97.83	45	97.38
60	50	99.84	50	97.67	50	99.64

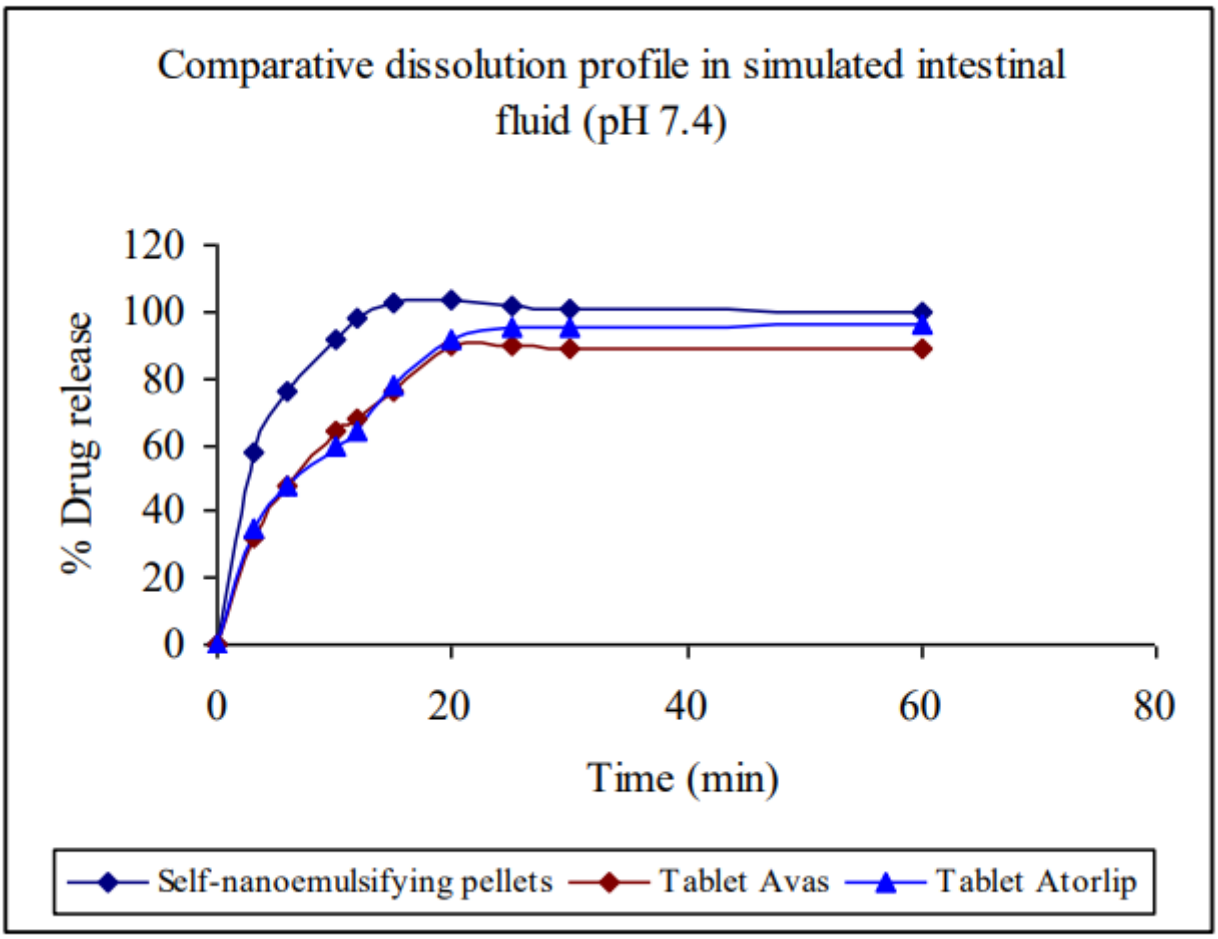

Figure 22: In vitro comparative dissolution study of of % cumulative drug release from selfnanoemulsifying pellets and marketed tablet Avas® (microlab) and Atorlip® (Cipla) in simulated intestinal fluid (pH 7.4)

Results and discussion

The dissolution study was performed using different dissolution medium to see the effect of pH on the dissolution profiles from the self-nanoemulsifying pellets. The result showed (Table 7.28, 7.29 and 7.28) that the release profile of the AT Calcium from the self-nanoemulsifying pellets was found to be almost equal in all dissolution media i.e in distilled water (99.35±0.69), in simulated gastric fluid (99.58±0.24) and in simulated intestinal fluid (97.88±0.31) authenticating the independency of pH influence on the drug release profile.

CONCLUSION

Keeping in view the poor solubility of the drug, the primary step was to ascertain its solubility for which an RP-HPLC method was devised and validated. The solubility of the AT Calcium was determined by using different oils as shown in the Table 6.1. After performing the solubility studies, the solubility of AT Calcium in the Sefsol 218 and Oleic acid mixture was found to be 130.06± 2.68 mg/ml, which was therefore selected as an oil phase for further studies. Similarly the Tween 20 and Carbitol were selected as a surfactant and co-surfactants respectively considering the influence on preliminary study on phase diagram.

The self-nanoemulsifying pellets were formulated and optimized, following the various preliminary steps. The backbone of this delivery system, is to construct different phase diagrams using different Smix ratio. As per earlier solubility studies the Sefsol 218 and Oleic acid were selected as an oil phase. The different surfactants and co-surfactants mixture were tried in order to see the effect on the phase boundary of nanoemulsion in its preliminary study. Finally Tween 20 and Carbitol were selected as surfactant and co-surfactant respectively considering the solubility profile of drug and influence on phase boundary.

After constructing the phase diagrams (Figure 6.2), different formulations were selected from each Smix ratio taking the lowest Smix concentration (Table 6.6 and 6.7). The selected formulations were screened on the basis of thermodynamic stability tests (Table 6.6 and 6.7) and the dispersibility tests in distilled water (Table 6.9 and 6.10). The selected formulations were further screened on the vigorous dispersibility tests using different dispersing medium i.e. distilled water and 0.1N HCl. As per the Table 6.9 and 6.10, it was not possible to go for the further studies by taking all the formulations passing with grade A, so to sort-out this problem five formulations initially were selected having least Smix concentration with grade A in both the dispersing media (Table 6.11)

In-vitro studies were performed to further screen the selected formulation to prepare self-nanoemulsifying pellets. In-vitro characterizations like refractive index (Table 6.12), viscosity (Table 6.13), and droplet size distributions (Table 6.15 to 6.18 and Figure 6.3 to 6.10) and TEM studies (Figure 6.11) were performed to screen the formulations. The mean droplet size of the formulations GM1, GM2, GM3 and GM4 were 42.8 nm, 195 nm, 107 nm and 81.6 nm respectively. The above in-vitro results reveal that the co-surfactants play a role of fluidizing the film layers around the oil water interface. As the concentrations of co-surfactant increases in the Smix the viscosity and the refractive index decreases linearly, but show very insignificant effect on the droplet nanosizing. The surfactant concentration of 1:1 as taken in GM1, was the least and it showed comparable results with other formulations. These findings show the typical behaviour of the surfactants and co-surfactant for the different oils. The formulations were also screened on the basis of dissolution performance in distilled water by dialysis bag method. The result shows the instant drug release from GM1 (99.65±1.29 in 6.5 hrs) formulations as compared to the other one. The GM1 formulations containing AT Calcium were also compared with the API suspension indicating the very significant differences in the dissolution profile (99.65±1.29) compared to suspensions (13.77±0.214) in 6.5 hrs. Considering the in-vitro characterizations GM1 formulations were selected for the pelletization purposes

The selected formulations GM1 were used for pellet preparations. For the preparations of the self-nanoemulsifying pellets, amount of MCC (640 mg) was optimized along with the binding fluid as water (Table 6.20 and 6.21). The parameters of the spheroniser were also optimized. The selected spheroniser speed was 1200 rpm (Table 6.22) with a spheronization time of 6 min (Table 6.23). The in-vitro characterization of prepared self-nanoemulsifying pellets were performed as shown in Table 6.24, 6.25 and 6.26. The particle size distributions of the pellets were done by sieving method indicating the particle size range form 0.84 to 1.41 mm. In-vitro dissolution of self-nanoemulsifying pellets was compared with the marketed tablet (Atorli) using different dissolution medium to see the effects of pH on dissolution profile of self-nanoemulsifying pellets.

The results showed that the dissolutionof self-nanoemulsifying pellets was independent of the pH (Tables 6.29, 6.30 and 6.31). The dissolution profile in simulated gastric fluid seemed to be prolonged (23.3±2.36 min) as compared to the one found in distilled water (13.3±4.7) and in simulated intestinal fluids (21.6±2.36).It was hence concluded that the intrinsic solubility of the AT Calcium might be the responsible factor for prolonged drug release profile and not the variability of nano-emulsifuing pellets in different dissolution media

CHAPTER 6

6.1 REFERNCES

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