FORMULATION AND EVALUATION OF PITAVASTATIN NANOSUSPENSION

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IN
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Submitted by

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Under the Guidance of

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This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

[M. DHANALAKSHMI]

Dedicated to

My parent's sister

and fiends

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The Joyness, satisfaction and euphoria that comes along with successful completion of any work would be incomplete unless we mention names of the people who made it possible, whose constant guidance and encouragement served as a beam of light crowned out effects.

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LIST OF ABBREVIATIONS:

Conc.	Concentration
^{0}C	Degree Centigrade
Mm	Millimeter
%	Percentage
DSC	Differential scanning colorimetry
nm	Nanometer
gm	Gram
mg	Milligram
hr	Hours
λ max	Absorption maxima
Min,	min Minute
PVP	Polyvinylpyrrolidone
µg,mcg	Micro gram
rpm	Rotations per minute
SLS	Sodium lauryl sulfate
UV spectroscopy	Ultra-Violet spectroscopy
R^2	Regression Coefficient
USP	United States Pharmacopoeia
IP	Indian pharmacopoeia
No.	Number

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INTRODUCTION

1.INTRODUCTION

Oral route has been commonly adopted and most convenient route for the drug delivery. Oral drug delivery system has received more attention in the pharmaceutical field, because of its more flexibility in designing the dosage form than the other drug delivery systems [1]. In recent years novel drug delivery systems like nanosuspensions draws a considerable attention in search for improving bioavailability of poorly soluble drugs [2].

It is estimated that more than 1/3 of the compounds being developed by the pharmaceutical industry are poorly water soluble. The solubility/dissolution behavior of a drug is key factor to its oral bioavailability. An improvement of oral bioavailability of poor water-soluble drugs remains one of the most challenging tasks of drug development. To overcome poor solubility, many approaches have been studied. They are generally salt formation, use of surfactant, use of prodrugs and micronization. In micronization, the particle size of a drug powder is reduced to a micron scale size (typically 2-10 micron), which increases the specific surface area and dissolution rates. However, many new drugs are so poorly soluble that micronization is not sufficient, which motivated the development of nanoscale systems. By decreasing the particle size from a micron to a nanometer scale, there is a significant increase in the surface area and related dissolution rate [3, 4].

Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase. Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions alone, or with a combination of pharmaceutical excipients. Nanosuspensions engineering processes currently used are precipitation [5], high pressure homogenization [6], and pearl milling [7], either in water or in mixtures of water and water miscible liquids or non-aqueous media [8].

Nanoprecipitation method presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. In this method, the drug is dissolved in an organic solvent such as acetone, acetonitrile, methanol or ethyl acetate. The organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and

homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. The super saturation is further accentuated by evaporation of drug solvent. This yields to the precipitation of the drug. High shear force prevents nucleus growth and Oswald's ripening [9].

Pitavastatin is a lipid lowering agent. Synthesis of cholesterol via competitive inhibition of the liver enzyme, this is a principal metabolite and an inhibitor of 3-hydroxy- 3-methylglutaryl-coenzyme-A (HMG Co-A) reductase, the enzyme that catalyses an early and Rate-limiting step in the biosynthesis of cholesterol [10]. Pitavastatin is a white to pale-yellow powder, crystalline, nonhygroscopic powder, insoluble in water and 0.1N HCl (30mg/ml and 60mg/ml, respectively). Pitavastatin belongs to BCS class-II drug category in which the drug is poorly soluble and highly permeable. It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limited absorption. Improvement of aqueous solubility in such case is a valuable goal to improve therapeutic efficacy. The dissolution rate is a function of the solubility and the surface area of the drug, thus, dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug [11, 12].

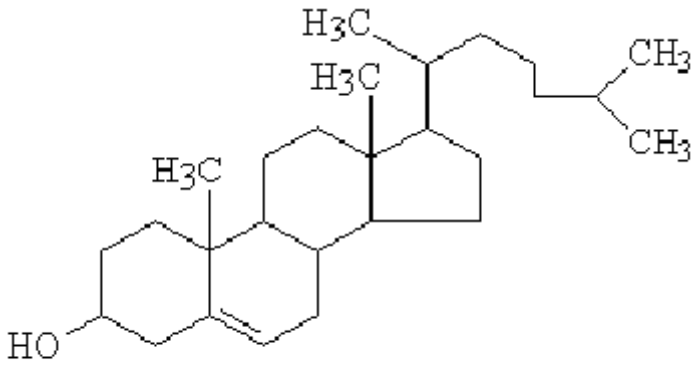
In this present study, an extensive literature survey indicates no reports available on the pharmaceutical approaches for improving the solubility of Pitavastatin. Therefore the present study aimed to develop nanosuspension of Pitavastatin by nanoprecipitation technique and evaluate for physicochemical and *in-vitro* release characterization.

2. REVIEW OF LITERATURE

2.1. CHOLESTEROL

Cholesterol is a waxy substance made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. Cholesterol is needed in the body to insulate nerves, make cell membranes and produce certain hormones, and it is an important lipid in some membranes. However, the body makes enough cholesterol, so any dietary cholesterol isn't needed. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. High cholesterol in serum is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke.

Chemical Structure of Cholesterol:



Cholesterol

Good Cholesterol and Bad Cholesterol:

HDL is called “good cholesterol” that is good for the cardiovascular system and LDL is called “bad cholesterol” that is bad for the cardiovascular system. These are the form in which cholesterol travels in the blood. LDLs have little protein and high levels of cholesterol and HDL has a lot of protein and very little cholesterol. LDL is the main source of artery clogging plaque. HDL actually works to clear cholesterol from the blood. The standard test of cholesterol is done after a 9-12 hours fast without food, liquids or pills. It gives information about total cholesterol, LDL, HDL and triglycerides (blood fats). The cholesterol content in blood is the key data for the health information of cholesterol related (Table 1).

Table 1. Initial classification based on total cholesterol, HDL, LDL and Triglyceride

CHOLESTEROL	CHOLESTEROL LEVEL	CATEGORY
Total Cholesterol	Less than 200 mg/dl	Desirable level.
	200 to 239 mg/dl	Borderline high for heart disease.
	240 mg/dl and above	High blood cholesterol. A person with this level has more than twice the risk of heart disease as someone whose cholesterol is below 200 mg/dl.
HDL Cholesterol	Less than 40 mg/dl	Low HDL cholesterol. A major risk factor for heart disease.
	40 to 59 mg/dl	The higher HDL level, the better.
	60 mg/dl and above	High HDL cholesterol. An HDL of 60 mg/dl and above is considered protective against heart disease.
LDL Cholesterol	Less than 100 mg/dl	Optimal
	100 to 129 mg/dl	Near or above optimal
	130 to 159 mg/dl	Borderline high
	160 to 189 mg/dl	High
	190 mg/dl and above	Very high
Triglyceride	Less than 150 mg/dl	Normal
	150-199 mg/dl	Borderline high
	200-499 mg/dl	High

Triglyceride:

Triglycerides are another fat in the bloodstream. High levels of triglycerides are also linked to heart disease. Triglyceride is the most common type of fat in the body. Many people who have heart disease or diabetes have high triglyceride levels. Normal triglyceride levels vary by age and sex. A high triglyceride level combined with low HDL cholesterol or high LDL cholesterol seems to speed up atherosclerosis, which is the buildup of fatty deposits in artery walls that increase the risk for heart attack and stroke. People should reduce the amount of saturated fat, trans-fat, cholesterol and total fat in

their diet. Some studies have shown a higher mortality in people with low cholesterol levels - that is, lower than 160 mg/dl. These deaths are from non-coronary causes (some cancers, chronic respiratory disease, liver disease and trauma). On the other hand, some evidence suggests that total cholesterol levels below 160 mg/dl are not dangerous. In many countries a major portion of the population has cholesterol levels in this range throughout life without serious health problems. Also, less than 6 percent of the American population has a cholesterol level below 160 mg/dl. It's rarely necessary to lower total cholesterol below that.

Hyperlipidemic Drugs:

Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides. They're transported in the blood as part of large molecules called lipoproteins. These are the five major families of blood (plasma) lipoproteins: (1) chylomicrons, (2) very low-density lipoproteins (VLDL), (3) intermediate-density lipoproteins (IDL), (4) low-density lipoproteins (LDL), (5) high-density lipoproteins (HDL). When hyperlipidemia is defined in terms of class or classes of elevated plasma lipoproteins, the term hyperlipoproteinemia is used. Hypercholesterolemia is the term for high cholesterol levels in the blood. Hypertriglyceridemia refers to high triglyceride levels in the blood.

Medications:

The main goal in lowering cholesterol is to lower your LDL and raise your HDL. To lower cholesterol, eat a heart-healthy diet, exercise regularly, and maintain a healthy weight. Some may also need to take cholesterol lowering medications. Cholesterol-lowering medicine is most effective when combined with a low-cholesterol diet and exercise program**.** The drugs of first choice for elevated LDL cholesterol are the HMG CoA reductase inhibitors, e.g. lovastatin, Pravastatin and simvastatin. Statin drugs are very effective for lowering LDL cholesterol levels and have few immediate short-term side effects [13]. They are easy to administer, have high patient acceptance and have few drug-drug interactions.

Type of hyperlipidemia:

1. Primary hyperlipidemia
2. Secondary hyperlipidemia

Anti-Hyperlipidemic drugs are mainly classified into 5 types [14]:

- **HMG-CoA Reductase Inhibitors (HMGs or statins):** Pravastatin, Simvastatin, pitavastatin, Atorvastatin, Fluvastatin, Lovastatin. (They are most potent LDL reducers)
- **Fibrates:** E.g. Fenofibrate, Gemfibrozil, Clofibrate
- **Anion –exchange resins(bile acid sequestrates):** E.g. Colesevelam, Colestipol, Cholestyramine
- **Nicotinic acid:** E.g. NIACIN.
- **Cholesterol absorption inhibitors:** E.g. Ezetimibe.
- **Other drugs** E.g. Alpha-tocopherol acetate (vitamin E), Omega-3 marine triglycerides (Maxepa), Orlistat.

Mechanism of action of statins:

- Block the rate-limiting enzyme for endogenous cholesterol synthesis, hydroxy-methylglutaryl Coenzyme A *(HMG CoA)* reductase.
- Increased synthesis of LDL-receptors (up regulation) in the liver
- Increased clearance of LDL from the circulation

Note: Plasma total cholesterol and LDL-cholesterol fall to attain a maximum effect 1 month after therapy.

Therapeutic uses:

These drugs are effective in lowering plasma cholesterol levels in all types of hyperlipidemia However, patients who are homozygous for familial hypercholesterolemia lack LDL receptors and therefore, benefit much less from treatment with these drugs. These drugs are often given in combination with other antihyperlipidemic drugs.

Pharmacokinetics of statins:

Pravastatin and Fluvastatin, Pitavastatin are almost completely absorbed after oral administration.

- Oral doses of lovastatin and simvastatin are from 30 to 50 percent absorbed.
- Pravastatin and Fluvastatin are active, whereas lovastatin and simvastatin must be hydrolyzed to their acid forms.
- Excretion takes place through the bile and feces
- Some urinary elimination also occurs.
- Their half-lives range from 1.5 to 2 hours.

Note:

1. Transient and minor abnormality of liver function tests.

2. Myopathy and rhabdomyolysis (disintegration or dissolution of muscle and elevation of muscle enzymes (creatine, phosphokinase, CPK) the risk is greater in:

- In patients with renal insufficiency
- In patients taking drugs such as cyclosporine, itraconazole, erythromycin, Gemfibrozil, or niacin. Plasma creatine kinase levels should be determined regularly.

ROUTE OF ORAL DRUG DELIVERY

Definition:-

A route of administration in pharmacy is the path by which a drug is taken into the body [15].

Oral route: -

In this route the drug is placed in the mouth and swallowed. It is also called per oral (p.o.)

Advantages of Oral route:

• Convenient - Can be self administered, pain free & easy to take

• Absorption - Takes place along the whole length of the gastro intestinal tract

• Cheap - Compared to most other parenteral routes.

Disadvantages of oral route:

• Sometimes inefficient - only part of the drug may be absorbed.

• First-pass effect - drugs absorbed orally are initially transported to the liver via the portal vein.

• Irritation to gastric mucosa – nausea and vomiting.

• Destruction of drugs by gastric acid and digestive juices.

• Effect too slow for emergencies.

• Unpleasant taste of some drugs.

• Unable to use in unconscious patient.

Table 2: Medication Routes and Administration: [16].

Forms	Acts In:	Advantages	Disadvantages	Technique
Tablet(pressed powder)	30-60 minutes	• Easy to administer • Inexpensive • Can be removed (lavage	• Not good in emergencies • Can irritate GI tract • Absorption is unpredictable	• Follow correct technique for ”pouring” meds • Do not touch meds • Person should be up- not laying down • Offer fluids to help swallowing • Make sure the person swallows the meds Do not force person to take meds If person refuses medication try again a short time later; keep meds secure and labeled until administered
Capsule (drug in a gelatin shell)	30-60 minutes			Same as above
Time released (medication in gelatin cap; also called Long Acting, Sustained	30-60 minutes	Drug is made to dissolve over a period of time and the effects last longer	Can’t crush or chew	Same as above

Release and/ or Spansule)				
Sprinkle (drug in a gelatin cap that can be opened to pour on food for administration)	30-60 minutes	Can open cap and pour on food to administer	Can't chew sprinkles	Same as above
Enteric coated (drug coated with hard shell so it will pass the stomach and dissolve in the intestines)	30-60 minutes	Will not cause stomach upset	Can't crush or chew	Same as above
Liquid Suspension (particles of drug suspended in a liquid agent)	30-60 minutes		Make sure you know if the person has an order to have liquids thickened to a specific consistency	• Wash hands • Make sure you know how many "ml" or "cc" you need to pour to get the dose you have to give • Gently shake the bottle • Have medication label facing into palm of hand

				• Use disposable medication cup for measuring liquid medications
Liquid Suspension (continued)	30-60 minutes			• Place cup on a flat surface at eye level • Pour the prescribed medication
Syrups (med is dissolved in liquid with sugar and flavoring	30-60 minutes	Same as Liquid Suspension	Same as Liquid Suspension	Same as Liquid Suspension
Elixir (Med is dissolved in liquid with sugar, flavoring and alcohol)	30-60 minutes	Same as Liquid Suspension	Same as Liquid Suspension	Same as Liquid Suspension

2.2. NANOTECHNOLOGY

Nanotechnology, the term derived from the Greek word Nano, meaning dwarf, applies the principles of engineering, electronics, physical and material science, and manufacturing at a molecular or submicron level. The materials at nanoscale could be a device or a system or these could be supra molecular structures, complexes or composites. An early promoter of nanotechnology, Albert Franks, defined it as 'that area of science and technology where dimensions and tolerances are in the range of 0.1nm to 100nm'. Nano technology is expected to make significant advances in the mainstream biomedical applications, including in the areas of gene therapy, drug delivery, imaging, and novel drug discovery techniques [17].

Nanotechnology is hailed as a new generation of technology with the potential to revolutionise many facets of the world we live in. This includes virtually all aspects of daily life, including health and health care, the manufacturing and use of materials and equipment, the environment and protection thereof. It is said to be able to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology will be smaller, cheaper, lighter yet more functional and require less energy and fewer raw materials to manufacture. However, the 'revolution' will not happen overnight and very large investments in research and development will be required in the process.

Nanotechnology can be defined as having the following features

- It involves research and technology development at the 1 nm –100 nm range.
- It creates and uses structures that have novel properties because of their small size.
- It builds on the ability to control or manipulate at the atomic and molecular scale.

At the nano-scale the interactions and physics between atoms display 'exotic' properties that are absent at larger scale because at this level atoms leave the realm of classical physical properties behind and enter the realm of quantum mechanics.

Table: 3 Comparative size illustrations

100 m=1 m=1000 mm	Human length (~1.7 m)
10-1 m=10 cm	
10-2 m=1 cm	
10-3 m=1 mm=1000 µm	Sand filter grain (0.5 mm=500 µm)
10-4 m=0.0001 m=100 µm	Hair diameter (75 µm=75000 nm)
10-5 m=10 µm	Red blood cells (5000 nm=5 µm)
E.coli (2000 nm= 2 µm)	
10-6 m=1 µm=1000 nm	10-6 m=1 µm=1000 nm
10-7 m=100 nm	Virus (50 nm) Pore size of UF membrane (50-200 nm) Colloidal particles (20-100 nm)
10-8 m=10 nm	DNA molecule (2 nm wide Typical protein (3 nm diameter)
10-9 m=1 nm	Buck ball(1 nm)

Nanotechnology includes a bewildering array of activities including: molecular manufacturing, supramolecular and self assembly/organization; biomimicry; Nanoparticle (e.g. Bucky balls and carbon Nano tubes), nanospheres, Nano cups and nanorods; nanobots (nanorobots); colloids, micelles, vesicles and Nano-emulsions; clathrate complexes and intercalation compounds.

The National Science Foundation in the USA predicts that the global marketplace for goods and services using nanotechnologies will grow to $1 trillion by 2015, and there are already over 500 products being sold that claim they are made with nanoscale or engineered nanomaterials. These include products like self-cleaning windows, automobile paint, sunscreens, and tennis rackets. In the future, a marriage of nano- and biotechnology

will likely create a whole new generation of drugs, biomedical devices, and other solutions to some of our most challenging medical problems [18].

Nanotechnology in drug delivery

The development of delivery systems for small molecules, proteins and DNA has been impacted to an enormous degree over the past decade by nanotechnology, and has led to the development of entirely new and somewhat unpredicted fields. For the pharmaceutical industry, novel drug delivery technologies represent a strategic tool for expanding drug markets. The technology can address issues associated with current pharmaceuticals such as extending product life (line extension), or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance [19]. This technology is permitting the delivery of drugs that are highly water- insoluble or unstable in the biological environment. Advantages of nano sizing of drugs has the potential to: Increase surface area, enhance solubility, increase rate of dissolution, increase oral bioavailability, more rapid onset of therapeutic action, decrease the dose needed, decrease fed/fasted variability and decrease patient to patient variability.

In recent trend, nano drug delivery may occur through gold nanospheres and rods, nanowires, nanotriangles, nanostars, nanocubes, and nanorice. The size of these nano configurations varies from 1 to 100 nm. Nanoplatforms include organic nanostructures, polymeric nanoparticle, lipid systems-liposomes, self assemblies-micelles, dendrimers, and carbon nanostructure-nanotubes. Inorganic nanostructures include metal nanoparticle and nanoshells, silicon nanostructure, nanocrystals, and quantum dots. Hybrid nanostructures, combining two to three of those previous listed can also be produced. Studies were described in which polymeric nanoparticles were used for tumor-targeted deliver. Gelatin-based engineered nanoparticles have been used for gene delivery and multifunctional nanoemulsions for oral and intravenous delivery. Gadolinium-loaded nanoemulsion has been used in animals for brain imaging, and this technology could easily be used for imaging within the eye to observe the results of various drug delivery modalities.

The benefits of nanotechnology are

- The lifespan of the blockbuster drugs can be resurrected by reformulating the drugs through novel drug delivery system.
- The effective patent protection can be enhanced.
- Drug delivery formulation involves low cost research compared to that for the discovery of new molecules.
- Minimizing use of expensive drugs would reduce the cost of the product.

Significance of drug delivery and targeting:

Although opportunities to develop nanotechnology based efficient drug delivery systems extend into all therapeutic classes of pharmaceuticals, the development of effective treatment modalities for the respiratory, central nervous system and cardio vascular disorders remains a financially and therapeutically significant need. Many therapeutic agents have not been successful because of their limited ability to reach to the target tissue. In addition, the faster growth opportunities are expected in developing delivery systems for anti cancer agents, hormones and vaccines because of safety and efficacy shortcomings in their conventional administration modalities. For example, in cancer chemotherapy, cytostatic drugs damage both malignant and normal cells alike. Thus, a drug delivery strategy that selectively targets the malignant tumor is very much needed. Additional problems include drug instability in the biological milieu and premature drug loss through rapid clearness and metabolism. Similarly, high protein binding of certain drugs such as protease inhibitors limits their diffusion to the brain and other organs. However, nanotechnology for drug delivery applications may not be suitable all drugs, especially those drugs that are less potent because the higher dose of the drug would make the drug delivery system much larger, which would be difficult to administer.

2.3. NANOSUSPENSION

A pharmaceutical nanosuspension is defined as "very finely disperaqueous vehicle, stabilized by surfactants, for either oral and topical use or parenteral and pulmonary administration, with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability". The diameter of the suspended particle is less than 1 μ min size (i.e. 0.1nm-1000 nm) [20, 21]. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm.3 An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nano size particles can increase dissolution velocity and saturation solubility because of the vapor pressure effect [22].

Need of Nanosuspensions

More than 40% of drugs are poorly soluble in water, so they show problems in formulating them in conventional dosage forms. Also, for class II drugs which are poorly soluble in aqueous and organic media, the problem is more complex [23]. Preparing nanosuspensions is preferred for such compounds that are insoluble in water (but are soluble in oil) with high log P value. Various approaches to resolve problems of low solubility and low bioavailability micronization, co-solvency, oily solution, salt formation- some other techniques are liposomes, emulsions, microemulsion, solid dispersion, ß- cyclodextrin inclusion complex etc. But, many of these techniques are not universally applicable to all drugs [23]. In these cases nanosuspensions are preferred. In case of drugs that are insoluble in both water and in inorganic media instead of using lipidic systems, nanosuspensions are used as a formulation approach. It is most suitable for the compounds with high log P value, high melting point, and high dose. Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster (e.g., oral or intravenous (IV) administration of the Nanosuspensions).

This is one of the unique advantages that it has over other approaches for enhancing solubility. It is useful for molecules with poor solubility, poor permeability or both, which

poses a significant challenge for the formulators. Major issues associated with poorly water-soluble compounds [24]**:**

- Poor bioavailability.
- Inability to optimize lead compound selection based on efficacy and safety.
- Fed/fasted variation in bioavailability.
- Lack of dose-response proportionality.
- Suboptimal dosing.
- Use of harsh excipients, i.e., excessive use of co-solvents and other excipients.
- Use of extreme basic or acidic conditions to enhance solubilization.

Although, all marketed products, currently are produced by so-called 'top-down techniques', in which the Nanoparticle are obtained through size reduction into the submicron-range, bottom-up techniques and especially controlled precipitation method, are methods of interest for nanozation of poorly soluble drugs. In this method, without any harsh conditions and only with simple equipments one could reduce the particle size to few hundred nanometers range. Therefore, whatever method which is used for the production of Nanosuspensions, a careful evaluation of the type and concentration of the stabilizer is a critical stage for the successful production of nanosuspensions. Both polymeric and surfactant stabilizers can be used for this purpose [25].

Nanosuspensions differ from Nanoparticle, which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid Nanoparticle (SLN), which are lipidic carriers of drug. The key difference from conventional formulations of suspensions is that the particle size distribution of the solid particles in Nanosuspensions is usually less than 1 μm (i.e. 0.1nm-1000nm), with an average particle size range between 200–600 nm. On the other hand, the particle diameter required in most good pharmaceutical suspensions, is 1 to 50 μm. In Nanosuspensions, the overall bioavailability is improved by an increase in surface area and saturation solubility via particle size reduction. This system cannot be achieved by the conventional milling techniques. (Figure 1). [20]

Major Advantages of Nanosuspensions [23] (Table 4)

- Its general applicability to most drugs and its simplicity.
- Can be applied for the poorly water soluble drugs.
- Can be given by any route.
- Reduced tissue irritation in case of subcutaneous/intramuscular administration.
- Rapid dissolution and tissue targeting can be achieved by IV route of administration.
- Oral administration of Nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability.
- The absorption from absorption window of the drugs can be increased, due to reduction in the particle size (Figure 2).
- Higher bioavailability and more consistent dosing in case of ocular administration and inhalation delivery (Figure 3 & 4).
- Drugs with high log P value can be formulated as Nanosuspensions to increase the bioavailability of such drugs.
- Improvement in biological performance due to high dissolution rate and saturation solubility of the drug.
- Ease of manufacture and little batch-to-batch variation.
- Long term physical stability (Due to absence of Ostwald ripening).
- Nanosuspensions can be incorporated in tablets, pellets, hydrogel and suppositories are suitable for various routes of administration.
- Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility.
- Possibility of surface-modification of Nanosuspensions for site specific delivery.
- Possibility of large-scale production, the pre-requisite for the introduction of a delivery system to the market.

Route of Administration	Disadvantages of Conventional Formulations	Benefits of Nanosuspensions
Oral	Slow onset of action/ poor absorption	Rapid onset of action/ improved solubility so improved bioavailability Reduced fed/fasted ratio
Ocular	Lachrymal wash off/ low bioavailability	Higher bioavailability/ dose consistency Lesser irritation
Intravenous	Poor dissolution/ non-specific action	Rapid dissolution/ tissue targeting Prolonged retention time in systemic circulation
Intramuscular	Low patient compliance due to pain	Reduced tissue irritation High bioavailability Rapid onset of action
Inhalation	Low bioavailability due to low solubility	Rapid dissolution/ high bioavailability/ dose regulation

Table 4: Advantages of Nanosuspensions over conventional formulations [20]:

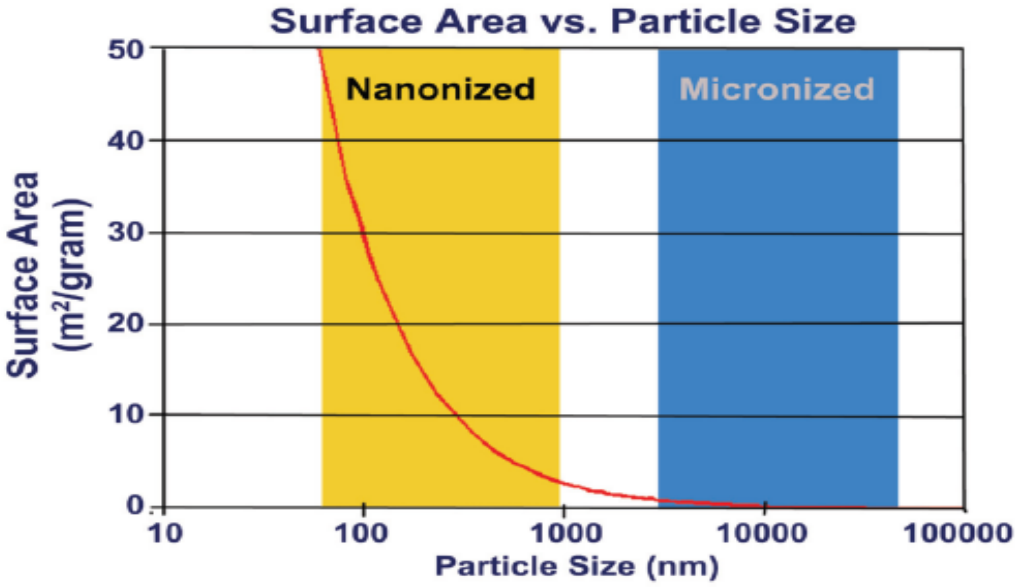


Figure 1[24]: the plot demonstrates the increase in surface area obtained when solids are fractured from the micron-size range (microparticles) to the nanometer-size particles used in the various Nanoparticle formulations to improve the performance of poorly water-soluble compounds.

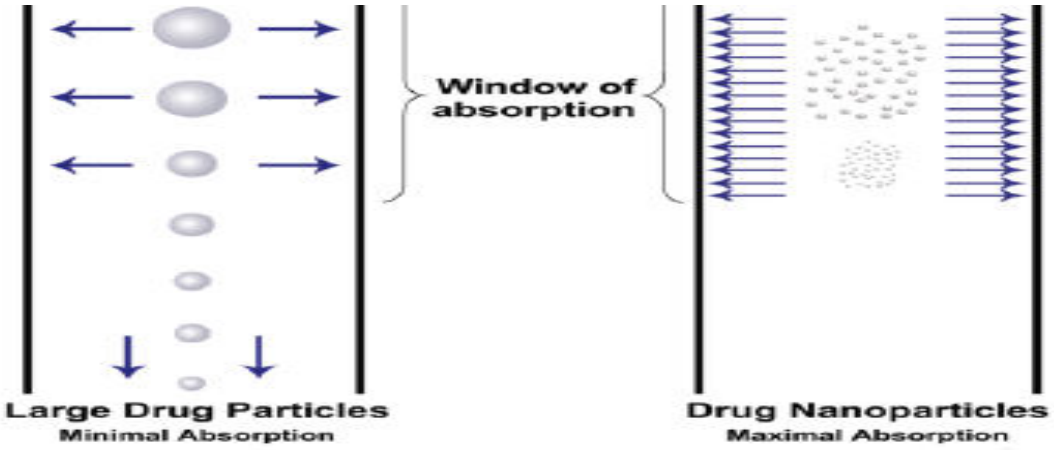


Figure 2[24]: The diagram demonstrates one of the primary issues associated with poorly water-soluble molecules whose bioavailability is dissolution-rate limited. On the left, large drug particles cannot adequately dissolve, which results in the inability to be absorbed. On the right, nanometer drug particles are rapidly dissolved during transit through the gut, thus maximizing absorption and improving bioavailability.

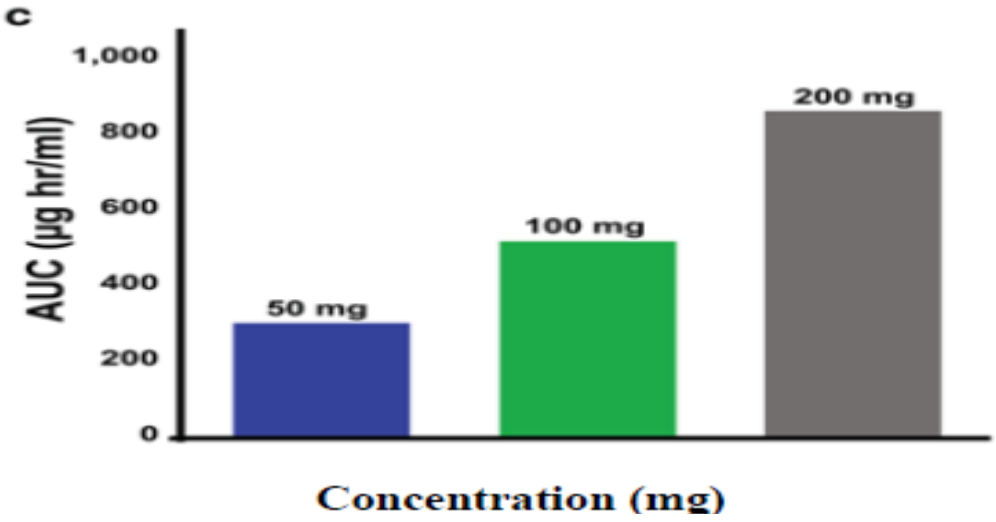


Figure 3[24]: A dose-escalation study is shown demonstrating dose proportionality for a Nanoparticle formulation of a poorly water-soluble compound.

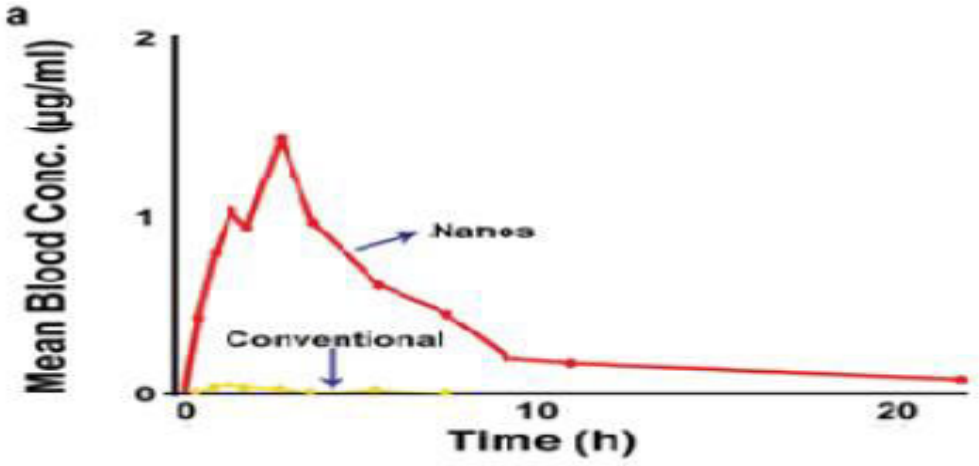


Figure 4[24]: The bioavailability of a poorly water-soluble model compound formulated as a Nanoparticle dispersion (red) or as a conventional crude suspension (yellow).

Formulation Considerations:

Stabilizer [23]:

Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nanosized particles can induce agglomeration or aggregation of the drug crystals. The main function of a stabilizer is to wet the drug particles thoroughly, and to prevent Ostwald's ripening and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behavior of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nanosuspension. The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case e.g. Cellulosics, Poloxamer, Polysorbates, Lecithin and Povidone. Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable nanosuspensions.

Organic solvents [23]:

Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or microemulsion as a template. As these techniques are still in their infancy, elaborate information on formulation considerations is not available.

The acceptability of the organic solvents in the pharmaceutical arena, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or microemulsion as templates (Table 5). Partially water-miscible organic solvents like glycols can be used as the internal phase of the microemulsion when the nanosuspensions are to be produced using a microemulsion as a template.

Surfactants [26]:

Surfactants are incorporated to improve the dispersion by reducing the interfacial tension. They also act as wetting or deflocculating agents e.g. Tween 80 and Span - widely used surfactants.

Co-surfactants:

The choice of co-surfactant is critical when using microemulsion to formulate nanosuspensions. Since co-surfactants can greatly influence phase behavior, the effect of co-surfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated e.g. Transcutol, glycofurol, ethanol and Iso-propanol - safely used as co-surfactants. Also, bile salts and dipotassiumglycerrhizinate can be used as co-surfactants.

Other additives [23]:

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety.

Table 5: Various examples of solvents used in nanosuspensions formulation

Type of solvent	Name	Remarks
Water-miscible solvents	Ethanol, Iso-propanol etc.	Pharmaceutically acceptable &less hazardous.
Partially water-miscible solvents	Ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, benzyl alcohol etc.	Preferred in the formulation over the conventional hazardous solvents, such as dichloromethane.

Properties of nanosuspensions:

Physical long-term stability [23]:

Another special feature of nanosuspensions is the absence of Ostwald ripening, which is suggestive of their long-term physical stability. Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles. Ostwald ripening is caused by the differences in dissolution pressure/saturation solubility between small and large particles. Molecules diffuse from the higher concentrated area around small particles (higher saturation solubility) to areas around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles. The diffusion process of the drug from the small particles to the large particles leaves an area around the small particles that is not saturated any more, consequently leading to dissolution of the drug from the small particles and finally completes disappearance of the small particles.

Internal structure of Nanosuspensions [20]:

The high-energy input during disintegration process causes structural changes inside the drug particles. When the drug particles are exposed to high-pressure homogenization, particles are transformed from crystalline state to amorphous state. The change in state depends upon the hardness of drug, number of homogenization cycles chemical nature of drug and power density applied by homogenizer.

Adhesiveness [20]:

There is a distinct increase in adhesiveness of ultra-fine powders compared to coarse powders. This adhesiveness of small drug nanoparticle can be exploited for improved oral delivery of poorly soluble drugs. A drastically remarkable report is that of the increase in bioavailability for danazol from 5 % (as macrosuspension) to 82% (as nanosuspensions).

Crystalline state and morphology [20]:

A potential change in the crystalline structure of nanosuspensions saying increasing the amorphous fraction in the particle or even creating completely amorphous particles is

a characteristic of consideration. The application of high pressures during the production of nanosuspensions was found to promote the amorphous state.

Increase in Saturation Solubility and Dissolution Velocity of drug [21]:

Dissolution of drug is increased due to increase in the surface area of the drug particles from micrometers to the nanometer size. According to Noyes-Whitney equation (Equation no.1), dissolution velocity increases due to increase in the surface area from micron size to particles of nanometer size.

$$dx/dt = [(D \times A)/ h] [Cs-X/V] \text{ -----Equation (1)}$$

Preparation Methods of nano- suspension:

There are different methods of Nanosuspensions preparation (Figure 6) [20]

- Homogenization in water (Disso Cubes).
- Media milling (Nanocrystals or Nanosystems).
- Homogenization in non-aqueous media (Nanopure).
- Combined precipitation and homogenization (Nanoedge).
- Nanojet technology.
- Emulsification-solvent evaporation technique.
- Hydrosol method.
- Supercritical fluid method.
- Dry co-grinding.
- Emulsion as template.
- Microemulsion as template.

Current techniques used to obtain drug nanoparticle can be divided into two categories:

Bottom up techniques:

It is the technique in which the nano size is obtained by increasing the size of particles from molecular range to nanorange [22]. The conventional methods of precipitation (‘Hydrosol’) are called Bottom up technology. Using a precipitation

technique, the drug is dissolved in an organic solvent and this solution is mixed with a miscible anti-solvent. In the water-solvent mixture, the solubility is low and the drug precipitates. Basic challenge is that during the precipitation procedure growing of the crystals need to be controlled by addition of surfactant to avoid formation of microparticles.

Advantage:

- The use of simple and low cost equipments.

Limitations:

- The drug needs to be soluble in at least one solvent and the solvent needs to be miscible with non-solvent.
- Moreover, it is not applicable to the drugs, which are poorly soluble in both aqueous and non-aqueous media.

Top down techniques:

The techniques in which Nano size range of particles is obtained by reduction in size of larger particles [22].

High Pressure Homogenization (Disso Cubes):

Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture. Disso cube technology was developed by Muller et al. in which, the suspension of the drug is made to pass through a small orifice that results in a reduction of the static pressure below the boiling pressure of water, which leads to boiling of water and formation of gas bubbles. When the suspension leaves the gap and normal air pressure is reached again, the bubbles shrink and the surrounding part containing the drug particles rushes to the center and in the process colloids, causing a reduction in the particle size. Most of the cases require multiple passes or cycles through the homogenizer, which depends on the hardness of drug, the desired mean particle size and the required homogeneity.

Scholer et al, prepared atovaquone nanosuspensions using this technique [27]. To produce a Nanosuspension with a higher concentration of solids, it is preferred to start homogenization with very fine drug particles, which can be accomplished by pre-milling.

Media milling (NanoCrystals):

This patent-protected technology was developed by Liversidge et al. (1992). Formerly, the technology was owned by the company nanosystems but recently it has been acquired by Élan Drug Delivery. In this method, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber (Figure 9). The milling chamber charged with polymeric media is the active component of the mill. The mill can be operated in a batch or recirculation mode. Crude slurry consisting of drug, water and stabilizer is fed into the milling chamber and processed into nano-crystalline dispersion and the milling media or pearls are then rotated at a very high shear rate. The milling process is performed under controlled temperatures. The typical residence time generated for a nanometer-sized dispersion with a mean diameter of <200nm is 30–60 min [23].

Principle [23]:

The high energy and shear forces generated as a result of the impaction of the milling media with the drug provide the energy input to break the microparticulate drug into nano-sized particles. The milling medium is composed of glass, zirconium oxide or highly cross-linked polystyrene resin. The process can be performed in either batch or recirculation mode. In batch mode, the time required to obtain dispersions with uni-modal distribution profiles and mean diameters <200 nm is 30–60 min. The media milling process can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very little batch-to-batch variation is observed in the quality of the dispersion.

Advantages:

- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation.
- Narrow size distribution of the final nano-sized product.

- Flexibility in handling the drug quantity, ranging from 1 to 400mg/ml, enabling formulation of very dilutes as well as highly concentrated nanosuspensions.

A comparison of the size of naproxen crystals before and after media milling.

Limitations:

The major concern is the generation of residues of milling media, which may be introduced in the final product as a result of erosion. This could be problematic when Nanosuspensions are intended to be administered for a chronic therapy. The severity of this problem has been reduced to a great extent with the advent of polystyrene resin-based milling medium. For this medium, residual monomers are typically 50 ppb and the residuals generated during the milling processing are not more than 0.005% w/w of the final product or the resulting solid dosage form.

Homogenization in non-aqueous media (Nanopure):

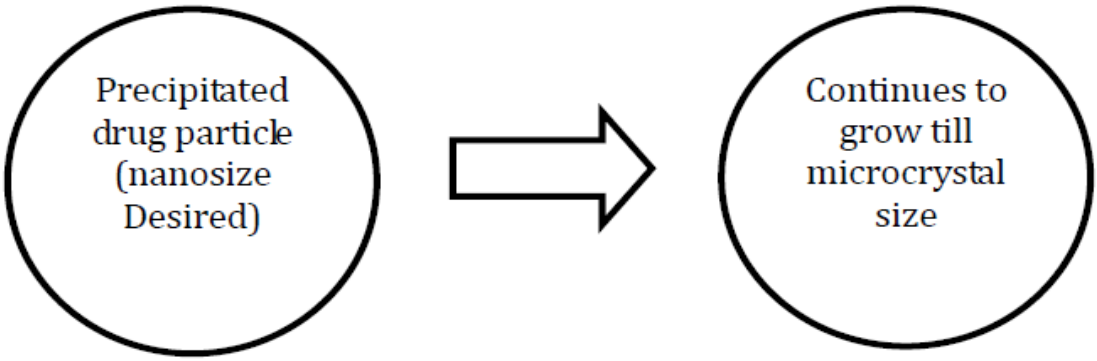
Nanopure is suspensions homogenized in water free media or water mixtures i.e. the drug suspensions in the non- aqueous media were homogenized at 0º C or even below the freezing point and hence are called "deep-freeze" homogenization. The results obtained were comparable to Disso cubes and hence can be used effectively for thermolabile substances at milder conditions. The Nanocrystals of the drug dispersed in liquid polyethylene glycol (PEG) or various oils can be directly filled as drug suspensions into HPMC capsules or gelatin.

Advantages:

- The dispersion medium need not be removed.
- Evaporation is faster and under milder conditions (when water and water miscible liquids are used).
- This is useful for temperature sensitive drugs.
- For i.v. injections, isotonic nanosuspensions are obtained by homogenizing in water-glycerol mixtures [22].

Combined precipitation and homogenization (Nanoedge):

The drug is dissolved in an organic solvent and this solution is mixed with a miscible anti-solvent for precipitation. In the water-solvent mixture, the solubility is low and the drug precipitates. Precipitation has also been coupled with high shear processing. This is accomplished by a combination of rapid precipitation and high-pressure homogenization. The nanoedge patented technology by Baxter depends on the precipitation of friable materials for fragmentation under conditions of high shear and/or thermal energy. Rapid addition of a drug solution to an anti-solvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded [28].



The basic principles of nanoedge are the same as that of precipitation and homogenization. A combination of these techniques results in smaller particle size and better stability in a shorter time. The major drawback of the precipitation technique, such as crystal growth and long-term stability, can be resolved using the Nanoedge technology [20]

Nanojet Technology:

This technique, called 'opposite stream or nanojet technology', uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure upto 4000 bar at the high velocity of 1000m/s[29]. The high shear force produced during the process results in particle size reduction. Equipment using this principle includes the M110L and M110S microfluidizers (Microfluidics). Dearn prepared nanosuspensions of atovaquone using the microfluidization process. The major

limitation of this technique is the high number of passes through the microfluidizers (up to 75 passes) and that the product obtained contains a relatively larger fraction of microparticles [20]. A limitation of this process is the large production time [29].

Emulsification-solvent evaporation technique:

This technique involves preparing a solution of drug followed by its emulsification in another liquid that is a non-solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using a high-speed stirrer [22].

Supercritical fluid method:

Supercritical fluid technology can be used to produce nanoparticle from drug solutions. The various methods attempted are rapid expansion of supercritical solution process (RESS), supercritical anti-solvent process and precipitation with compressed anti-solvent process (PCA). The RESS involves expansion of the drug solution in supercritical fluid through a nozzle, which leads to loss of solvent power of the supercritical fluid resulting in precipitation of the drug as fine particles. In the PCA method, the drug solution is atomized into a chamber containing compressed CO_2. As the solvent is removed, the solution gets supersaturated and thus precipitates as fine crystals. The supercritical anti- solvent process uses a supercritical fluid in which a drug is poorly soluble and a solvent for the drug that is also miscible with the supercritical fluid. The drug solution is injected into the supercritical fluid and the solvent gets extracted by the supercritical fluid and the drug solution gets supersaturated. The drug is then precipitated as fine crystals [22].

Limitations:

- Use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques.
- Particle nucleation overgrowth due to transient high supersaturation, which may also result in the development of an amorphous form or another undesired polymorph.

Dry co-grinding:

Nanosuspensions prepared by high pressure homogenization and media milling using pearl-ball mill are wet–grinding processes. Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported. Itoh*et al.* reported the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinyl pyrrolidone (PVP) and Sodium dodecyl sulfate (SDS). Many soluble polymers and co-polymers such as PVP, Polyethylene glycol (PEG), Hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used. Physicochemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug. Recently, nanosuspensions can be obtained by dry milling techniques. Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable amorphous solid can be obtained [30].

Emulsion as template [23]:

Apart from the use of emulsions as a drug delivery vehicle, they can also be used as templates to produce nanosuspensions. The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents can be used as the dispersed phase of the emulsion. There are two ways of fabricating drug nanosuspensions by the emulsification method. In the first method, an organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants to form an emulsion. The organic phase is then evaporated under reduced pressure so that the drug particles precipitate instantaneously to form a nanosuspensions stabilized by surfactants. Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the nanosuspensions by controlling the size of the emulsion. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally, organic solvents such as methylene chloride and chloroform were used. However, environmental hazards and human safety concerns about residual solvents

have limited their use in routine manufacturing processes. Relatively safer solvents such as ethyl acetate and ethyl formate can still be considered for use.

Another method makes use of partially water-miscible solvents such as butyl lactate, benzyl alcohol and triacetin as the dispersed phase instead of hazardous solvents. The emulsion is formed by the conventional method and the drug nanosuspension is obtained by just diluting the emulsion. Dilution of the emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of a nanosuspension. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of di-ultrafiltration in order to make it suitable for administration.

However, if all the ingredients that are used for the production of the nanosuspensions are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspensions.

Advantages:

- Use of specialized equipment is not necessary.
- Particle size can easily be controlled by controlling the size of the emulsion droplet.
- Ease of scale-up if formulation is optimized properly.

Limitations:

- Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique.
- Safety concerns because of the use of hazardous solvents in the process.
- Need for di-ultrafiltration for purification of the drug nanosuspensions, which may render the process costly.
- High amount of surfactant/stabilizer is required as compared to the production techniques described earlier.
- The production of drug nanosuspensions from emulsion templates has been successfully applied to the poorly water-soluble and poorly bioavailable anti-cancer drug mitotane, where a significant improvement in the dissolution rate of the drug (five-fold increase) as compared to the commercial product was observed.

Microemulsion as template/Lipid emulsion [23]:

Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant and co-surfactant. Their advantages, such as high drug solubilization, long shelf-life and ease of manufacture, make them an ideal drug delivery vehicle. Taking advantage of the microemulsion structure, one can use microemulsions even for the production of nanosuspensions. Oil-in-water microemulsions are preferred for this purpose. The internal phase of these microemulsions could be either a partially miscible liquid or a suitable organic solvent, as described earlier. The drug can be either loaded in the internal phase or pre-formed microemulsions can be saturated with the drug by intimate mixing. The suitable dilution of the microemulsion yields the drug nanosuspensions by the mechanism described earlier. The influence of the amount and ratio of surfactant to co-surfactant on the uptake of internal phase and on the globule size of the microemulsion should be investigated and optimized in order to achieve the desired drug loading. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of di-ultrafiltration in order to make it suitable for administration. However, if all the ingredients that are used for the production of the nanosuspensions are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspensions.

The advantages and limitations are the same as for emulsion templates. The only added advantage is the need for less energy input for the production of nanosuspensions by virtue of microemulsions. Also the limitation will include large amounts of surfactant or stabilizers are required [31]. E.g. Griseofulvin nanosuspensions which is prepared by using water, butyl lactate, lecithin and the sodium salt of tauro-deoxycholate, where a significant improvement in the dissolution rate of the drug (three-fold increase) as compared to the commercial product was observed. It was found that the nature of the co-surfactant affected the dissolution rate of the drug nanosuspensions, as anticipated. However, this technique is still in its infancy and needs more thorough investigation. For a comparative advantages & limitations with examples, see table 6.

Table 6: Advantages and disadvantages of various preparation techniques of nanosuspensions [28]

Method	Advantages	Limitations	Drug
High-pressure Homogenization	Widely applying regions, ease of scale-up and little batch to batch variation, narrow size distribution in the final product, allowing aseptic production of nanosuspensions for parenteral administration and flexibility in handling the drug quantity.	Pretreatment of micronized drug particles and pre-suspending materials Before subjecting it to homogenization.	Albendazole. Amphotericin B. Aphidicolin. Atovaquone. Azithromycin. Bupravaquone. Clofazamine. Fenofibrate. Glucocorticoid drugs.
Milling	Same as those for high-pressure homogenization.	Potential erosion of material from the milling pearls.	Cilostazol. Danazol. Naproxen.
Microprecipitation	Low need of energy, stable products and simple process.	Narrowly applying space, wide size distribution and potential toxicity of non-aqueous solvents.	Breviscapine. Griseofulvin. Ibuprofen. Mitotane.
Emulsion and microemulsion	Low need of energy, stable products, simple process, small size of particles and uniform particle distribution.	High concentration undesired surfactants and residual solvents.	___
Dry Co-grinding[21]	Easy process No organic solvent Require short grinding time	Generation of residue of milling media	Clarithromycin. Glibenclamide. Glisentide. Griseofulvin. Naproxen. Nifedipine. Phenytoin. Pranlukast.

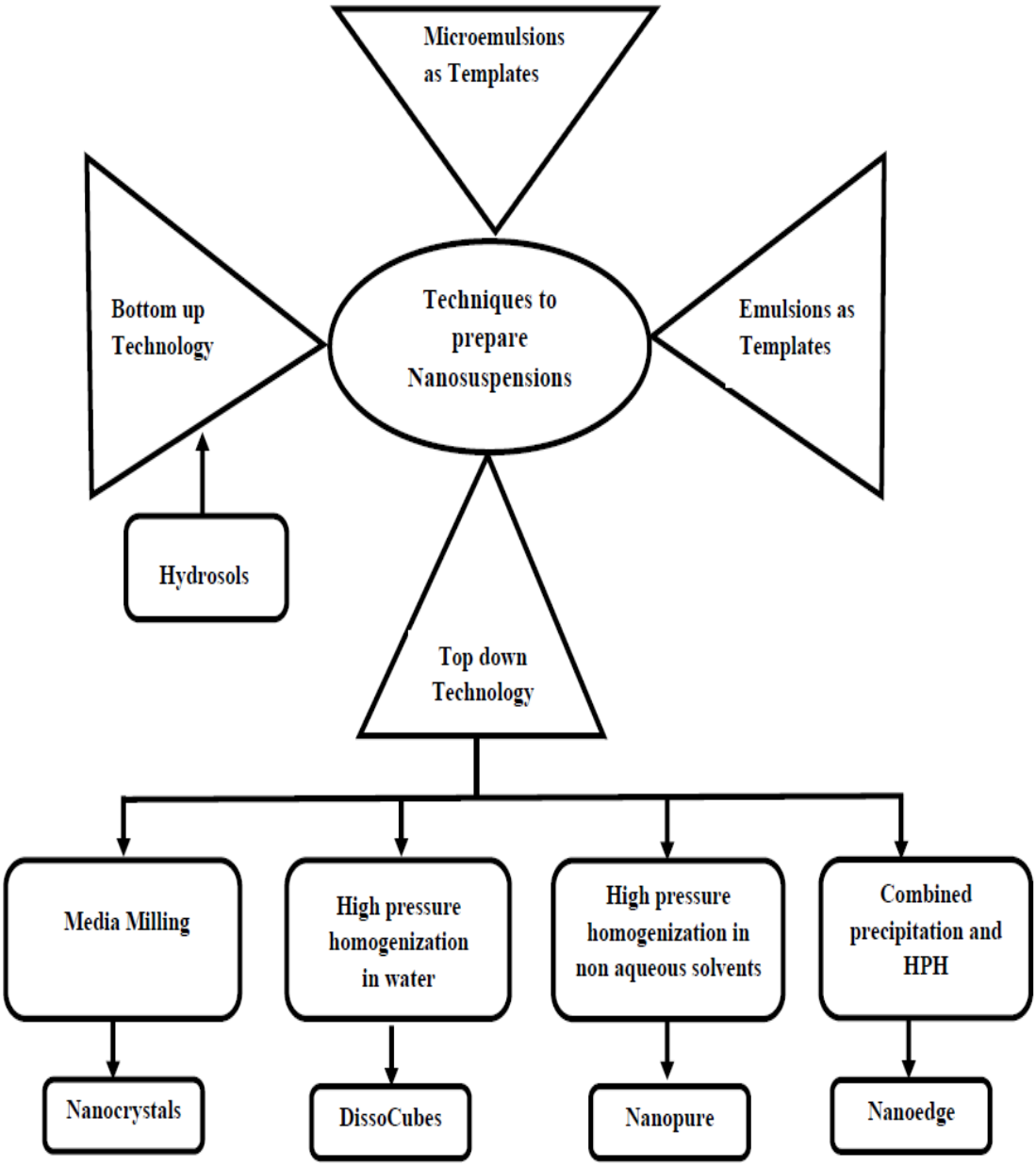


Figure 5: Various methods for preparation of nanosuspensions [22].

Pharmaceutical application of nanosuspensions [27]:

Intravenous administration:

The parenteral route of administration provides a quick onset of action, rapid targeting and reduced dosage of the drug. It is the preferred route for drugs undergoing first-pass metabolism and those that are not absorbed in the GIT or degraded in the GIT. One of the important applications of nanosuspensions technology is the formulation of intravenously administered products. IV administration results in several advantages, such as administration of poorly soluble drugs without using a higher concentration of toxic co solvents, improving the therapeutic effect of the drug available as conventional oral formulations and targeting the drug to macrophages and the pathogenic microorganisms residing in the macrophages.

Bioavailability enhancement:

The poor oral bioavailability of the drug may be due to poor solubility, poor permeability or poor stability in the gastrointestinal tract (GIT).Nanosuspensions resolve the problem of poor bioavailability by solving the twin problems of poor solubility and poor permeability across the membrane. Bioavailability of poorly soluble oleanolic acid, a hepato protective agent, was improved using a Nanosuspensions formulation. The therapeutic effect was significantly enhanced, which indicated higher bioavailability. This was due to the faster dissolution (90% in 20 min) of the lyophilized Nanosuspensions powder when compared with the dissolution from a coarse powder (15% in 20 min).

Pulmonary administration:

Aqueous Nanosuspensions can be nebulized using mechanical or ultrasonic nebulizers for lung delivery. Because of their small size, it is likely that in each aerosol droplet at least one drug particle is contained, leading to a more uniform distribution of the drug in lungs. They also increase adhesiveness and thus cause a prolonged residence time. Budenoside drug nanoparticles were successfully nebulized using an ultrasonic nebulizer.

Ocular administration:

Ocular delivery of the drugs as nanosuspensions to provides a sustained release of drug. Pignatello et al. prepared Eudragit retard nanosuspensions of cloricromene for

ocular delivery. They observed that the drug showed a higher availability in rabbit aqueous humor and the formulation appeared to offer a promising means of improving the shelf-life and the bioavailability of this drug after ophthalmic application.

Drug targeting:

Nanosuspensions can also be used for targeting as their surface properties and changing of the stabilizer can easily alter the *in-vivo* behavior. The drug will be up taken by the mononuclear phagocytic system to allow regional-specific delivery. This can be used for targeting anti-myco bacterial, fungal or leish manial drugs to the macrophages if the infectious pathogen is persisting intracellularly.

Mucoadhesion of the nanoparticles:

Nanoparticles orally administered in the form of a suspension diffuse into the liquid media and rapidly encounter the mucosal surface. The particles are immobilized at the intestinal surface by an adhesion mechanism referred to as "bioadhesion." From this moment on, the concentrated suspension acts as a reservoir of particles and an adsorption process takes place very rapidly. The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption. The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT.

Targeted drug delivery:

The need to target drugs to specific sites is increasing day by day as a result of therapeutic and economic factors. Nanoparticulate systems have shown tremendous potential in targeted drug delivery, especially to the brain. Successful targeting of the peptide dalarg in to the brain by employing surface modified poly isobutl cyanoacrylate nanoparticles has been a major achievement in targeted delivery. Likewise nanosuspensions can be used for targeted delivery as their surface properties and in-vivo behavior can easily be altered by changing either the stabilizer or the milieu. Their versatility and ease of scale-up and commercial production enables the development of commercially viable nanosuspensions for targeted delivery. Overall nanosuspensions have indicated a good potential in targeted drug delivery but this has yet to be fulfilled.

Oral drug delivery:

Oral drug delivery is the quick onset of action, which is seen in case of naproxen, an NSAID. In spite of the tremendous potential of nanosuspensions in oral delivery, formulating compounds as nano-crystalline dispersions is not of value when metabolic and/or permeation-related issues affect bioavailability. However, in future, it should be possible to engineer nanosuspensions by using the agents that enhance permeation and/or minimize gut-related metabolic issues. This amalgamated approach would facilitate delivery of the compounds belonging to BCS Class IV that exhibit poor water solubility and poor membrane permeability.

Characterisation of nanosuspensions [27]:

Nanosuspensions are characterized for appearance, color, odor, assay, related impurities, particle size, zeta potential, crystalline status, dissolution studies and *in- vivo* studies. Among this, the most important characterization techniques were discussed (Figure 11) [31].

Mean particle size and size distribution:

The mean particle size and the span of particle size distribution (polydispersity index, PI) are two important characteristic parameters because they affect the saturation solubility, dissolution rate, physical stability, even in-vivo behaviour of nanosuspensions. It is indicated that saturation solubility and dissolution velocity show considerable variation with the changing, particle size of the drug. Particle size distribution determines the physiochemical behaviour of the formulation, such as saturation solubility, dissolution velocity and physical stability. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and coulter counter multisizer. PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1– 0.25 indicates a fairly narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution. The coulter-counter gives the absolute number of particles per volume unit for the different size classes, and it is a more efficient and appropriate technique than LD for quantifying the contamination of nanosuspensions by microparticulate drugs.

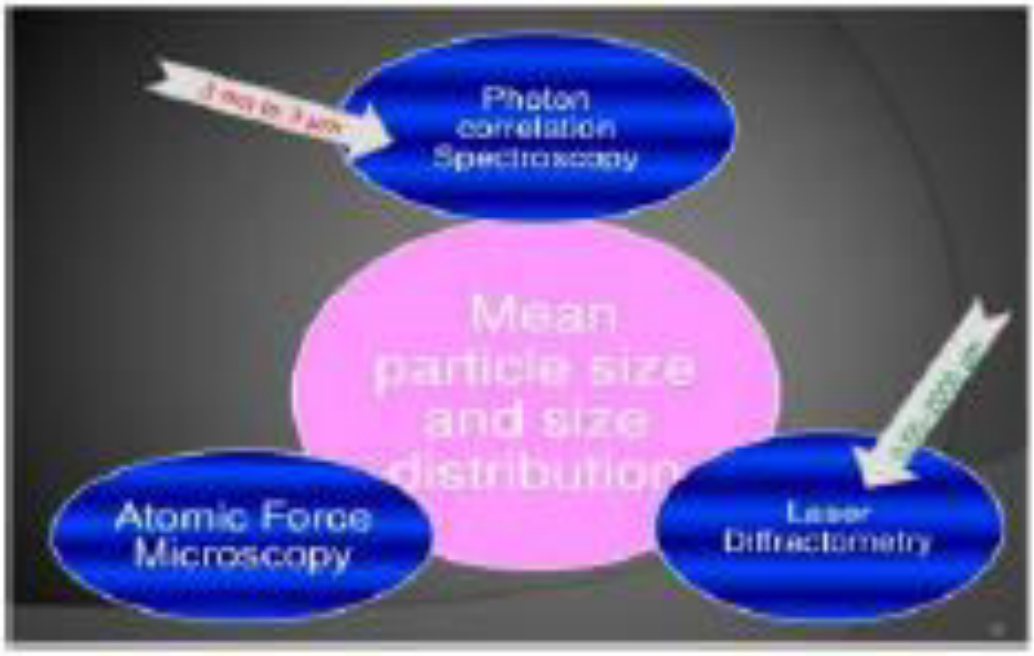


Fig 6: Mean Particle size.

Surface charge (zeta potential):

Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanosuspensions. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

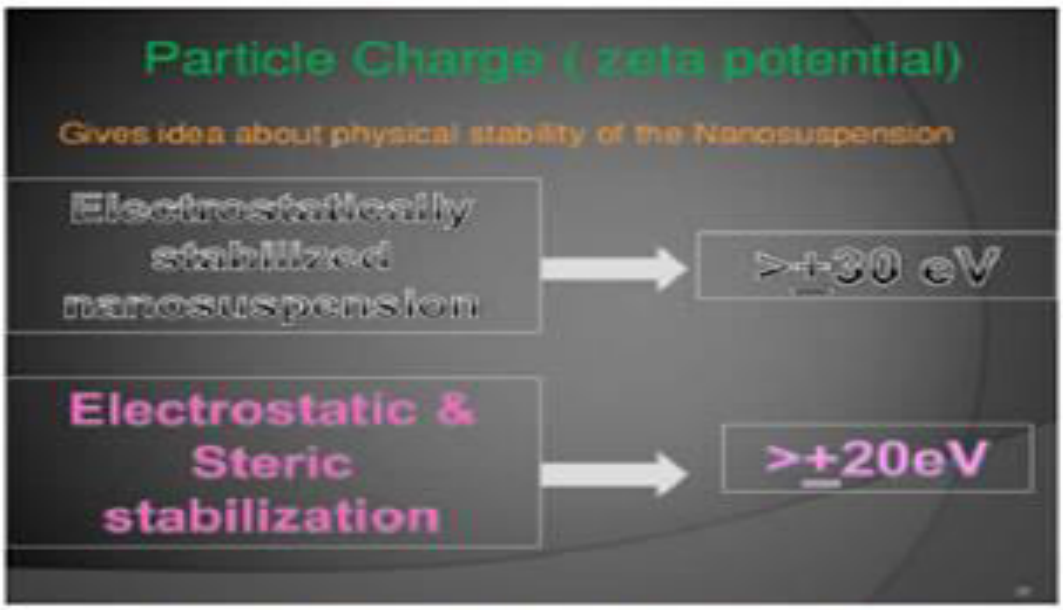


FIG 7 Surface Charge (Zeta Potential).

Crystalline state and particle morphology:

The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing. Nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high-pressure homogenization. The changes in the solid state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis and supplemented by differential scanning calorimetry. In order to get an actual idea of particle morphology, scanning electron microscopy is preferred.

Saturation solubility and dissolution velocity:

Nanosuspensions have an important advantage over other techniques, that it can increase the dissolution velocity as well as the saturation solubility. The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflects the advantages that can be achieved over

conventional formulations, especially when designing the sustained-release dosage forms based on nanoparticulate drugs.

Dissolution Studies:

An USP dissolution apparatus (Electro lab, India) Type II (paddle method) at rotation speed of 50 rpm was used for *in vitro* testing of drug dissolution from the various formulations obtained after each size reduction step.

Stability of nanosuspensions:

The high surface energy of nanosized particles induces agglomeration of the drug crystals. The main function of the stabilizer is to wet the drug particles thoroughly to prevent Ostwald ripening and agglomeration of the nanosuspensions and form a physically stable formulation by providing a steric or an ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, Poloxamer, Polysorbates, lecithin, polyoleate and povidone. Lecithin may be preferred in developing parenteral nanosuspensions.

Drug content:

Drug content of nanosuspensions formulation can be carried out by extracting the nanosuspensions in suitable solvent mixture, like methanol: THF (1:1) mixture, shaken well, and then centrifuged. The supernatants can be separated and diluted with same solvent mixture and the absorbance can be measured at suitable λmax. The drug content then can be calculated using the calibration curve [21].

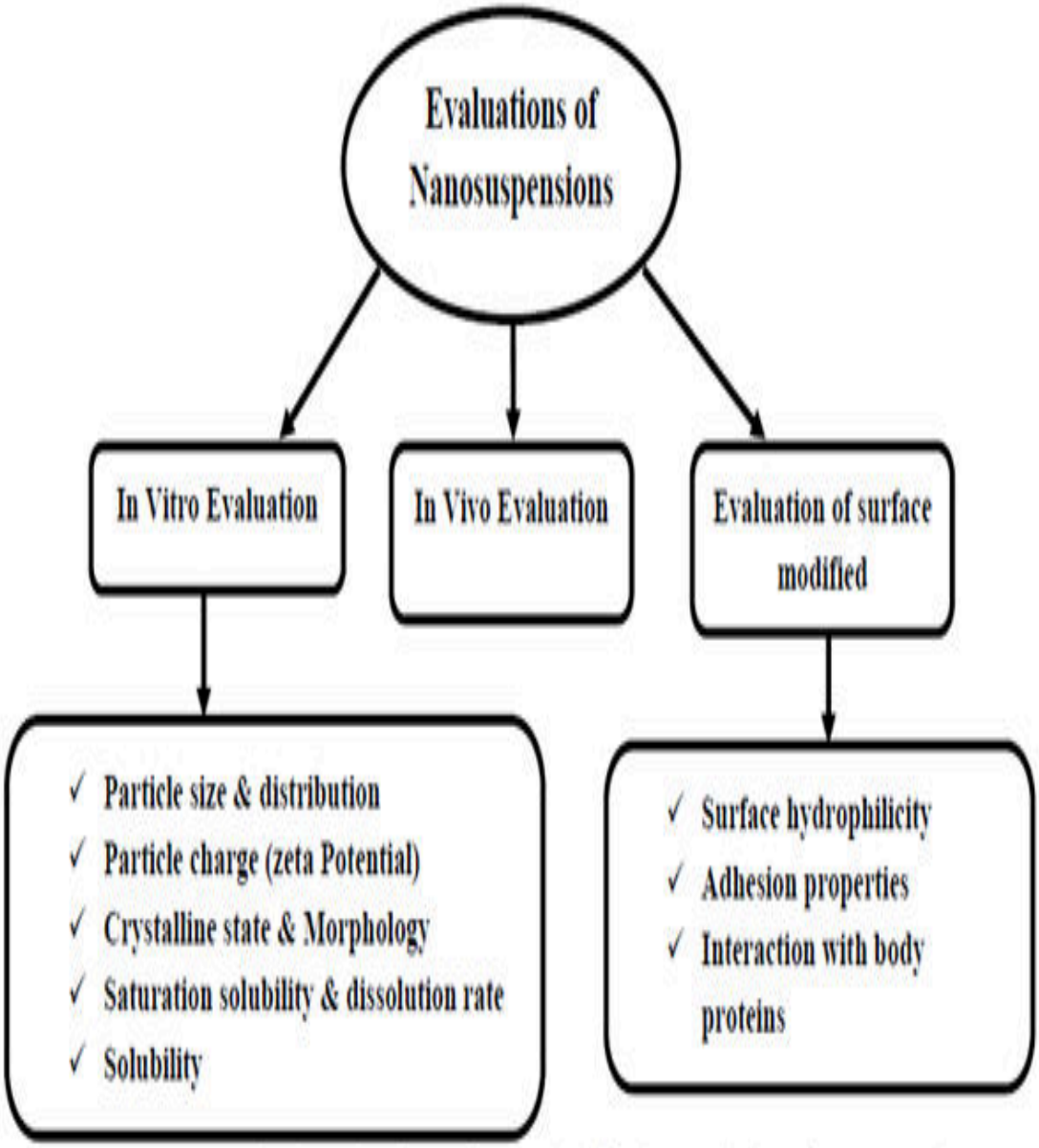


Fig 8 Flowchart showing various methods for characterization of nanosuspensions

2.4. REVIEW OF PREVIOUS WORKS

Mullar RH et al., reported nanosuspensions contain submicron colloidal dispersion of particles in liquid phase stabilized by surfactants and the reduction of drug particles into submicron range leads to significant increase in the dissolution rate and enhanced bioavailability [32].

Ali HS et al. prepared Nanosuspensions by two technologies such as top-down and bottom-up technology. In the "top-down" technology, mechanical comminution process is involved where larger drug particles are reduced to smaller size as in milling (jet mills and pear-ball mills) and homogenization (high pressure homogenizers). In the "bottom-up" technology, molecules are dissolved and then precipitated by non-solvent addition as in supercritical fluid technology, spray freezing into liquid process, evaporative precipitation in to aqueous solutions and liquid solvent change process. Although "top down" approaches are widely employed, the drawbacks associated with mechanical attrition processes such as time consumption, intensive energy use, introduction of impurities, inadequate control of particles size and electrostatic effects, promote greater interest towards "bottom up" preparation of Nanoparticles[33].

Kristl J et al., Due to low water solubility Nanosuspensions of Celecoxib was investigated. Emulsion-diffusion technique was used to formulate the Nanosuspensions. Celecoxib dissolved in ethyl acetate was added to aqueous solution of stabilizers (Tween 80 and a combination of PVP K-30) and homogenized [34].

Pignatello R et al., prepared Cloricromene (AD6) loaded polymeric Nanoparticle suspensions for ophthalmic application using inert polymer resins (Eudragit RS100 and RL100) by quasi emulsion solvent diffusion technique by varying some formulation parameters. These AD6 loaded Nanosuspensions showed improved shelf life and bioavailability of drug after ophthalmic application [35].

Fadda AM et al., Diclofenac is a potent nonsteroidal anti-inflammatory drug with a very low aqueous solubility and gastrolesive actions. To improve dissolution profile and oral bioavailability of Diclofenac, Diclofenac Nanosuspensions were formulated by homogenization technique and results showed that drug dissolution rate in Nanosuspensions was highly enhanced [36].

Mullar RH et al., Bupravaquone is a new naphthoquinone antibiotic used in the treatment of Cryptosporodiumparvum infection. The bioavailability of Bupravaquone is limited through oral administration so to overcome the problem of high elimination rate caused by diarrhoea, Bupravaquone was formulated as a mucoadhesive Nanosuspensions by high pressure homogenization [37].

Zhonggui H et al., 10-hydroxycamptothecin is a potent antitumor agent, has poor solubility in water and organic solvents and it was found that lactone ring readily open and converted to carboxylate form under physiologic and alkaline condition. So it was prepared as Nanosuspensions with active form of 10-hydroxycamptothecin by microprecipitation, high pressure homogenisation method [38].

Agnihotri SM et al., reported Polymeric Nanoparticles suspensions were prepared from poly (lactide-co-glycolide) and poly (lactide-co-glycolide-leucine) {poly [Lac (Glc-Leu)]} biodegradable polymers and loaded with Diclofenac sodium to improve the ocular availability of the drug. Nanoparticle suspensions were prepared by emulsion and solvent evaporation technique and system showed a better size distribution for ophthalmic application. *In vitro* release tests showed an extended-release profile of Diclofenac sodium from the Nanoparticles suspensions [39].

Jin S et al., identified Nanosuspensions as the most promising delivery system for poorly soluble drugs due to improved bioavailability, high drug loading, and targeting capability. Used different manufacturing processes including solidification and solid modification technique for the production of Nanosuspensions [40].

Arunkumar N et al., Atorvastatin calcium is used in the treatment of hypercholesterolemia. Because of low solubility of Atorvastatin crystals it is formulated as Nanosuspensions by high pressure homogenization technique in which the crystalline form is converted to amorphous form which showed significant increase in solubility [41].

Zhang D et al., Azithromycin is clinically effective against Gram-positive and Gram-negative bacteria. Because of low bioavailability, Azithromycin was prepared as Nanosuspensions by high pressure homogenization to increase its saturation solubility and dissolution velocity. In order to enhance the stability of the Nanosuspensions, it was converted to freeze-dried powder by lyophilization [42].

Hongyu P et al., reported solid-in-oil Nanosuspensions as a potent carrier of Diclofenac sodium for enhancing percutaneous absorption by Transdermal delivery [43].

Akanksha T et al., described Nanosuspensions as effective treatment of ocular diseases by improving bioavailability16.

Thakar Det al developed and characterized the Nanosuspensions of telmisartan by bead milling process. In this study, the media used is ZnO_2 beads. A variety of surface active agents were tested for their stabilizing effects. Formulation factors evaluated were ratio of polymer to drug, whereas process parameters were milling time and concentration of ZnO_2 beads. Responses measured in this study include particle size, measurement, particle size distribution and zeta potential [44].

HecqJ and Co-Workers reported Nanosuspensions of poorly water-soluble drugs such as Nifedipine (20g/ml). In order to enhance these characteristics, preparation of Nifedipine Nanoparticles has been achieved using high pressure homogenization. Through the DSC and XRD study, it has been shown that initial crystalline state is maintained following particle size reduction and that the dissolution characteristics of Nifedipine Nanoparticles were significantly increased in regards to the commercial product. These approaches have a general applicability to many poorly water-soluble drug entities [45].

RuolenXiong and Co-Workers prepared injectable nimodipine Nanosuspensions by high-pressure homogenization. Characterization of the product was performed for SEM and DSC. Irritability study in rabbits showed that this formulation provided less local irritation and phlebitis risks than the commercial ethanol product, which represented a promising new drug formulation for intravenous therapy of subarachnoid hemorrhage related vasospasm [46].

Pandya M Vikram*et al* formulated and characterized Nanosuspensions of simvastatin using pluronic F127 and ZnO_2 beads by wet milling technique. The results showed that Nanosuspensions prepared with the higher concentration of pluronic F127 and the higher quantities of ZnO_2 reduce the particle size and enhanced the dissolution rate of the formulation. This preparation of simvastatin nanosuspensions increased bioavailability, dissolution rate, solubility [47].

Thakar D et al developed and characterized the Nanosuspensions of telmisartan by bead milling process. In this study, the media used is ZnO2 beads. A variety of surface active

agents were tested for their stabilizing effects. Formulation factors evaluated were ratio of polymer to drug, whereas process parameters were milling time and concentration of ZnO2 beads. Responses measured in this study include particle size, measurement, particle size distribution and zeta potential [48].

ChetanDetroja et al enhanced the oral bioavailability of practically insoluble candesartan cilexetil by preparing Nanosuspensions using ZnO2 beads and converted to solid state by spray drying. DSC and XRD analysis showed that crystalline state of CC remained unchanged in SDCN. Dissolution studies in phosphate buffer pH 6.5 containing 0.7% Tween 20 showed SDCN was almost completely dissolved exhibiting higher dissolution velocity and solubility. In-vivo performance, showed increase in dissolution velocity and saturation solubility leads to enhancement of bioavailability of SDCN when compared to bulk candersartancilexetil suspension. Thus, the results conclusively demonstrated a significant enhancement in antihypertensive activity of candesartan when formulated as Nanosuspensions [49].

HetalParesh et al developed Nanosuspensions of olmesartan medoxomil using media milling technique. The results indicated that the initial crystalline state is preserved following particle size reduction and that the saturation solubility, dissolution velocity and diffusion rate of the drug from the Nanosuspensions is significantly higher than that of the plain drug suspension as well as from the marketed tablet formulation[50].

3. AIM AND OBJECTIVE

The aim of this work is to formulate and develop nanosuspensions containing a hypercholesterolemia drug and carrier's urea, PVP 30, β-cyclodextrin and Tween 80 & SLS.

Objective of the Study:

The main objective of the present study is to carry out formulation and evaluation of nanosuspensions of hypercholesterolemia by using suitable polymers to improve its bioavailability.

The objectives of the present study as follows

- Preformulation studies for selection of suitable excipients to develop the dosage form based on physicochemical properties of drug and excipients.
- Screening of excipients for compatibility and efficacy for developing the formulation.
- Carry out Preformulation study of Pitavastatin drug.
- Preparation of nanosuspensions drug delivery system for enhancing the solubility and thus bioavailability of drug.
- Evaluation of nanosuspensions drug delivery system, *in vitro*.
- Optimize the formulation using experimental design technique regarding particle size, particle size distribution, zeta potential, stability, release profile, etc.
- Evaluation of formulated product and identification of defects.
- Study the stability of the formulation following ICH guidelines.

The following parameters were examined:

1. Pre-formulation studies.

   a) Drug and carrier interaction by FT-IR spectroscopy.

   b) Drug and carrier interaction by Differential Scanning Calorimetry (DSC)

2. Preparation of standard curve of Pitavastatin in 0.1N HCL Buffer

3. Preparation of Pitavastatin Nanosuspensions by precipitation method.

4. Evaluation of Pitavastatin Nanosuspensions.

   a) Drug and carrier interaction by FT-IR spectroscopy.

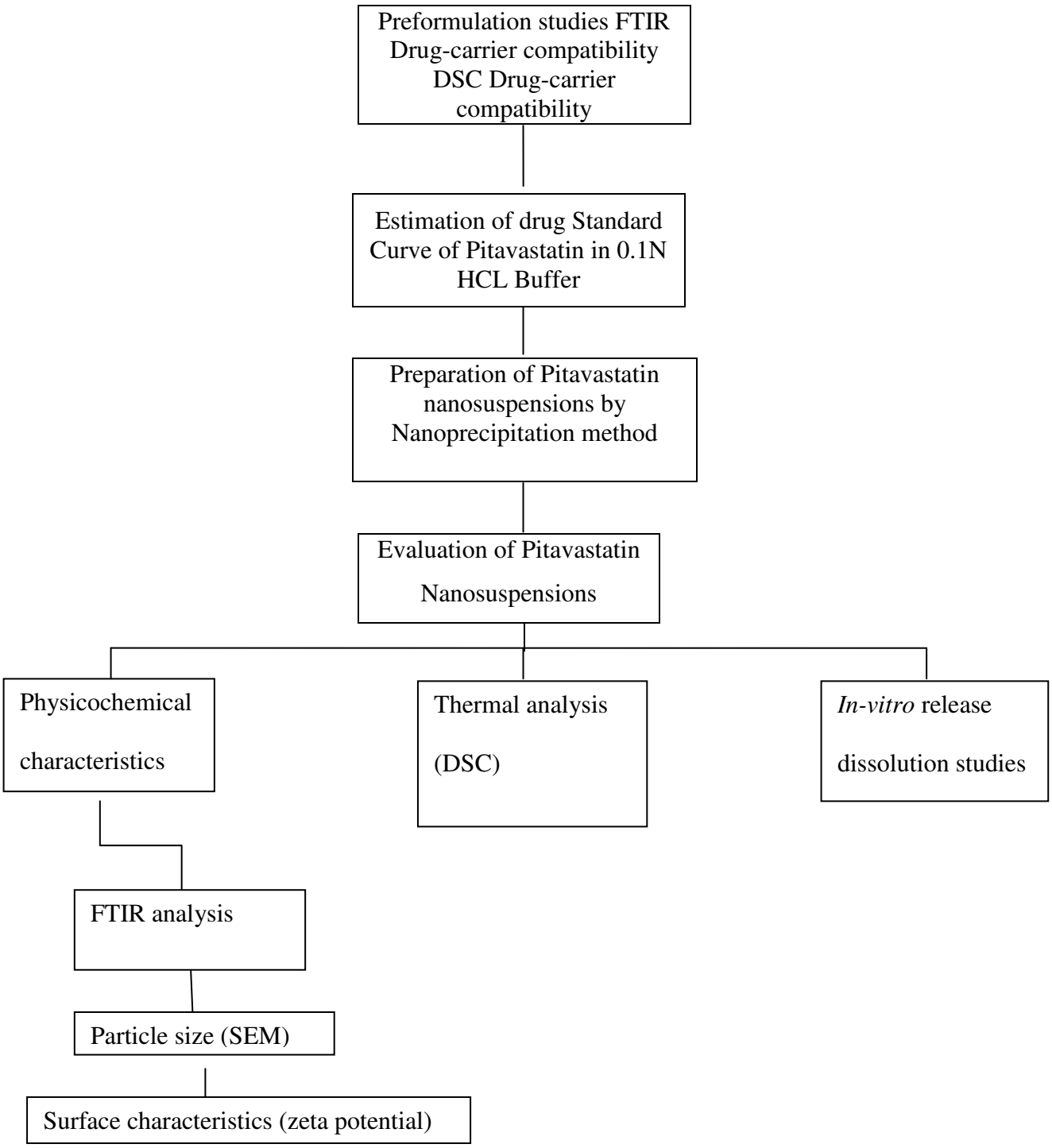
   b) Particle size determination by Scanning Electron Microscopy (SEM)

   c) Surface characteristic by Zeta potential analyzer.

   d) Thermal analysis by Differential Scanning Calorimetry (DSC).

   e) *In-vitro* dissolution studies

4. PLAN OF WORK



5. PROFILES

5.1. DRUG PROFILE

5.1.1. LIVALO (Pitavastatin):

Category **:** Hypercholesterolemia

Empirical formula **:** $C_{50}H_{46}CaF_2N_2O_8$

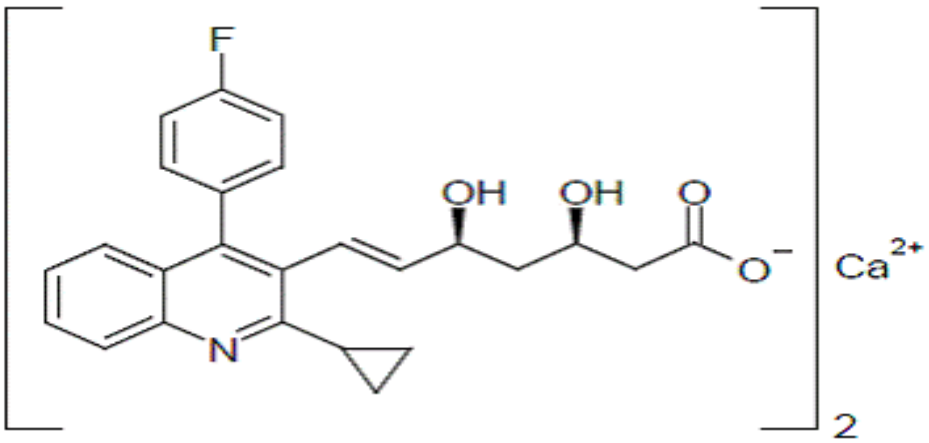
Molecular weight **:** 880.98.

Physical properties **:** Odorless and occurs as white to pale-yellow powder.

Solubility:

It is freely soluble in pyridine, chloroform, dilute hydrochloric acid, and tetrahydrofuran, soluble in ethylene glycol, sparingly soluble in octanol, slightly soluble in methanol, very slightly soluble in water or ethanol, and practically insoluble in acetonitrile or diethyl ether.

Chemical structure:



Chemical name:

Pitavastatin is (+) monocalcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3, 5dihydroxy-6-heptenoate}.

Mechanism of action:

Pitavastatin is lipid-lowering agent that works to control the synthesis of cholesterol via competitive inhibition of the liver enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. As a result, a compensatory increase in LDL-receptor expression can be observed which facilitates an increase LDL catabolism.

Pharmacokinetics:

Absorption : Pitavastatin was absorbed in the small intestine but very little in the colon.

Distribution **:** 148 L

Protein binding **:** >99% protein bound in human plasma, mainly to albumin and alpha 1-acid glycoprotein.

Metabolism:

Pitavastatin is mainly metabolized by liver. It undergoes glucuronidation by uridine 5-diphosphate glucuronosyl transferases (UGT1A3 and UGT2B7) to form the major circulating metabolite, pitavastatin lactone. The cytochrome P450 system has little involvement with the metabolism of pitavastatin. There is some metabolism by CYP2C9 and to a lesser extent, CYP2C8. Studies suggest that concomitant therapy with drugs that are involved with the cytochrome P450 system will not affect the pharmacokinetics of pitavastatin.

Route of elimination : 79% in feces and 15% excreted in urine.

Bioavailability **:** 51%;

Half-life elimination : Plasma elimination half-life = 12 hours.

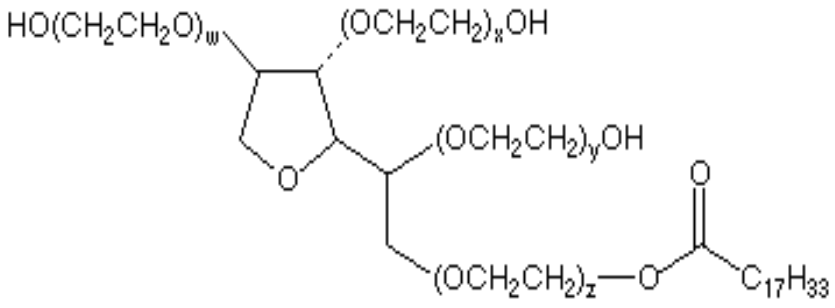
Clearance: CL/F (apparent clearance), 4 mg, healthy male Korean subjects = 23.6 L/h.

Adverse reactions: Toxicity the most frequent adverse reactions (rate ≥2.0% in at least one marketed dose) were myalgia, back pain, diarrhea, constipation and pain in extremity.

5.2. POLYSORBATE 80

Synonyms[82]: Atlas E, Aarmotan PMS 20, capmul POE-O, cremophor PS-80, crillet4, crillet 50, Emrite 6120 & Tween 80

Chemical structure:



Polysorbate 80
(Sum of w, x, y, and z is 20)

Description: Yellow oily liquid with characteristic odour and a warm and somewhat bitter taste.

Functional category: Dispersing agents, emulsifying agent, non ionic surfactant, solubilizing agent, suspending agent & wetting agent

Typical properties:

HLB value : 15

Solubility : Soluble in ethanol and water

Specific gravity : 1.08

Acidity/ alkalinity : P^H=6-8 for a aqueous solution

Flash point : 149^0c

Viscosity **:** 425mPaS

Incompatibilities: Discoloration or precipitation occurs with various substances, especially phenols, tannins, tars and tar like materials. The anti microbial activity of parabens is reduced in the presence of Polysorbates.

Stability and storage conditions:

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases .Polysorbates is hygroscopic and also prolonged storage can lead to the formation of peroxides. Polysorbates can be stored in a well closed container, protected from light in a cool, dry place.

Safety: Polysorbates are used in oral, parenteral and topical formulations and generally regarded as non-toxic and non- irritant materials. There are however cases of hypersensitivity following their topical and intramuscular use when heated to decomposition, the Polysorbates emit acrid smoke and irritating fumes.

Moderately toxic by IV route: Mildly toxic by ingestion.

Applications:

1. Widely useful as emulsifying agents in the preparation of stable oil in water pharmaceutical emulsions.

2. Also useful as solubilizing agents for a variety of substances including essential oils and oils soluble vitamins.

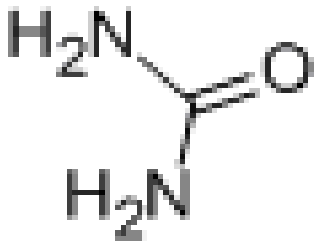
3. They can be used as wetting agents in the formulation of oral and parenteral formulations.

4. They have been found to be useful in improving the oral bio availability of drug molecules that are substrates for the p-glycol protein.

5.3. UREA

Synonyms:URE;UREA;UREUM;B-I-K;Hyanit;UreaAr;UreaGr;UreaBp;UreaIp;Panafil

Chemical structure:



Description: A compound formed in the liver from ammonia produced by the deamination of amino acids. It is the principal end product of protein catabolism and constitutes about one half of the total urinary solids.

Chemical Formula **:** CH_4N_2O

Formula Weight : 60.06

Solubility : H2O: 8 M at 20 °C

Pharmacodynamic: As humectants, urea draws water into the striatum corneum.

5.4. Polyvinylpyrrolidone

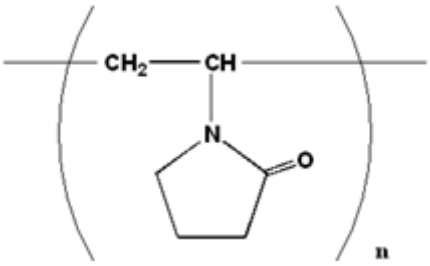
Synonym: Poly [1-(2-oxo-1-pyrrolidinyl) ethylene]; polyvidone; Polyvinylpyrrolidone; PVP; 1-vinyl-2-pyrrolidinone polymer

Chemical name **:** 1-Ethenyl-2-pyrrolidinone homo polymer.

Empirical Formula : (C_6H_9NO) n

Molecular Weight **:** 2500-3000.

Structural Formula:



Description: povidone occurs as a fine, white to creamy-white colored, odorless.

Typical properties

Acidity/alkalinity : pH=3.0-7.0(5%w/v aqueous solution).

Flow ability : 20g/s for povidone k-15;

1620g/s for povidone k-29/32.

Melting point : Softens at 150^0c.

Moisture content:

Povidone is very hygroscopic, significant amounts of moisture being absorbed at low relative humidities.

Solubility:

Freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether hydrocarbons, and mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution, which is a function of the polymer.

Viscosity (dynamic): The viscosity of aqueous povidone solutions depends on both the concentration and the molecular weight of the polymer employed.

Functional Category: Disintegrent; dissolution aid; suspending agent; tablet binder

Applications:

It is used as a binder in many pharmaceutical tablets it simply through the body when taken orally.

- It is used in pleurodesis.
- It is used as an adhesive in glue stick and hot-melt adhesive.
- As an emulsifier and Disintegrent for solution polymerization
- Used in aqueous metal quenching.
- As a thickening agent in tooth whitening gels.

5.5. Sodium lauryl sulfate

Description:

Sodium Lauryl Sulfate (SLS) is an anionic surfactant naturally derived from coconut and/or palm kernel oil. It usually consisting of a mixture of sodium alkyl sulfates, mainly the lauryl. SLS lowers surface tension of aqueous solutions and is used as fat emulsifier, wetting agent, and detergent in cosmetics, pharmaceuticals and toothpastes. It is also used in creams and pastes to properly disperse the ingredients and as research tool in protein biochemistry. SLS also has some microbicidal activity.

Structure:

Synonyms:

Dodecyl alcohol, hydrogen sulfate, sodium salt

Dodecyl sodium sulfate

Dodecyl sulfate sodium

Dodecyl sulfate sodium salt

Lauryl sodium sulfate

Lauryl sulfate sodium

Lauryl sulfate sodium salt

Lauryl sulfate, sodium salt

Lauryl sodny [Czech]

Categories : Surface-Active Agents

Chemical Formula : $C_{12}H_{25}NaO_4S$

Mechanism of action:

Like other surfactants, SLS is amphiphilic. It thus migrates to the surface of liquids, where its alignment and aggregation with other SLS molecules lowers the surface tension. This allows for easier spreading and mixing of the liquid. SLS has potent protein denaturing activity and inhibits the infectivity of viruses by solubilizing the viral envelope and/or by denaturing envelope.

Pharmacodynamic: SLS is an anionic surfactant. Its amphiphilic properties make it an ideal detergent.

5.6. β –CYCLODEXTRIN

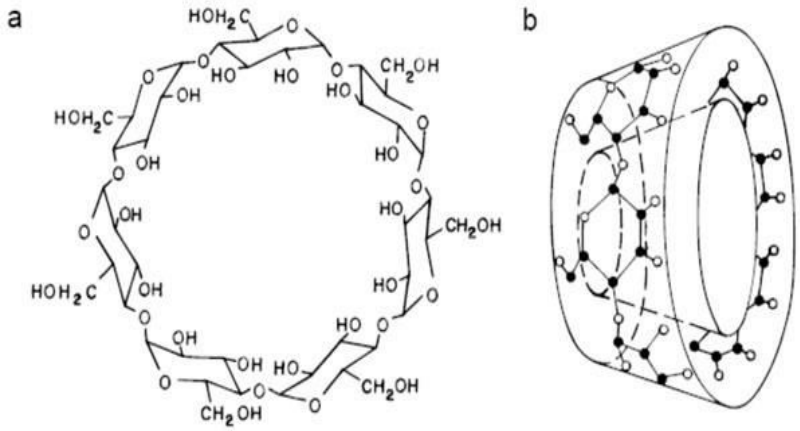
Synonym : Beta – cyclodextrin, βCD, BCD, β-sachar Dextrin cyclodextrin B, cycloamyloses, Cyclomaltoses and Schardinger dextrins

Chemical formula : $(C_6H_{10}O_5)_7$

Molecular weight : 1135.00

Solubility : Sparingly soluble in water, freely soluble in hot Water Slightly soluble in alcohol

Structure :



The chemical structure (A) and the toroidal shape (B) of the cyclodextrin molecule

Table.7: Some characteristics of (α, β, γ, δ).

Type of CD	Cavity diameter A	Molecular weight	Solubility(g/100ml)
α – CD	4.7 – 5.3	972	14.5
β - CD	6.0 – 6.52	1135	1.85
γ - CD	7.5 – 8.3	1297	23.2
δ - CD	10.3 -11.2	1459	8.19

Applications of cyclodextrin

Since each guest molecule is individually surrounded by a cyclodextrin (derivative) the molecule is micro-encapsulated from a microscopically point of view. This can lead to advantageous changes in the chemical and physical properties of the guest molecules.

i. Stabilization of light or oxygen-sensitive substances.

ii. Modification of the chemical reactivity of guest molecules.

iii. Fixation of very volatile substances.

iv. Improvement of solubility of substances.

v. Modification of liquid substances to powders.

vi. Protection against degradation of substances by microorganisms.

vii. Masking of ill smell and taste.

viii. Masking pigments or the color of substances.

6. MATERIALS

6.1. INGREDIENTS USED:

Table 8: Ingredients used for the experiment

S.No	Name of the ingredient	Manufacturer/Suppliers
1.	Pitavastatin	Spectrum lab,hyd
2.	Urea	RankemPVT. Ltd,India
3.	PVP-k30	RankemPVT. Ltd,India
4.	β-cyclo dextrin	Rankem PVT. Ltd,India
5.	Tween-80	RankemPVT. Ltd,India
6.	SLS	RankemPVT. Ltd,India
7.	Methanol	Rankem PVT. Ltd,India

6.2. INSTRUMENTS USED:

Table 9: Instruments used for the experiment

S.No	NAME OF THE INSTRUMENT	MANUFACTURING COMPANY
1.	Digital balance	Shimadzu ELB 300
2.	Ultrasonic bath sonicator	Bandelinsono plus model HD,2070
3.	Magnetic stirrer with hot plate,	Eltek MS 2012
4.	Uv-spectophotometer	Perkin elmer lambda25
5.	Dissolution apparatus US	Labindia DS-800
6.	FT-IR spectrophotometer	Perkin elmer spectrum RX1
7.	Scanning electron microscope	Jobel model JSM 6400,Tokyo
8.	Zeta potential analyser	Zetasizer 3000 HS,Malvern instrument, UK
9.	Differential scanning calorimetry	DSC DA 609 Shimadzu, japan

6.3METHODS

1 .PREFORMULATION STUDIES [51, 52]

Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Preformulation studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

Fourier Transform Infra Red Spectroscopy (FTIR)

Compatibility study of Pitavastatin with the carrier's urea, PVP, β-cyclodextrin, and mixture of urea, PVP and β-cyclodextrin used to formulate nanosuspension was determined by FTIR Spectroscopy using Perkin Elmer RX1. Spectral analysis of pitavastatin, urea, PVP and β-cyclodextrin and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients. The compatibility study on FTIR was carried by JASCO FT/IR 4100, MD, and USA in the frequency range 4000-400cm^{-1}

Differential Scanning Calorimetry (DSC):

Differential scanning calorimetric curve of pure Pitavastatin, PVP, urea β-cyclodextrin and mixture of drug and carriers measurement were carried out by using a thermal analysis instrument equipped with a liquid nitrogen sub ambient accessory. First, a suspension sample of about 10 mg was placed in an aluminum pan, hermetically sealed before being placed in the calorimeter thermocouples. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C, Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

6.4. ESTIMATION OF PURE PITAVASTATIN

Pitavastatin can be estimated spectrophotometrically at 241nm in the range of 3-18 mcg/ml as per Beer Lambert's law.

Preparation of 0.1 N HCl:

Dissolve 8.5 ml of concentrated HCl in 1000 ml of distilled water.

Preparation of standard drug solution:

Stock solution:

10 mg of Pitavastatin was dissolved in 10 ml of 0.1 N HCl, to get a solution of 1000 µg/ml concentration.

Standard solution:

1 ml of stock solution was made to 10 ml with 0.1 N HCl thus giving a concentration of 100 µg/ml. Aliquot of standard drug solution ranging from 0.3ml, 0.6 ml, 0.9 ml, 1.2 ml,1.5ml and 1.8 ml were transferred into 10 ml volumetric flask and were diluted up to the mark with 0.1 N HCl. Thus the final concentration ranges from 3-18 µg/ml. Absorbance of each solution was measured at 241 nm against 0.1 N HCl as a blank. A plot of concentrations of drug versus absorbance was plotted.

PREPARATION OF PITAVASTAIN NANOSUSPENSION BY NANOPRECIPITATION :

The nanosuspension was obtained by the precipitation process. The drug pitavastatin was initially dissolved in 5 ml of methanol it is organic phase. The organic phase was slowly added drop wise with syringe in 10ml of 0.1N HCL aqueous phase containing carrier urea, PVP, β-cyclodextrin and surfactants SLS and Tween 80 kept at room temperature with speed of 900-1000rpm. Speeds using Magnetic stirrers until all the drug solution completely added in to surfactant solution and methanol completely evaporate from the solution.

Fig: 9 Photograph of the nanosuspension showing bluish opalescence.

Table 10: Formulation of pitavastatin nanosuspensions

FORMULATION	PITAVASTATIN	UREA	PVP30	β-CD	SLS	TWEEN 80	WATER	METHANOL
F0	10mg	---	---	---	---	---	10ml	5ml
F1	10mg	20mg	---	---	---	---	10ml	5ml
F2	10mg	---	20mg	---	---	---	10ml	5ml
F3	10mg	---	---	20mg	---	---	10ml	5ml
F4	10mg	20mg	---	---	5mg	---	10ml	5ml
F5	10mg	---	20mg	---	5mg	---	10ml	5ml
F6	10mg	---	---	20mg	5mg	---	10ml	5ml
F7	10mg	20mg	---	---	---	0.1ml	10ml	5ml
F8	10mg	---	20mg	---	---	0.1ml	10ml	5ml
F9	10mg	---	---	20mg	---	0.1ml	10ml	5ml
F10	10mg	30mg	---	---	10mg	---	10ml	5ml
F11	10mg	---	30mg	---	10mg	---	10ml	5ml
F12	10mg	---	---	30mg	10mg	---	10ml	5ml
F13	10mg	30mg	---	---	---	0.2ml	10ml	5ml
F14	10mg	---	30mg	---	---	0.2ml	10ml	5ml
F15	10mg	---	---	30mg	---	0.2ml	10ml	5ml

EVALUATION OF PITAVASTAIN NANOSUSPENSION

Fourier Transform Infra Red Spectroscopy (FTIR)

Compatibility study of Pitavastatin with the carrier's urea, PVP, β-cyclodextrin, and mixture of urea, PVP and β-cyclodextrin used to formulate nanosuspensions was determined by FTIR Spectroscopy using Perkin Elmer RX1. Spectral analysis of Pitavastatin, urea, PVP and β-cyclodextrin and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients. The compatibility study on FTIR was carried by JASCO FT/IR 4100, MD, and USA in the frequency range 4000-400 cm^{-1}.

Scanning Electron Microscopy (SEM):

Surface morphology of the specimen will be determined by using a scanning electron microscope (SEM), Model JSM 84 0A, JEOI, Japan. The samples are dried thoroughly in vaccum desicator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current about 80mA.The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and working distance WD=39mm.

Zeta potential measurement:

Zeta potential of the suspension is measured by Malvern Zetasizer. The zeta sizer mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the center of the sample cell, and the scattered light at an angle of about 130 is detected. Zetasizer software produces a

frequency spectrum from which the electrophoretic mobility hence the zeta potential is calculated.

Thermal Analysis by Differential Scanning Calorimetry (DSC):

DSC scans of the prepared lyophilized powdered drug sample and pure drug samples were recorded using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminum pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

Drug entrapment efficiency (DEE)

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5 _C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 241 nm using UV spectrophotometer against blank/control Nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated [53].

The entrapment efficiency (EE %) could be achieved by the following equation:

$$\text{Entrapment efficiency}\,(\%) = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Particle Size Analysis:

The particle size analysis was carried out using Microtac blue wave particle size analyzer. Before measurement the samples has to be diluted with de-ionized water to obtain a suitable Concentration for measurement. The results obtained for particle size distributions were used to confirm the formation of nano-sized particles.

Saturation Solubility Studies:

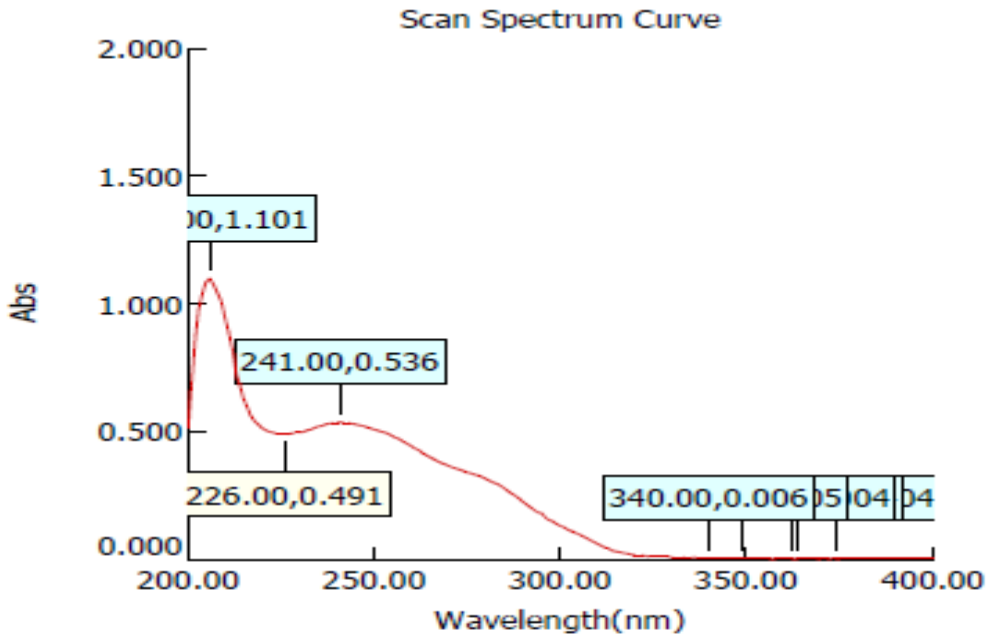
Saturation solubility measurements were assayed through ultraviolet absorbance determination at 241 nm using shimadzu UV-Visible spectrophotometer. The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of lyophilized nanosuspensions. 10 mg of unprocessed pure drug and

nanosuspensions equivalent to 10 mg of Pitavastatin were weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples were collected after the specified time interval, and it is filtered and analyzed. The diluted samples were analyzed using UV spectrophotometer at 241nm.

Dissolution study:

In vitro drug release studies were performed in USP apparatus-Type II using paddle method at rotation speed of 50 rpm. Dissolution was carried out in 0.1N HCL as a dissolution medium. The volume and temperature of the dissolution medium were 900 ml and 37.0 + 0.5oC. 5 ml of sample was withdrawn periodically (after 5minutes) and replaced with an equal volume of fresh 0.1N HCL up to 60min. Samples were suitably diluted and filtered through a filter paper (0.22 μm, Whatman Inc., USA). The filtrate was then subject to the UV analysis against the blank (distilled water). Percent cumulative release of SS was calculated based on the standard UV calibration curve at 241nm (Systronic 2203, Japan).

7. RESULT



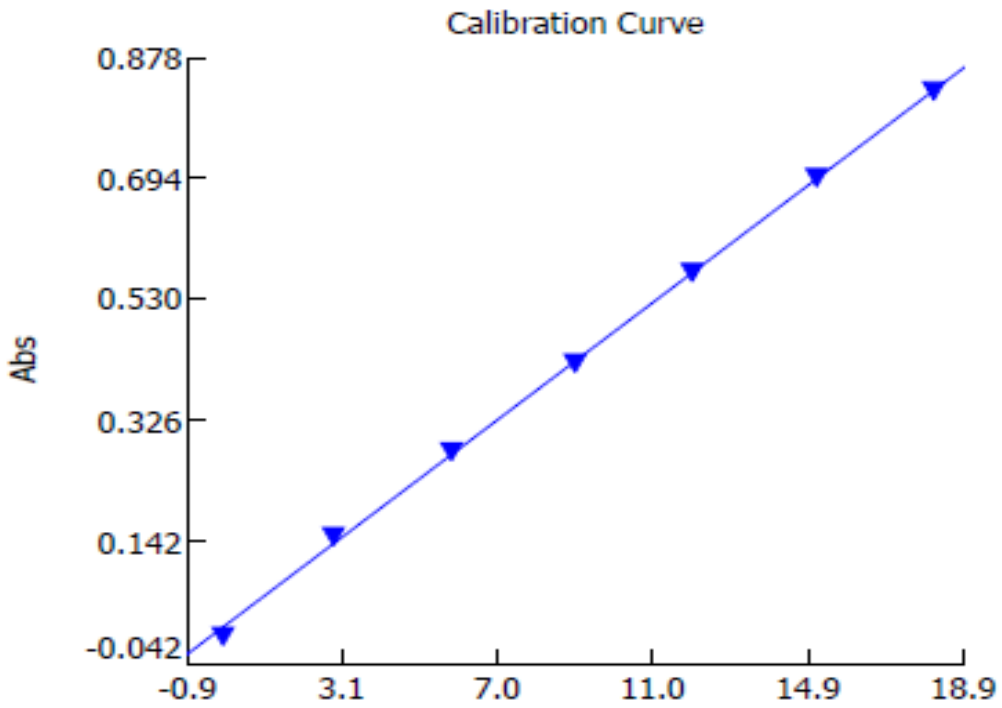
1	Peak	374.00	0.004
2	Peak	364.00	0.004
3	Peak	362.00	0.004
4	Peak	349.00	0.005
5	Peak	340.00	0.006
6	Peak	241.00	0.536
7	Peak	206.00	1.101
1	Valley	226.00	0.491

GRAPH NO: 1 λ_{max} OF PITAVASTATIN

TABLE 11: DATA FOR STANDARD CALIBRATION CURVE OF PITAVASTAIN DRUG IN0.1N HCL BUFFER:

S.No	Concentration (µg/ml)	Absorbance (241nm)
1.	0	0
2.	3	0.151
3.	6	0.287
4.	9	0.425
5.	12	0.562
6.	15	0.699
7.	18	0.837
	Slope	0.04603
	Regression	0.9999

STANDARD CURVE FOR PITAVASTATIN IN 0.1N HCL AT 241nm



Graph No: 2 Con. [µg/ml]

Preformulation study:

Identification:

Determination of melting point:

Melting point of pitavastatin was found to be in the range of 190-192^{0}c as reported in the literature, thus indicating purity of sample. If any impurity will present, will cause variation in the melting point of a given drug substance. The melting point of the obtained drug was found to be 191$^0_{C.}$ Hence the obtained drug was found to be pure without any impurities.

Solubility:

Pitavastatin is very slightly soluble in water. Very soluble in pyridine, chloroform, dilute hydrochloric acid, tetrahydrofuran , soluble in ethylene glycol, sparingly soluble in octanol, slightly soluble in methanol.

Drug entrapment efficiency:

The formulations showed drug entrapment in the range of 84–92 %. Formulation (Fp15) containing drug concentration 80 mg/ml stabilised with β-cyclodextrin showed highest entrapment up to 92 % w/v. Formulations containing β-cyclodextrin showed better entrapment in comparison to PVP, urea.

Particle Size Analysis:

The particle size distribution studies showed that all the formulation particle size was in the range of 443-446 nm and where as unprocessed drug shows 50.5± 0.07μm sizes. All the formulations having a particle size in the nanometre range and showing ideal surface morphology.

Saturation Solubility Studies:

The saturation solubility studies indicating that nanosuspensions showing maximum solubility Compared to unprocessed drug which are due to the amorphous nature of drug.

Table 12: Results of solubility and drug entrapment efficiency.

Studies of Formulation and Pure Drug

Sample	Solubility (µg/ml)	Drug entrapment
F0	132	----
F1	209	54
F2	253	57
F3	287	60
F4	312	63
F5	334	67
F6	378	70
F7	402	73
F8	436	75
F9	628	78
F10	499	81
F11	521	84
F12	548	86
F13	592	87
F14	475	90
F15	651	92

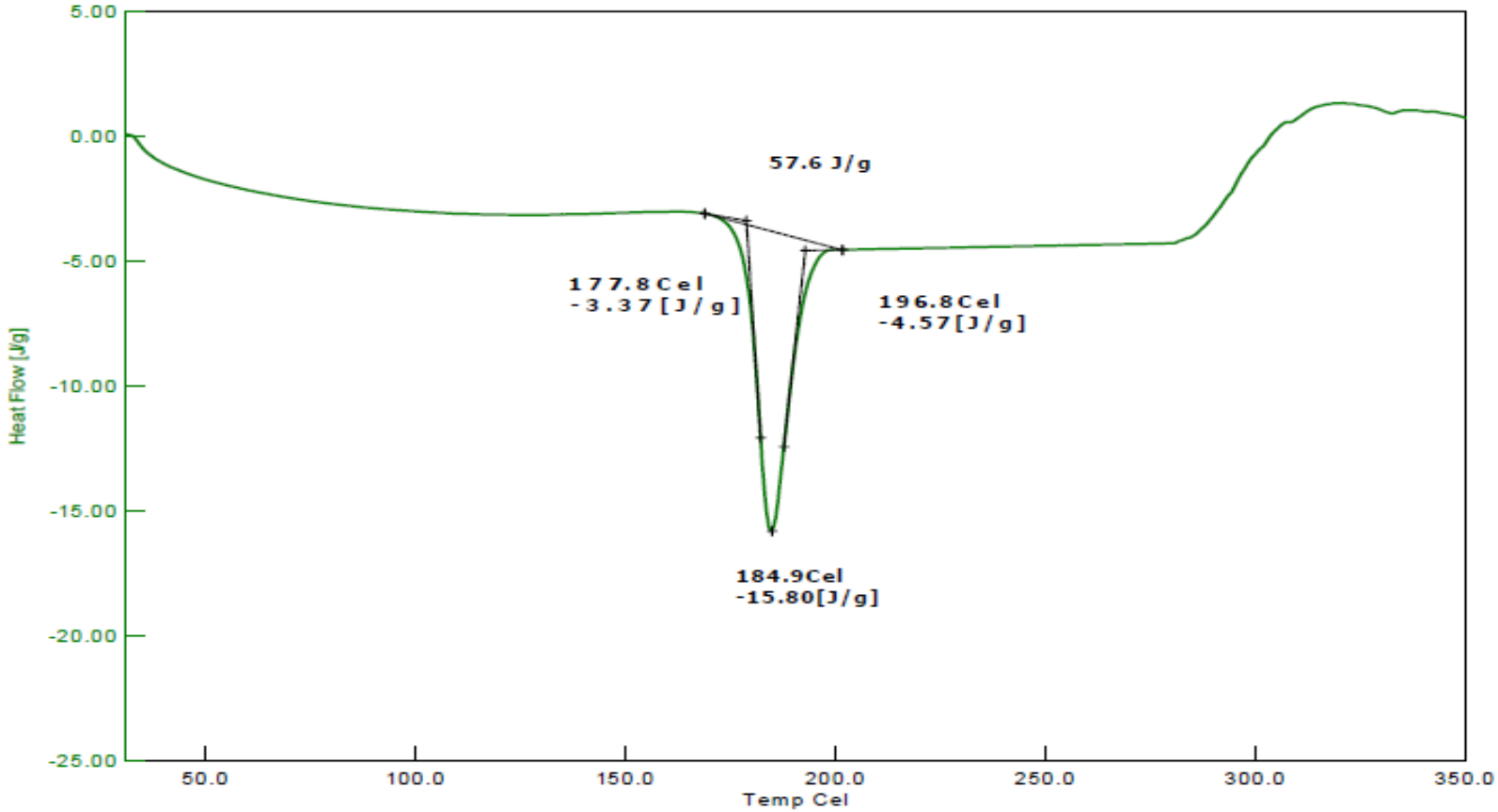
Differential scanning colorimetry:

The physical state of raw pitavastatin and lyophilized drug nanoparticles was examined by DSC. The DSC of pitavastatin shows an endothermic curve at its melting point 184.9°C (ΔH = -15.80 J/g) and the precipitated drug shows an endothermic peak at 300. 7°C (ΔH = -16.43J/g). The complete absence of pitavastatin peak indicates that pitavastatin is present as amorphous after being precipitated as nanoparticles it reduced crystallinity.

Module: DSC
Data Name: SUN-02
Measurement Date: 07/26/2013
Sample Name: PITAVA
Sample Weight: 4.314 mg
Reference Name: Aluminium
Reference Weight: 0.000 mg

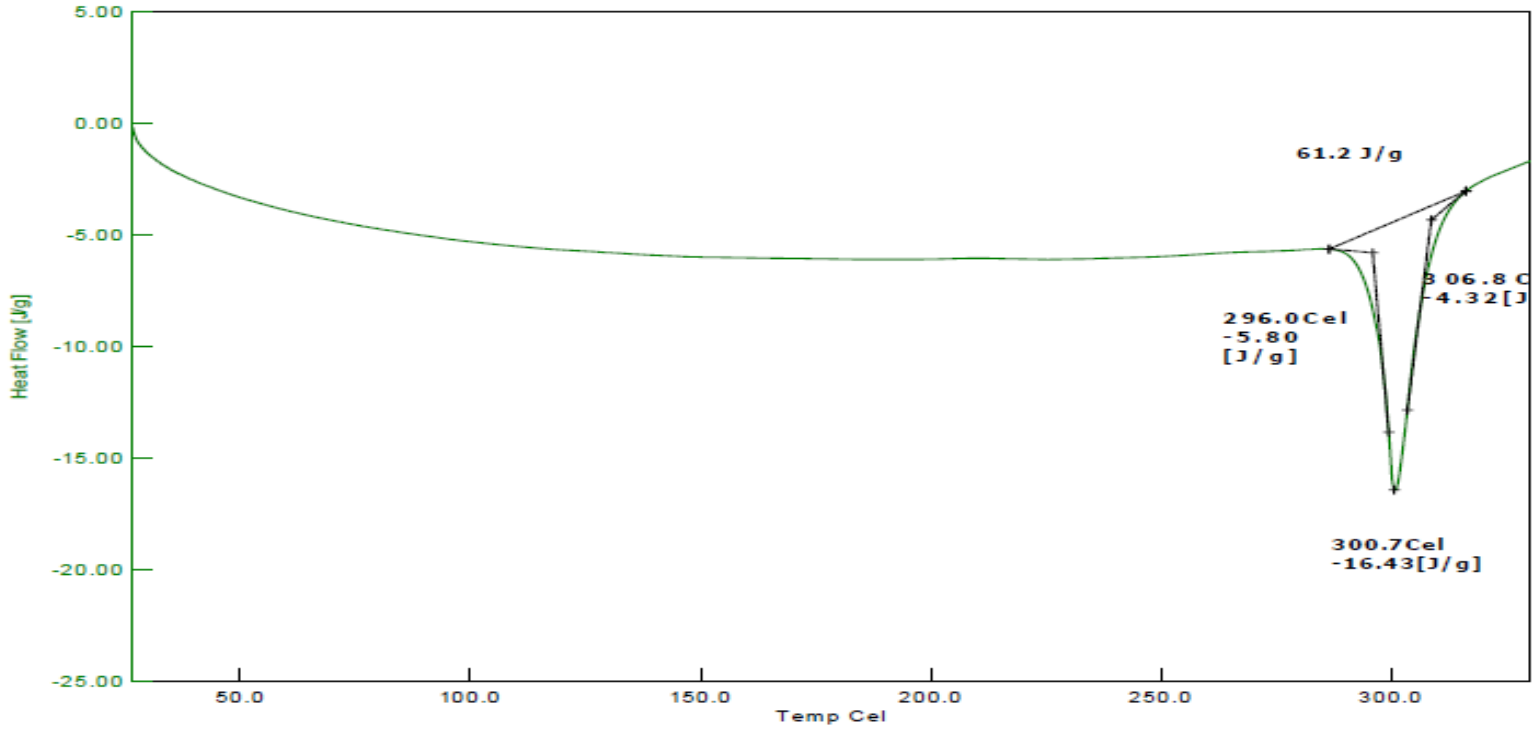
DSC

Comment:
Operator: EXSTAR
Gas1: Nitrogen
Gas2:
Pan: Aluminium



Module: DSC
Data Name: SUN
Measurement Date: 07/26/2013
Sample Name: B-CD
Sample Weight: 2.631 mg
Reference Name: Aluminium
Reference Weight: 0.000 mg

Comment:
Operator: EXSTAR
Gas1: Nitrogen
Pan: Aluminium



Compatibility studies:

FT-IR spectroscopy was carried out to study the compatibility of pure pitavastatin drug with β-cyclodextrin carrier and Tween 80 used in the formulation of nanosuspensions. The physical mixture has characteristic IR peaks at 1026.14 C-F(alkyl halide), 1153.07 C-F(alkyl halide) ,696.33 C-Cl(alkyl halide), 753.22 C-Cl(alkyl halide).The pitavastatin has characteristic IR peaks at 1520.07 C=C(Aromatic), 3738.54 O-H(Alcohol), 1436.76-C-H(Alkane), 1321.31 C-N(Amine).The β-cyclodextrin has characteristic IR peaks at 3392.14 N-H(Amine), 2927.62 C-H(Alkane), 1029.36 C-F(alkyl halide), 577.98 C-Br(Alkyl Halide).The tween80 has characteristic peaks IR at 3382.76 O-H(Alcohol), 2922.28 C-H(Alkane). 2339.80 C-N (Nitrile), 1540.30 C=C (Aromatic).

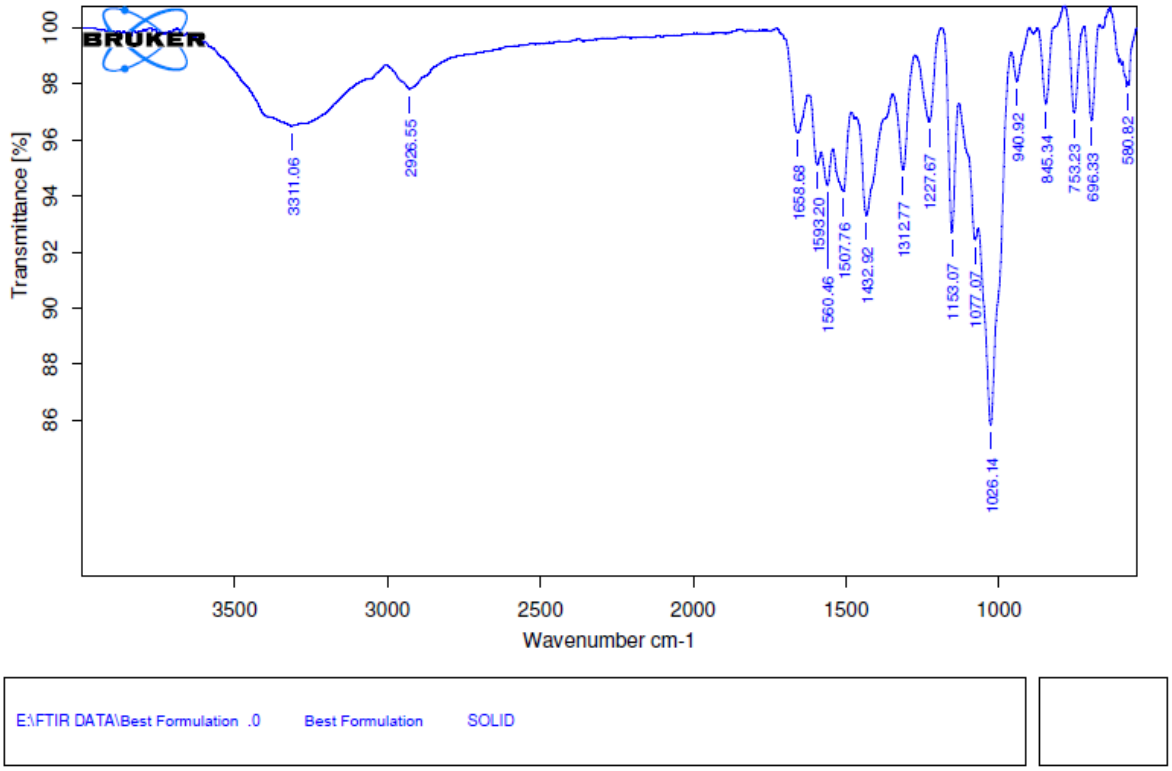


Table: 13 pitavastatin nanosuspension of β-cyclodextrin & Tween 80

S.No	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching /Deformation
	Pitavastatin nanosuspensions of β-cyclodextrin and tween80	1026.14	C-F(alkyl halide)	Stretching
		1153.07	C-F(alkyl halide)	Stretching
		696.33	C-Cl(alkyl halide)	Stretching
		753.22	C-Cl(alkyl halide)	Stretching

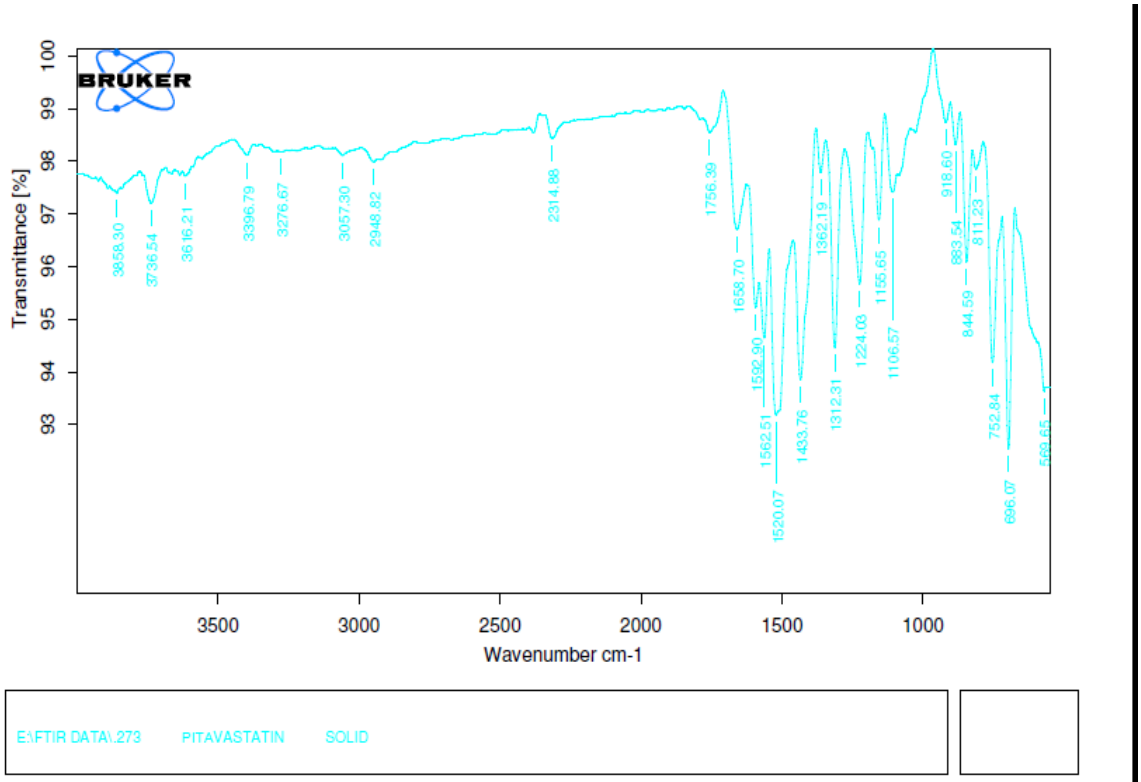


Table 14: Pure pitavastatin

S.No	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching /Deformation
1	Pitavastatin	1520.07	C=C(Aromatic)	Stretching
		3738.54	O-H(Alcohol)	Stretching
		1436.76	-C-H(Alkane)	Stretching
		1321.31	C-N(Amine)	Stretching

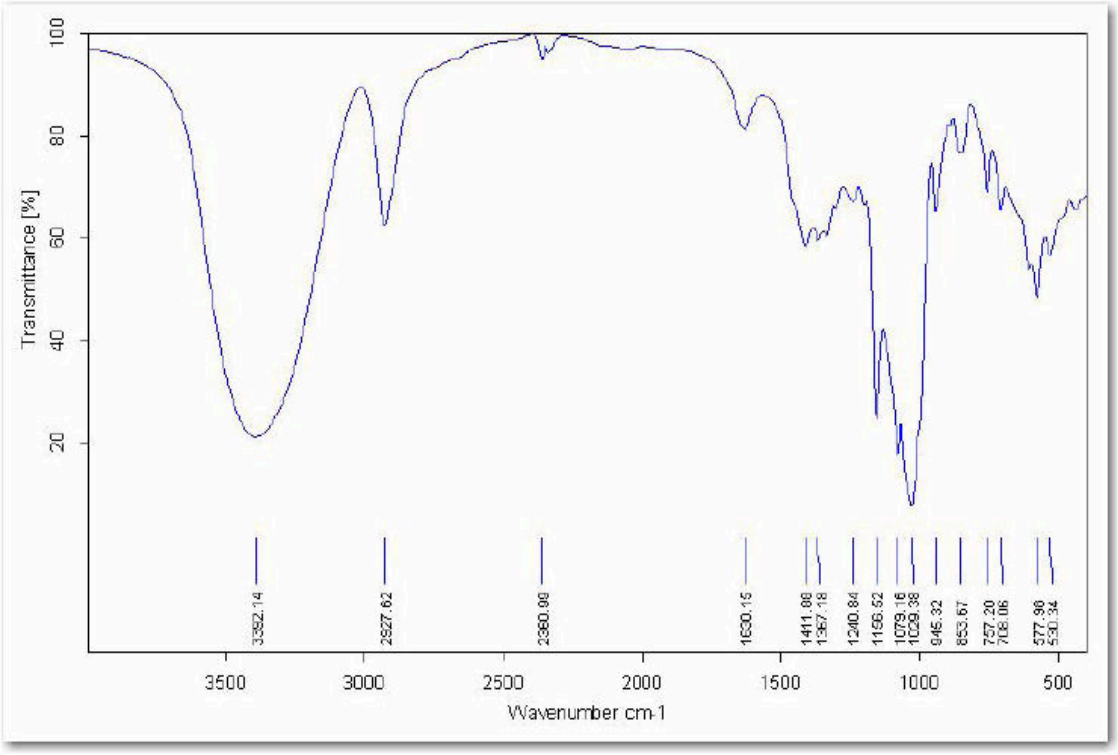


Table 15: β-cyclodextrin

S.No	IR Spectrum	Peaks($cm^{-1)}$	Groups	Stretching /Deformation
1	β-cyclodextrin	3392.14	N-H(Amine)	Stretching
		2927.62	C-H(Alkane)	Stretching
		1029.36	C-F(alkyl halide)	Stretching
		577.98	C-Br(Alkyl Halide)	Stretching

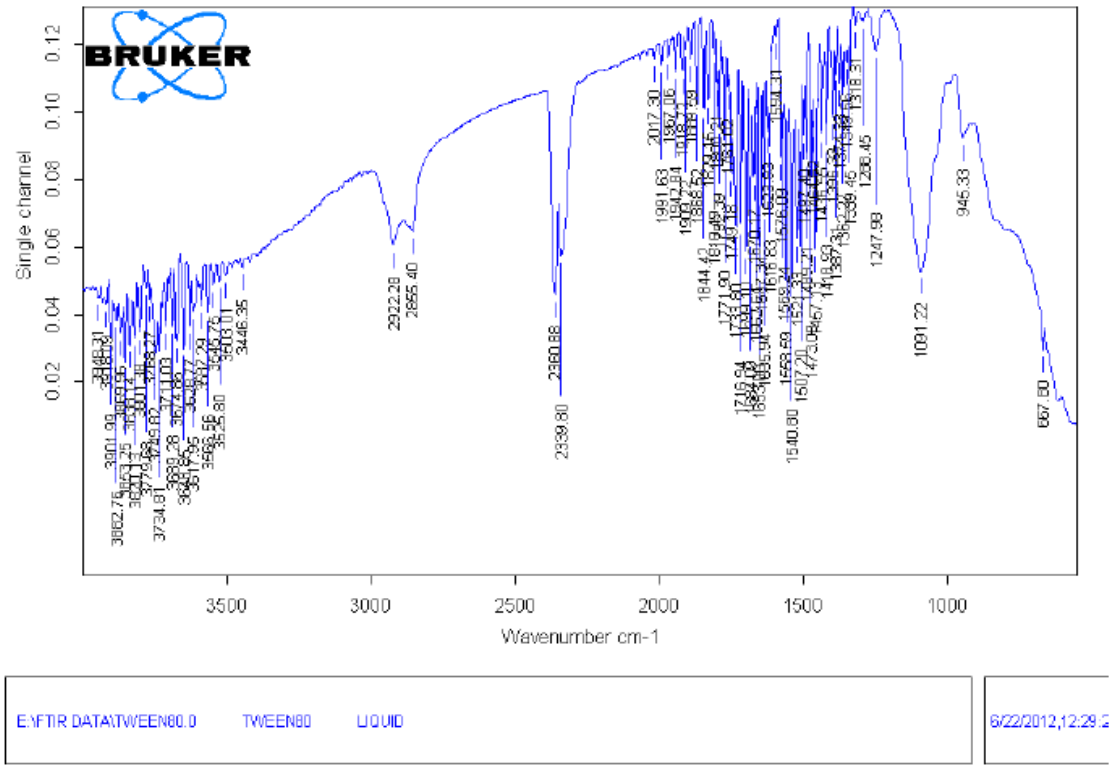
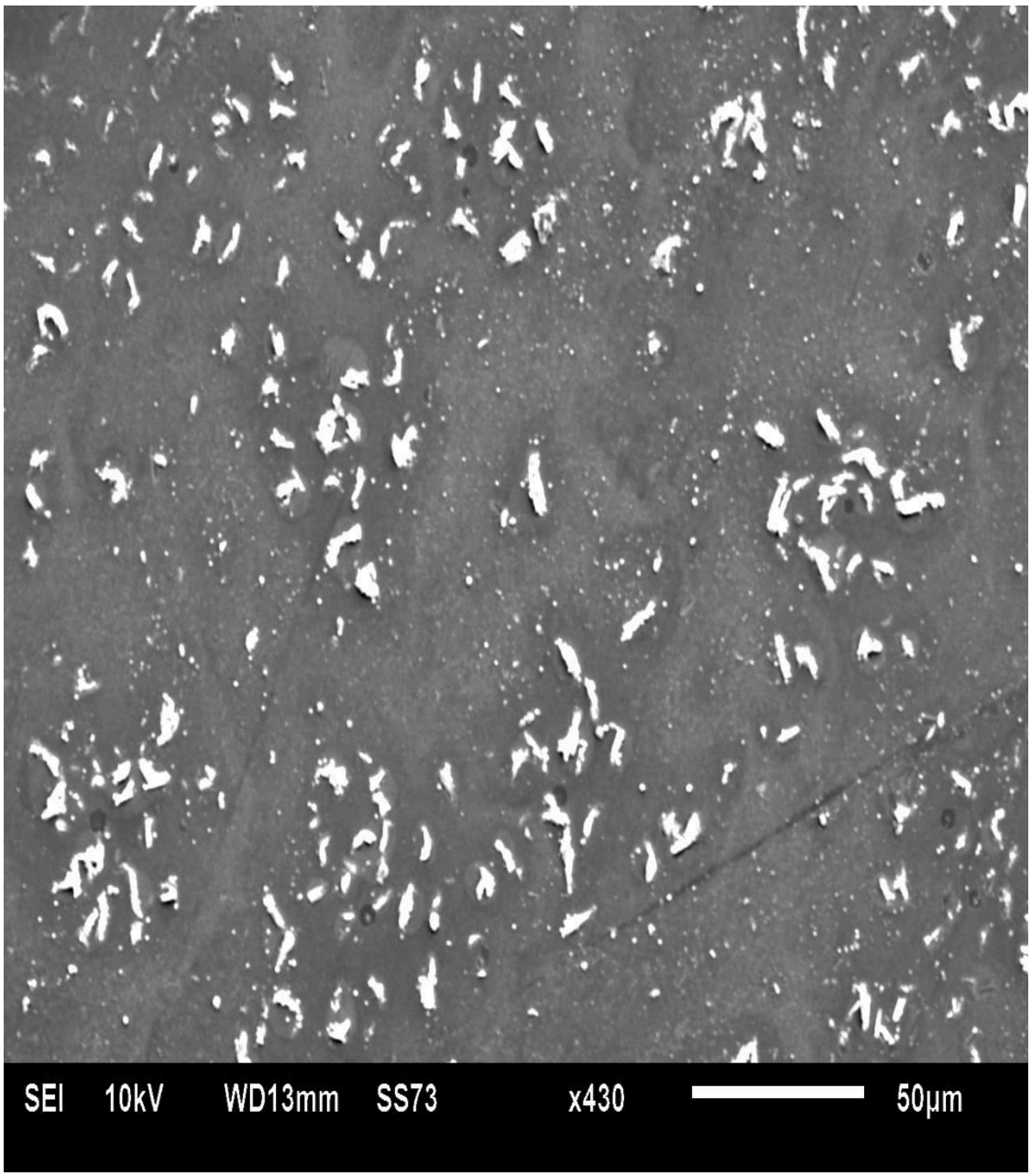


Table 16: Tween 80

S.No	IR Spectrum	Peaks(cm^{-1})	Groups	Stretching /Deformation
1	Tween80	3382.76	O-H(Alcohol)	Stretching
		2922.28	C-H(Alkane)	Stretching
		2339.80	C-N(Nitrile)	Stretching
		1540.30	C=C(Aromatic)	Stretching

Shape and surface morphology:

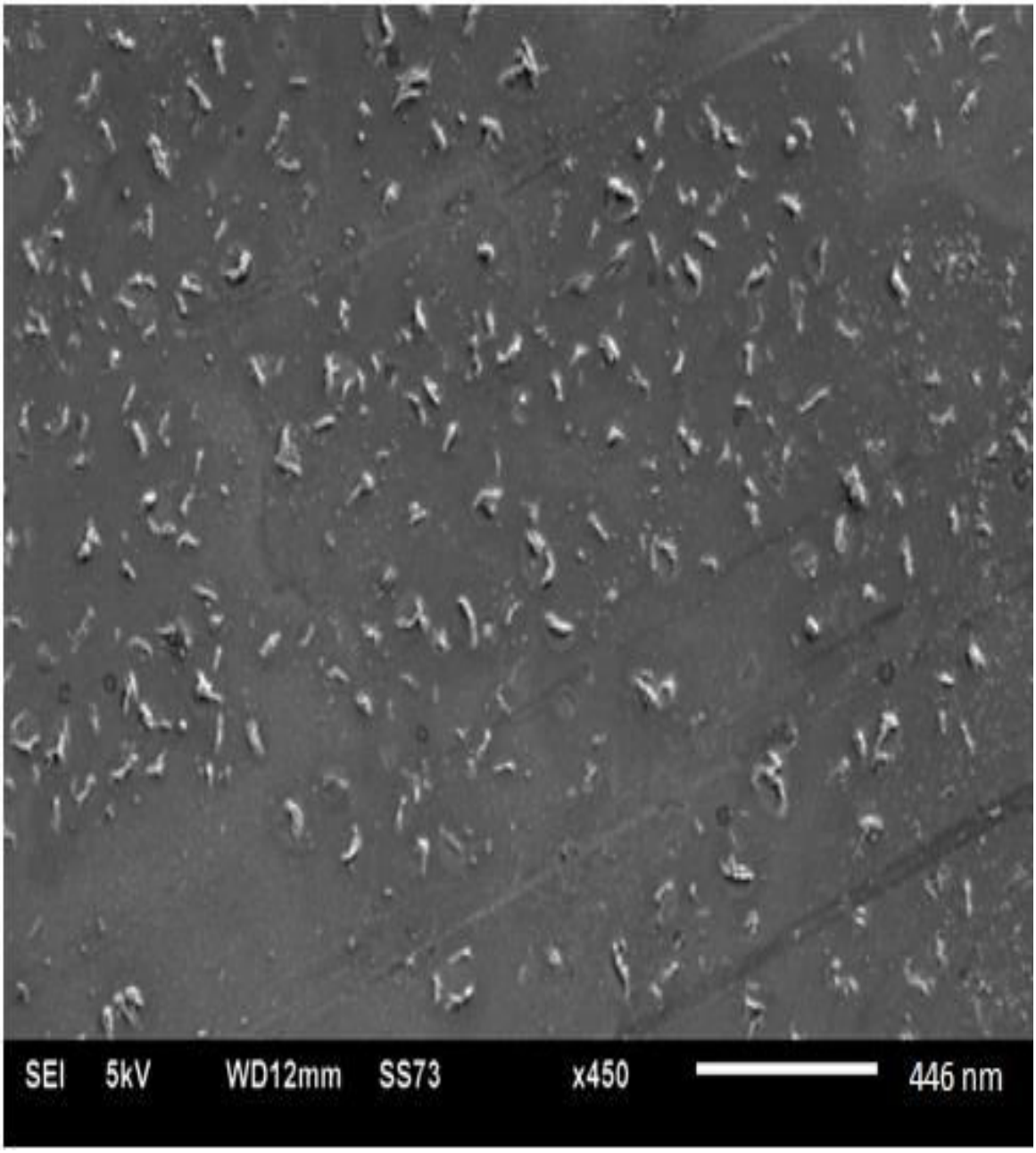
Nanoparticle surface morphology and shape were visualized using SEM (JSM-T330A, JEOL). The drug loaded nanoparticles were found to be spherical with smooth surface.



SCANNING ELECTRON MICROSCOPY OF PURE PITAVASTATIN

SCANNING ELECTRON MICROSCOPY FOR

FORMULATION15



ZETA POTENTIAL:

Zeta potential is a term related to the stability of samples for molecules and particles that are small enough, high zeta potential will confer stability i.e.it resist aggregation .Here zeta potential of the prepared nanosuspension was found to be -34.4,which would not allow aggregation

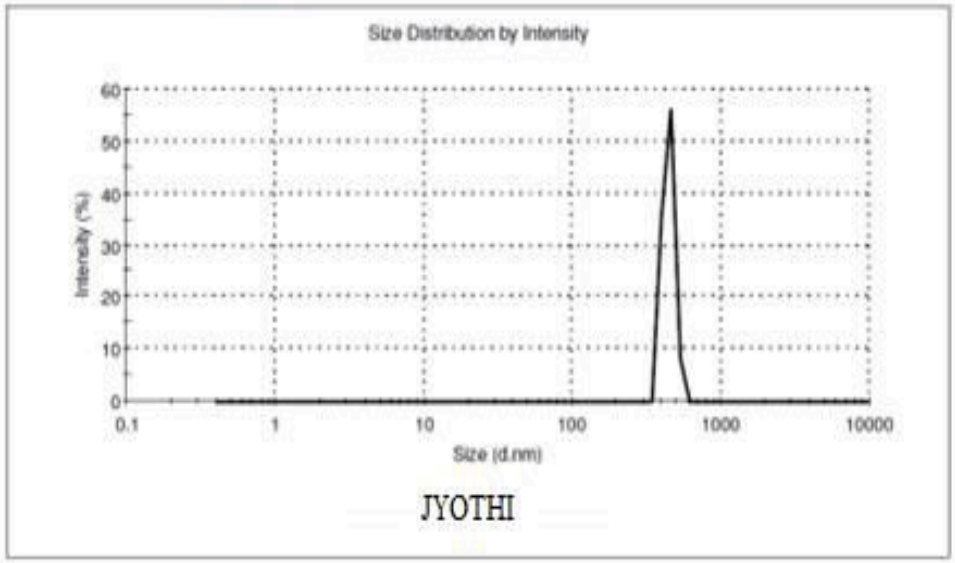
System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	168.4	**Measurement Position (mm):**	5.50
Cell Description:	Clear disposable zeta cell	**Attenuator:**	6

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	446 nm	**Peak 1:**	442.3	100.0	39.50
PdI:	0.623	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.826	**Peak 3:**	0.000	0.0	0.000

Result quality : GOOD



Malvern Instruments Ltd
www.malvern.com

DTS Ver. 5.10
Serial Number : MAL1031371

File name: NAVIN NEW
Record Number: 17
13 Jun 2013 4:30:15 Ph

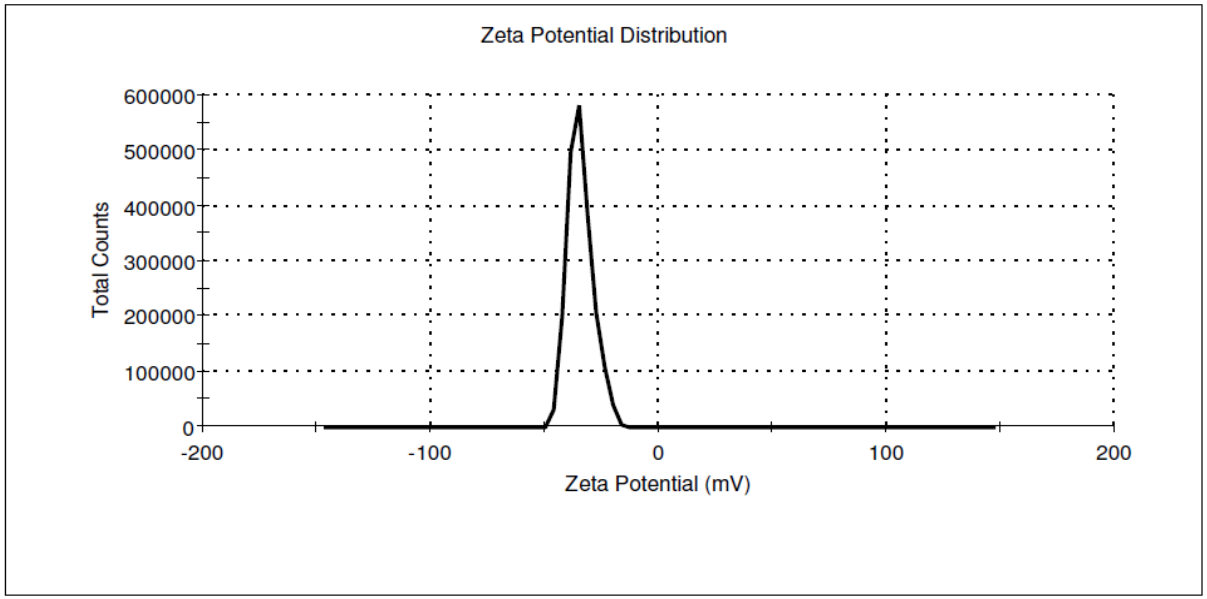
System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	365.1	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	5

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-34.4	**Peak 1:**	-34.4	100.0	5.52
Zeta Deviation (mV):	5.52	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	1.06	**Peak 3:**	0.00	0.0	0.00

Result quality : Good



JYOTHI

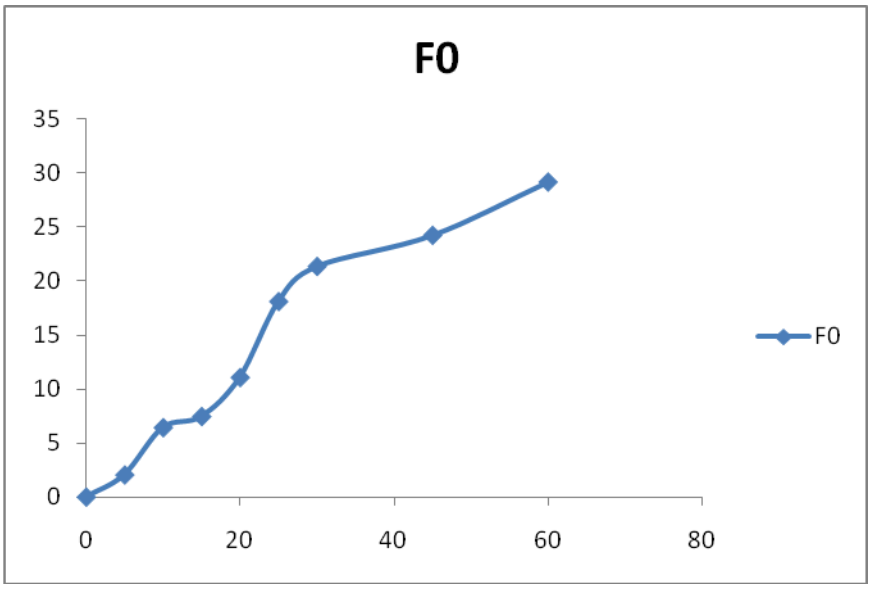
IN-VITRO DRUG RELEASE CHARACTRISTICS OF PITAVASTAIN NANOSUSPENSION

In-vitro release behavior of Pitavastatin is summarized in tables; *in-vitro* drug release from the nanosuspensions in 0.1N HCL buffer was performed. The *in-vitro* drug release profile of nanosuspensions formulations obtained from experiment was shown in table.

The release rate profiles were drawn as the percentage pitavastatin dissolved from the nanosuspensions and pure drug versus time. Dissolution studies of pure pitavastatin and all other prepared nanosuspensions (F0- F15) were carried out in 0.1N HCL. T90% (time to dissolve 90% drug) values calculated from release profile are reported. From this data, it was evident that onset of dissolution of pure pitavastatin was very low. Dissolution of pitavastatin nanosuspensions was affected by different surfactant concentrations and organic to aqueous solvent ratio. It can be observed that, 80% of the pitavastatin nanosuspensions were dissolved in 5min; while in the same period, 2.16% of the raw pitavastatin was dissolved. According to Noyes–Whitney equation, the dissolution rate is directly proportional to its surface area exposed to the dissolution medium. The increase dissolution for drug nanoparticles could thus be mainly ascribed to their greater surface area in comparison with raw drug.

Table 17: Dissolution profile of formulation (F0) in 0.1N HCL

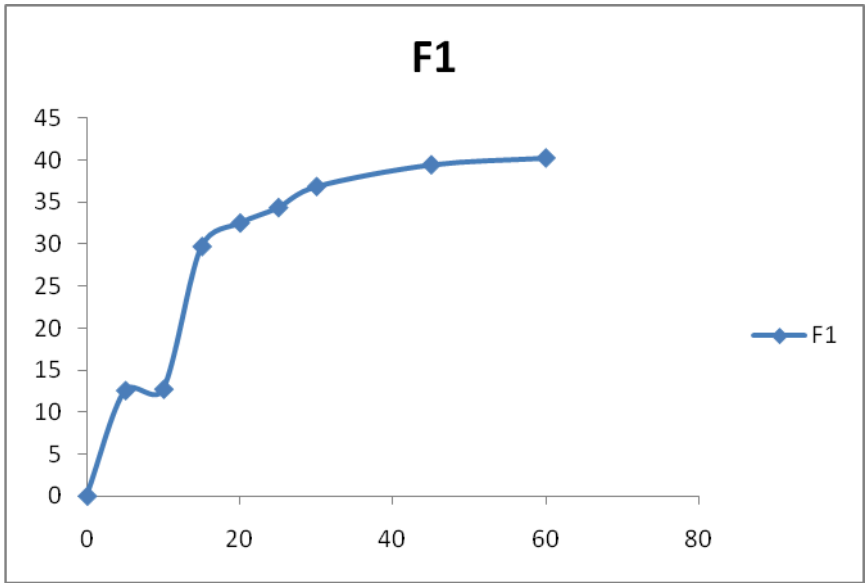
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial2	Trial3	Mean ± SD
0	0	0	0	0
5	2.16	2.01	1.98	2.05±0.09643
10	6.49	6.57	6.23	6.43±0.17776
15	7.47	7.82	7.09	7.46±0.35529
20	11.01	10.89	11.33	11.07±0.22774
25	18.10	18.24	17.98	18.10±0.13012
30	21.05	21.33	21.68	21.35±0.31564
45	24.00	24.47	24.23	24.23±0.23501
60	29.11	29.37	29.00	29.16±0.19



Graph No.3: *In-Vitro* Drug Release of F_0

Table 18: Dissolution profile of formulation (F1) in ph 0.1N HCL

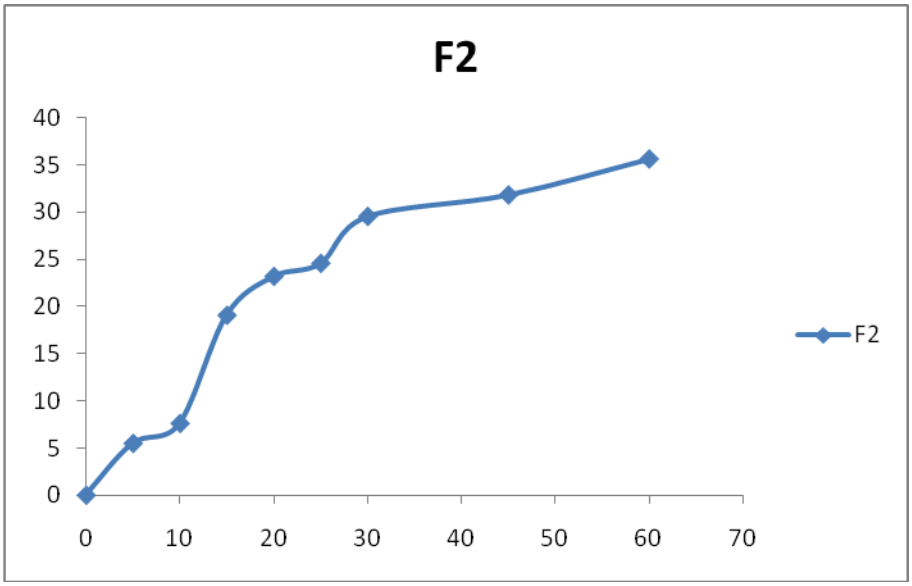
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	12.59	12.37	12.73	12.56±0.18147
10	25.96	25.65	25.57	25.72±0.20599
15	29.90	29.73	29.48	29.70±0.21126
20	32.46	32.89	32.17	32.50±0.36226
25	34.23	34.69	34.09	34.33±0.31390
30	36.98	36.69	36.86	36.84±0.14571
45	39.34	39.19	39.78	39.43±0.30664
60	40.33	40.15	40.25	40.24±0.09018



Graph No.4: *In-Vitro* Drug Release

Table 19: Dissolution profile of formulation (F2) in ph 0.1N HCL

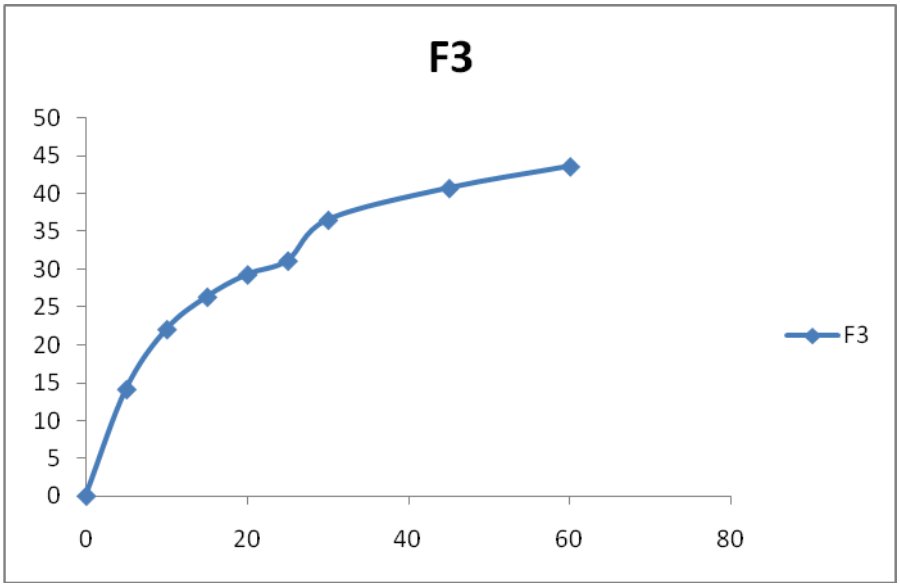
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	5.50	5.32	5.69	5.50±0.18502
10	7.47	7.60	7.76	7.61±0.14525
15	19.28	19.04	18.94	19.08±0.17473
20	23.21	23.15	23.26	23.20±0.05507
25	24.59	24.92	24.23	24.58±0.34510
30	29.90	29.43	29.35	29.56±0.29715
45	31.87	31.74	31.92	31.84±0.09291
60	35.80	35.52	35.65	35.65±0.14011



Graph No.5: *In-Vitro* Drug Release of F_2

Table 20: Dissolution profile of formulation (F3) in 0.1N HCL

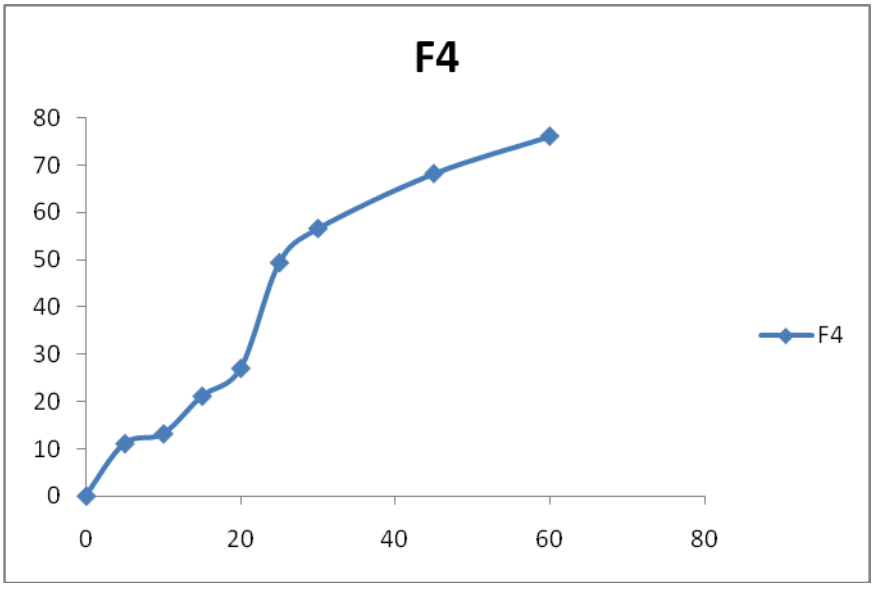
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	14.16	14.22	13.95	14.11±0.14177
10	22.03	22.19	21.87	22.03±0.16
15	26.55	26.37	26.09	26.33±0.23180
20	29.51	29.08	29.27	29.28±0.21548
25	31.28	31.15	30.92	31.11±0.18230
30	36.39	36.67	36.47	36.51±0.14422
45	40.92	40.74	40.49	40.71±0.121594
60	43.67	43.60	43.48	43.58±0.09609



Graph No.6: *In-Vitro* Drug Release of F_3

Table 21: Dissolution profile of formulation (F4) in 0.1N HCL

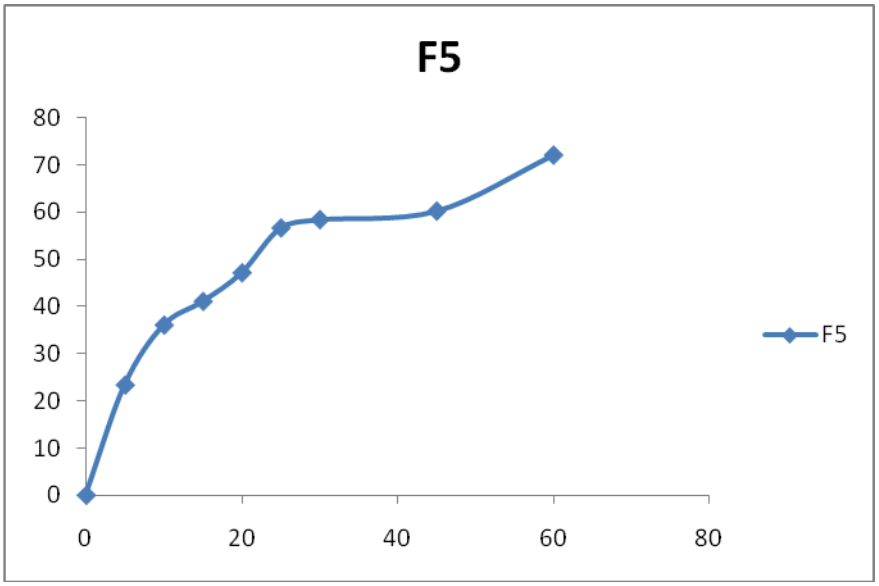
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	11.23	11.09	10.97	11.09±0.13012
10	13.37	12.92	13.19	13.16±0.22649
15	21.24	21.11	21.00	21.11±0.12013
20	27.15	26.87	26.99	27.00±0.14047
25	49.57	49.32	49.18	49.35±0.19756
30	56.66	56.42	56.74	56.60±0.16653
45	68.46	68.27	67.96	68.23±0.25238
60	76.33	76.13	75.94	76.13±0.195021



Graph No.7: *In-Vitro* Drug Release of F

Table 22: Dissolution profile of formulation (F5) in 0.1N HCL

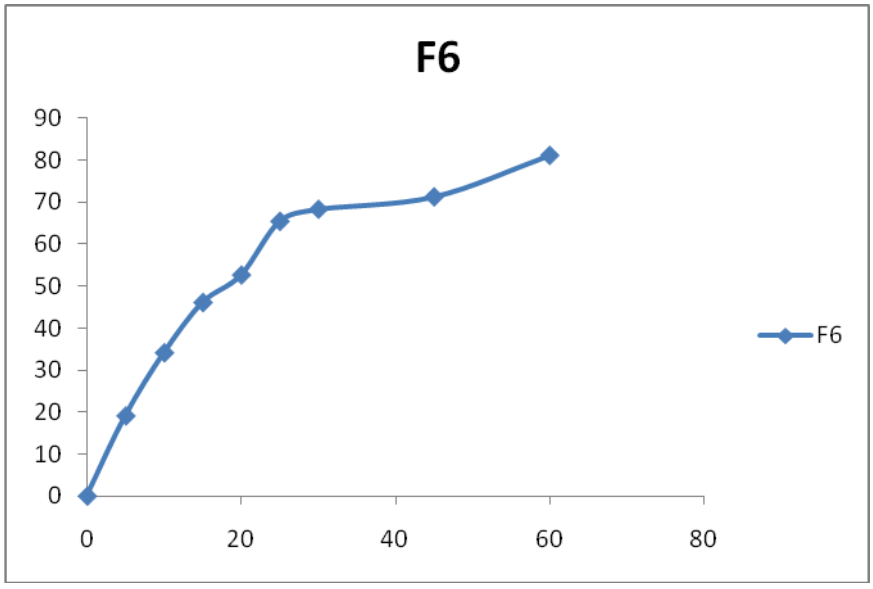
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	22.62	22.11	22.43	22.38±0.25774
10	36.2	35.90	36.17	36.09±0.16522
15	41.31	41.13	40.89	41.11±0.21071
20	47.21	46.94	47.39	47.18±0.22649
25	56.46	56.91	56.72	56.69±0.22590
30	58.62	58.29	58.50	58.47±0.16703
45	60.59	59.97	60.27	60.27±0.31005
60	72.40	72.13	71.89	72.14±0.25514



Graph No.8: *In-Vitro* Drug Release

Table 23: Dissolution profile of formulation (F6) in 0.1N HCL

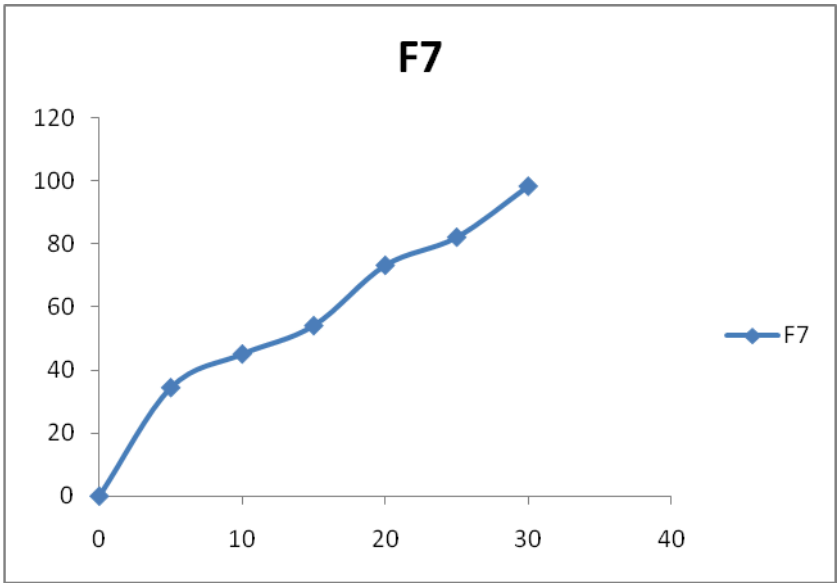
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	19.28	18.93	19.01	19.07±0.18339
10	34.62	33.92	33.72	34.08±0.47258
15	46.03	45.73	46.36	46.04±0.31511
20	52.72	52.43	52.60	52.58±0.14571
25	65.31	65.52	65.22	65.35±0.15394
30	68.46	67.92	68.29	68.22±0.27610
45	71.21	70.89	71.43	71.17±0.2753
60	81.45	80.67	80.97	81.03±0.39344



Graph No.9: *In-Vitro* Drug Release of F_6

Table 24: Dissolution profile of formulation (F7) in 0.1N HCL

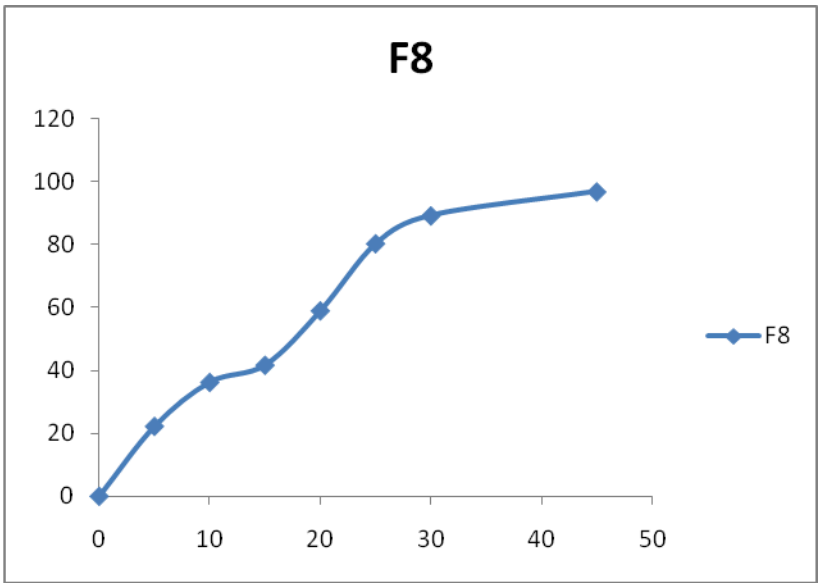
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	34.62	34.26	34.41	34.43±0.18083
10	45.25	44.96	45.09	45.10±0.14525
15	54.10	53.87	54.37	54.11±0.25026
20	73.18	73.69	72.92	73.26±0.39170
25	82.23	82.36	81.96	82.18±0.20404
30	98.36	98.57	98.19	98.37±0.19035



Graph No.10: *In-Vitro* Drug Release of F_7

Table 25: Dissolution profile of formulation (F8) in 0.1N HCL

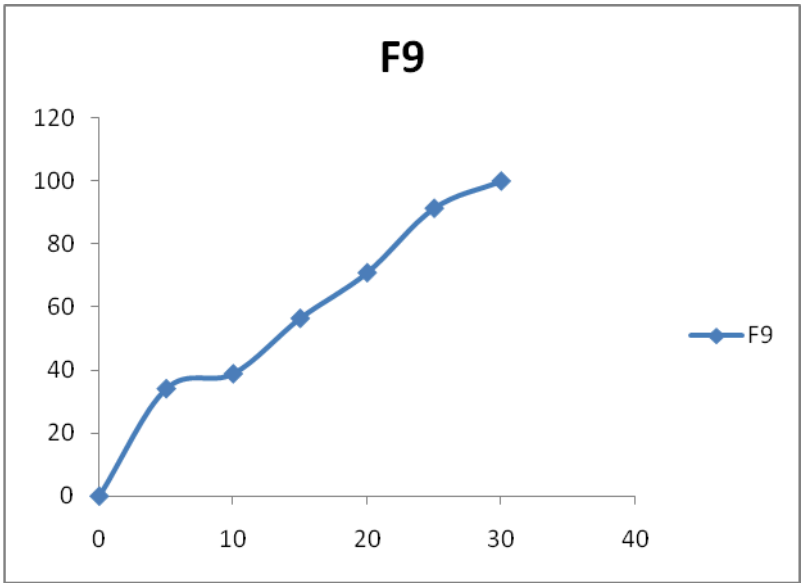
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	22.62	22.25	21.90	22.25±0.36004
10	36.39	35.87	36.46	36.24±0.32233
15	41.70	41.53	41.87	41.70±0.17
20	59.02	58.87	59.21	59.03±0.17039
25	80.46	80.66	80.05	80.39±0.31096
30	89.51	89.33	88.92	89.25±0.30237
45	97.00	96.73	96.92	96.88±0.13864



Graph No.11: *In-Vitro* Drug Release of F_8

Table 26: Dissolution profile of formulation (F9) in 0.1N HCL

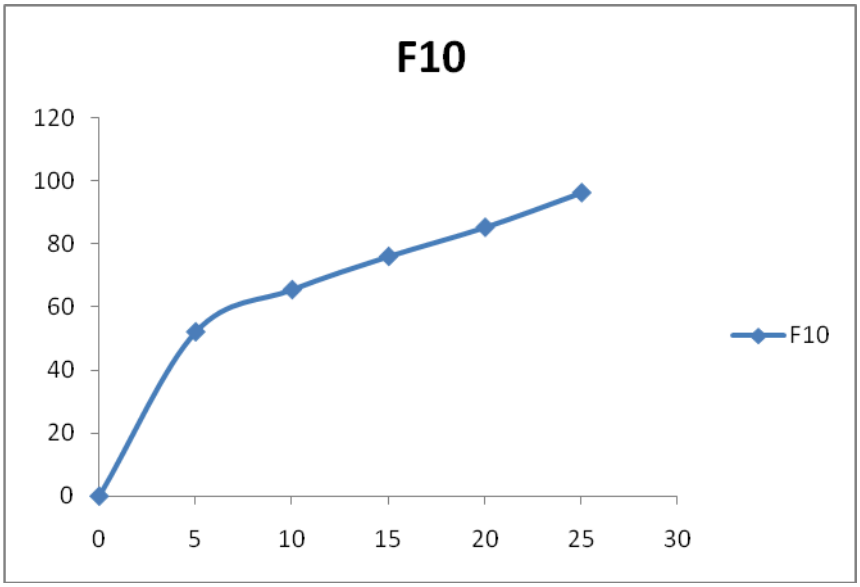
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	34.42	34.19	33.78	34.13±0.32419
10	39.15	38.81	38.69	38.88±0.23860
15	56.85	56.43	56.19	56.49±0.33406
20	71.21	71.09	70.67	70.99±0.28354
25	91.48	91.84	91.09	91.47±0.37509
30	100.33	100.10	99.89	100.10±0.22007



Graph No.12: *In-Vitro* Drug Release of F

Table 27: Dissolution profile of formulation (F10) in 0.1N HCL

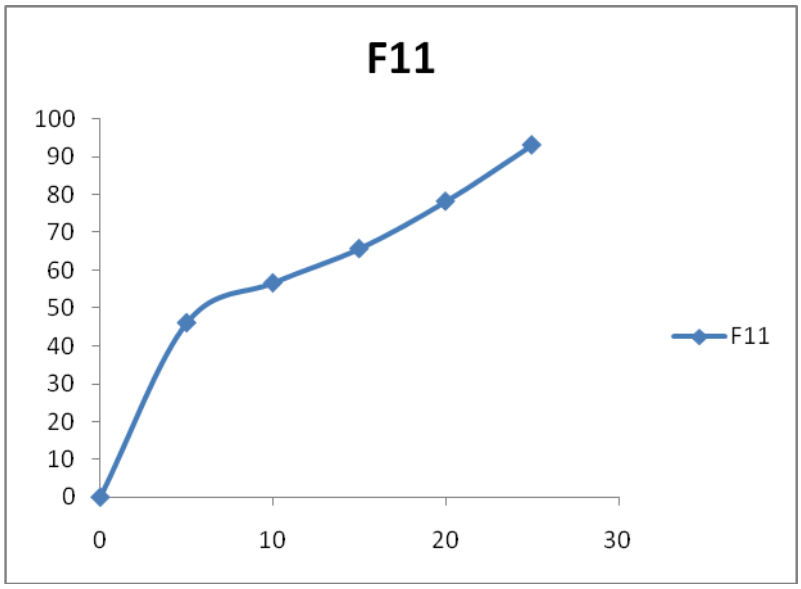
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	52.13	51.87	52.57	52.19±0.35383
10	65.90	65.61	65.23	65.58±0.33600
15	76.13	75.94	76.29	76.12±0.13868
20	85.58	85.15	85.37	85.36±0.21501
25	96.20	96.54	96.38	96.37±0.17009



Graph No.13: *In-Vitro* Drug Release of F_{10}

Table 28: Dissolution profile of formulation (F11) in 0.1N HCL

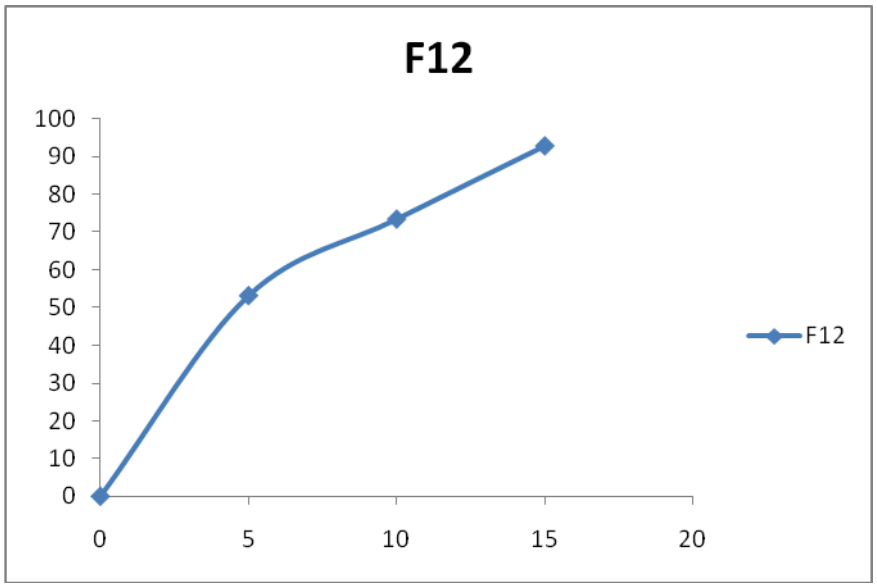
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	46.03	45.90	46.23	46.05±0.56092
10	56.66	56.23	56.87	56.58±0.32624
15	65.90	65.61	65.40	65.63±0.25106
20	78.10	77.87	78.43	78.13±0.28148
25	93.05	92.76	93.23	93.01±0.237135
30	100.53	100.37	100.19	100.36±0.170098



Graph No.14: *In-Vitro* Drug Release of F_{11}

Table 29: Dissolution profile of formulation (F12) in 0.1N HCL

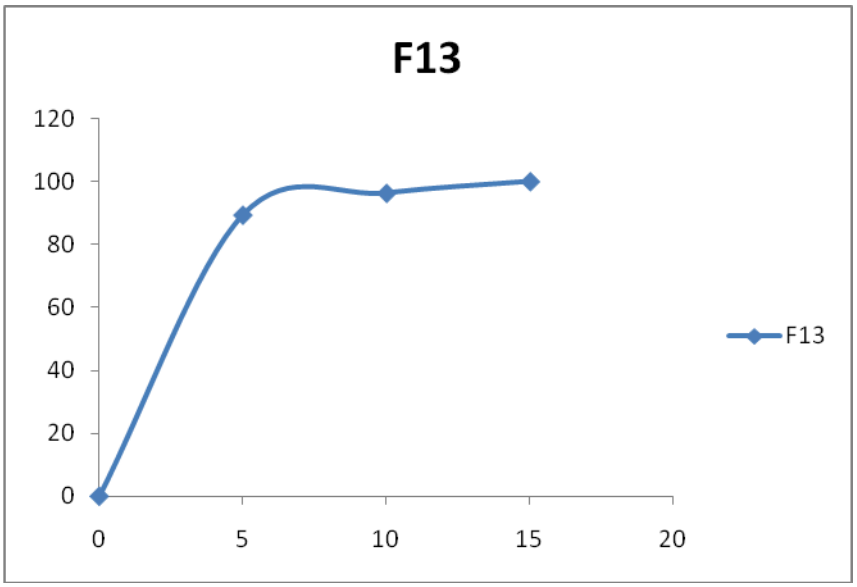
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	53.31	53.19	52.96	53.15±0.17857
10	73.18	73.69	73.41	73.42±0.25540
15	92.81	93.26	92.66	92.81±0.38940
20	103.28	103.09	102.87	103.11±0.21548



Graph No.15: *In-Vitro* Drug Release of F_{12}

Table 30: Dissolution profile of formulation (F13) in 0.1N HCL

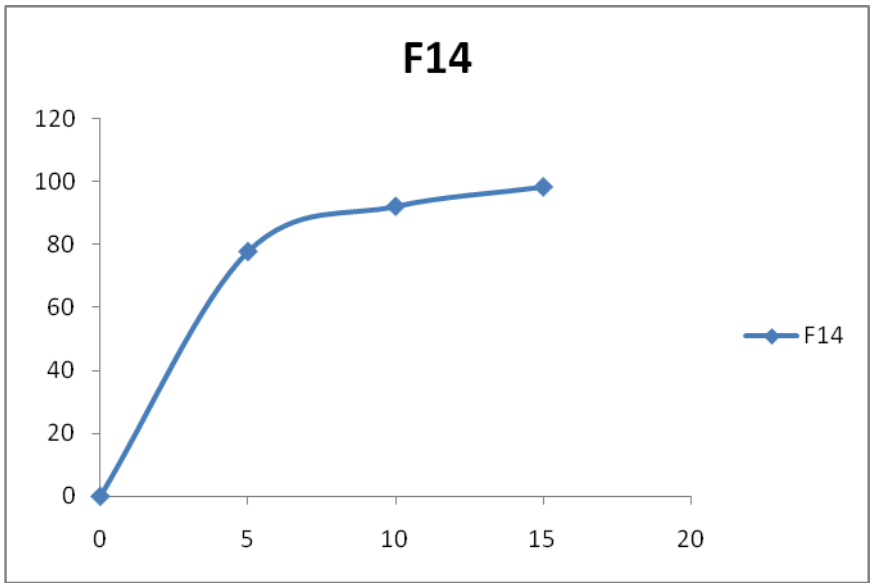
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	89.51	89.63	89.15	89.43±0.24979
10	96.40	96.19	96.58	96.39±0.19519
15	100.14	99.89	100.24	100.09±0.18027



Graph No.15: *In-Vitro* Drug Release of F_{13}

Table 31: Dissolution profile of formulation (F14) in 0.1N HCL

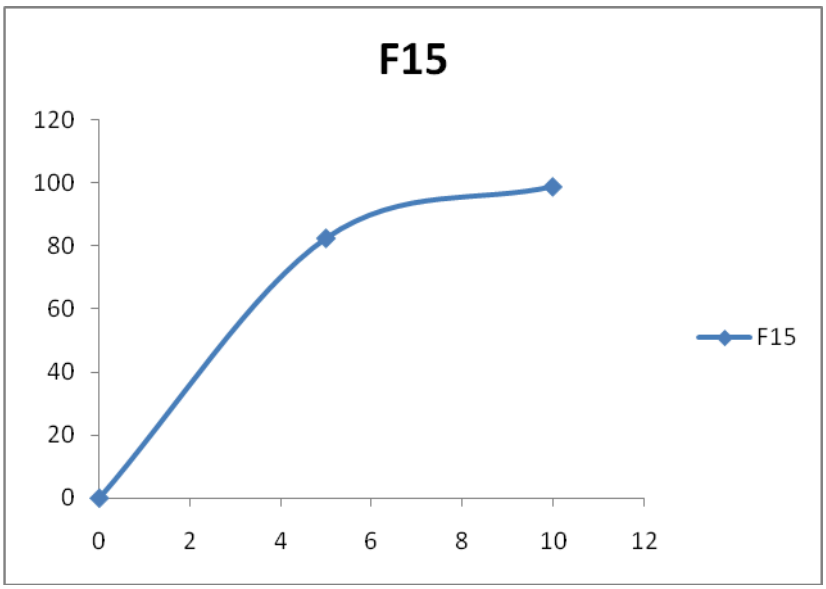
Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	78.30	77.90	78.02	77.74±0.20526
10	92.07	91.93	92.39	92.12±0.22479
15	98.17	98.28	98.39	98.28±0.11



Graph No.17: *In-Vitro* Drug Release of F_{14}

Table 32: Dissolution profile of formulation (F15) in 0.1N HCL

Time (min)	Cumulative Percent Drug Release			
	Trial 1	Trial 2	Trial 3	Mean ± SD
0	0	0	0	0
5	82.88	82.54	82.27	82.56±0.30566
10	98.87	99.01	98.93	98.93±296.81



Graph No.18: *In-Vitro* Drug Release of F_{15}

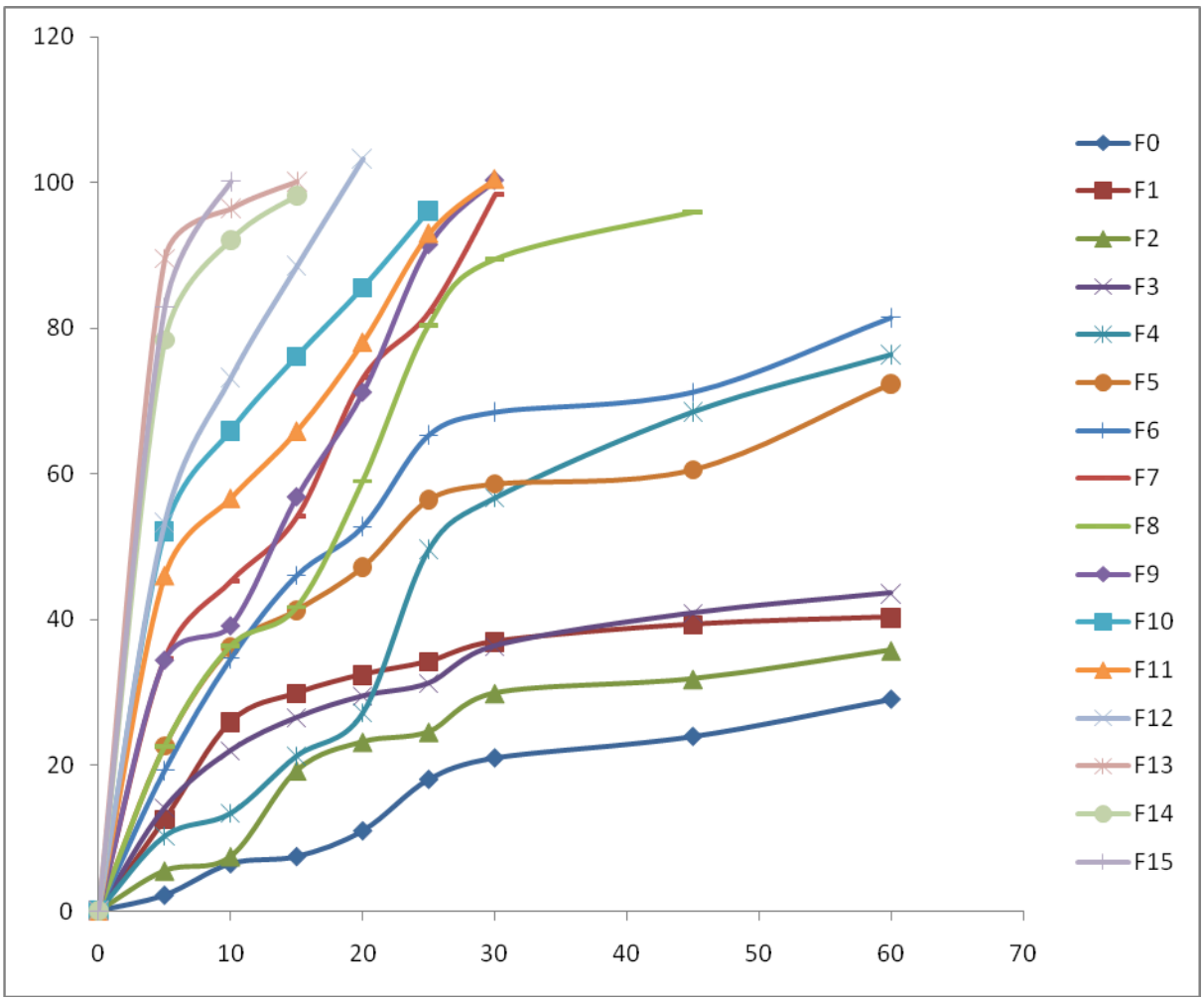
Table no: 33 Comparative cumulative % drug release of formulations F0-F_{15}

Cumulative Percent Drug Release (%)					
TIME	**F0**	**F1**	**F2**	**F3**	**F4**
0	0	0	0	0	0
5	2.05±0.09643	12.56±0.18147	5.50±0.18502	14.11±0.14177	11.09±0.13012
10	6.43±0.17776	25.72±0.20599	7.61±0.14525	22.03±0.16	13.16±0.22649
15	7.46±0.35529	29.70±0.21126	19.08±0.17473	26.33±0.23180	21.11±0.12013
20	11.07±0.22774	32.50±0.36226	23.20±0.05507	29.28±0.21548	27.00±0.14047
30	18.10±0.13012	34.33±0.31390	24.58±0.34510	31.11±0.18230	49.35±0.19756
45	21.35±0.31564	36.84±0.14571	29.56±0.29715	36.51±0.14422	56.60±0.16653
60	24.23±0.23501	39.43±0.30664	31.84±0.09291	40.71±0.121594	68.23±0.25238

Cumulative Percent Drug Release (%)					
TIME	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0
5	22.38±0.25774	19.07±0.18339	34.43±0.18083	22.25±0.36004	34.13±0.32419
10	36.09±0.16522	34.08±0.47258	45.10±0.14525	36.24±0.32233	38.88±0.23860
15	41.11±0.21071	46.04±0.31511	54.11±0.25026	41.70±0.17	56.49±0.33406
20	47.18±0.22649	52.58±0.14571	73.26±0.39170	59.03±0.17039	70.99±0.28354
25	56.69±0.22590	65.35±0.15394	82.18±0.20404	80.39±0.31096	91.47±0.37509
30	58.47±0.16703	68.22±0.27610	98.37±0.19035	89.25±0.30237	100.10±0.22007
45	60.27±0.31005	71.17±0.2753		96.88±0.13864	
60	72.14±0.25514	81.03±0.39344			

Cumulative Percent Drug Release (%)						
TIME	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
0	0	0	0	0	0	0
5	52.19±0.35383	46.05±0.56092	53.15±0.17857	89.43±0.24979	77.74±0.20526	82.56±0.30566
10	65.58±0.33600	56.58±0.32624	73.42±0.25540	96.39±0.19519	92.12±0.22479	98.93±296.81
15	76.12±0.13868	65.63±0.25106	92.81±0.38940	100.09±0.18027	98.28±0.11	
20	85.36±0.21501	78.13±0.28148	103.11±0.21548			
25	96.37±0.17009	93.01±0.237135	53.15±0.17857			
30		100.36±0.170098				
45						

COMPARATIVE STUDY OF NANOSUSPENSION FORMULATIONS



Graph No.19: *In-Vitro* Drug Release of F_1.F_{15}

8. DISCUSSION

Pitavastatin –lipid lowering drug used in the treatment of hypercholesterolemia currently available as tablet dosage form belongs to BCS class-II drug having low solubility and high permeability. Therefore it is poorly bioavailable any attempt that can enhance the solubility of pitavastatin can show improved bioavailability of drug. Considering this the present study aimed to prepare nanosuspension of pitavastatin and evaluate the formulation for its physico-chemical properties and *in-vitro* release studies.

Among the various pharmaceutical approaches, nanoparticle approach assumes significance today as this approach influences physico-chemical properties as well as biological property of the drug. Based on this factor pitavastatin in the present study was attempted as nanosuspension. Different approaches have been reported for the preparation of nanosuspension. In the present study nanoprecipitation method was chosen because the method of preparation is simple to follow at laboratory scale, can ensure small particle formulation besides other advantages. Many factors influence the particle size of nanosuspension, of which particularly the influence of carriers of nanosuspension is of importance. In this study the pitavastatin nanosuspension were prepared using different carriers such as urea,PVP30,β-cyclodextrin,SLS and Tween 80,the proportion of these carriers in the formulations were selected upon the earlier study on simvastatin.(Vikram M. Pandya et al 2011).Formulations F0-F15 were developed using different carriers. A Preformulation study by FTIR spectral analysis revels that there was no chemical interaction between the drug and carriers.

The particle size of pitavastatin was significantly reduced from 50µm to 446nm for the formulation F15.The least particle size was observed with formulation F15 containing β-cyclodextrin and Tween 80.Our finding suggest that beyond the particle size change some other factors related to the carrier used may be predominantly influencing the solubility of pitavastatin. Overall analysis by pitavastatin nanosuspension suggests the particle size or the solubility of nanosuspension appears dependent on the carrier characteristics and the concentration of the carriers.

The drug entrapment of the nanosuspension was found to high (92%) with F15 containing β-cyclodextrin and Tween 80 whereas the least drug entrapment was observed in F1 (54%) containing urea. All the carriers showed drug entrapment above 54%.An

increase interaction between the drug and the carrier results in increased in drug entrapment.

The dissolution profiles of the various formulations are shown in table 33 and graph 19. Each formulation showed variable percentage drug dissolved in over time. Pure Pitavastatin showed 29.1% drug release in 60 mins indicating poor solubility characteristics and therefore slow dissolution of the drug. Among the nanosuspensions the formulation F15 showed highest dissolution of drug 98.93% in the shortest time. The least percentage drug release (35.65%) was observed in formulation F2 containing PVP30.

The poor dissolution from PVP30 carrier may be due to increase in viscosity of the environmental media broughtout by PVP30 that acts as a barrier to diffusion and dissolution of drug in the environment. From the dissolution profile of nanosuspensions it is understood the carrier characteristics appear significantly influencing the dissolution of the drug. Comparison of the dissolution profile of various formulations reveals that F15 appears the best formulation as it showed highest percentage drug release in shortest time .β-cyclodextrin present in the F15 along with Tween 80 as stabilising agent may be influencing a great extent the solubility and therefore the dissolution of pitavastatin .The mechanism of the dissolution of the best formulation F15 was examined for their surface characteristics and DSC thermogram. The DSC thermogram of F15 showed the shifting of the endothermic peak of Pitavastatin from 184-300 °C indicating amorphous formulation of drug in the nanosuspension .Amorphous form of drug is more soluble as compared to its crystalline form and therefore the enhanced solubility and dissolution in F15 may be due to the existence of the drug in amorphous state in nanosuspension.

A stable nanosuspension should have zeta potential greater than ±25mv, since higher value of zeta potential produce repulsive force between the particles in nanosuspension, thus prevent aggregation of particles dispersed and thus stabilise the nanosuspension. From this study it is evident that the zeta potential of the formulation F15 was found to be -34.4 mV and thus ensuring the stability of formulation F15 nanosuspension.

9. CONCLUSION

The results of the present study demonstrate that nanoprecipitation technique was employed to produce nanoparticles of pitavastatin, a poorly water-soluble drug, for the improvement of solubility and dissolution velocity. In this process, the particle size of pitavastatin can be obtained in the nano-size ranges, by adjusting the operation parameters. The best nanosuspension of pitavastatin can be obtained by 10mg, β-cyclodextrin 30mg, and 0.2% w/v Tween 80. Thus it can be concluded that the dissolution of pitavastatin is significantly increased when it is nanosized and thus the bioavailability can be increased compared with the pure pitavastatin.

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