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FORMULATION AND EVALUATION OF PITAVASTATIN BY NANOSUSPENSION

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ABSTRACT

Oral Nanosuspension of Pitavastatin was prepared by Precipitation method using various polymers such as SLS, PVP-K30, Poloxamer 407. All the formulation were evaluated for entrapment efficieny, zeta potential, SEM analysis, invitro dissolution studies. All the prepared formulations were found to be having entrapment efficiency within acceptable limits in the range of 92-101% respectively. IR spectroscopic studies indicated that there are no drug-excepient interactions. When compared to other all the formulations F9 is the best formulation which showed 97.52% of drug released respectively with in 45 min and follows First order release kinetics.

INTRODUCTION

Nanotechnology opens up new vistas of research in the development of novel drug delivery systems [1]. "Nano" word comes from the Greek word 'nanos" which means dwarf[9]. Nano means it is the factor of 10^{-9} or one billionth. Nanosuspension is submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 µm stabilized by surfactants and polymers prepared by suitable methods for drug delivery applications [2]. Nanosuspension has revealed their potential to solve the problem associated with the delivery of poorly water soluble and poorly water and lipid soluble drugs. It enhances the absorption and bioavailability and help to reduce the dose of conventional oral dosage forms. For a long duration of time micronization of poorly soluble drugs by colloid mills or jet mills was preferred the overall particle size [3]

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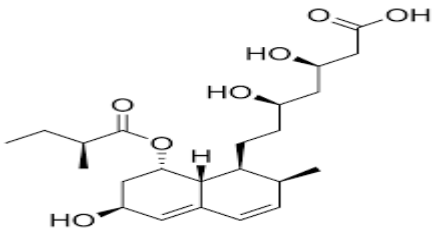
distribution ranges from 0.1µm to approximately 25µm, only negligible amount being below 1µm in the nanometer range.

Drug Profile
Pitavastatin
Structure



Synonyms: Pitavastatia, Pitavastatin, Pitavastatina, Pitavastatine, Pitavastatinum
Categories: Agents Causing Muscle Toxicity, Anticholesteremic Agents, Cytochrome P-450 CYP2C8 Substrates
CAS number: 147511-69-1
Weight: Average: 421.4608; **Monoisotopic:** 421.168936466

Chemical Formula: $C_{25}H_{24}FNO_4$
IUPAC Name: (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid

Pharmacodynamics

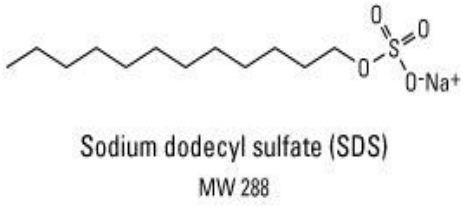
Pitavastatin is an oral antilipemic agent which inhibits HMG-CoA reductase. It is used to lower total cholesterol, low density lipoprotein-cholesterol (LDL-C), apolipoprotein B (apoB), non-high density lipoprotein-cholesterol (non-HDL-C), and trigleride (TG) plasma concentrations while increasing HDL-C concentrations. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease [4].

Mechanism of action

Pitavastatin is a statin medication and a competitive inhibitor of the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis.3 Pitavastatin acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increase hepatic uptake of LDL, thereby reducing circulating LDL-C levels [5].

Volume of distribution: 148 L29
Protein binding: Pitavstatin is more than 99% protein bound in human plasma, mainly to albumin and alpha 1-acid glycoprotein.
Half life: The mean plasma elimination half-life is approximately 12 hours.

Polymer Profiles
Sodium Lauryl Sulphate
Structure

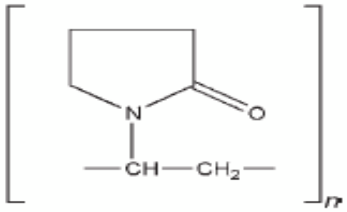


Sodium dodecyl sulfate (SDS)
MW 288

IUPAC Name: Sodium dodecyl sulfate
Weight:288.379
Chemical Formula: C12H25NaO4S
Pharmacodynamics: SLS is an anionic surfactant. Its amphiphilic properties make it an ideal detergent.
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.
Molar mass: 288.372 g/mol
Appearance: white or cream-colored solid
Melting point: 206 °C (403 °F; 479 K)

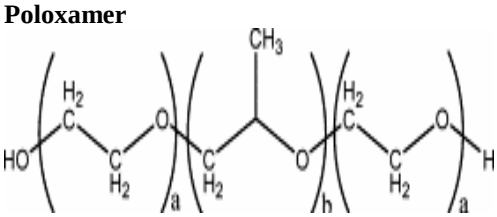
PVP K30
Synonyms: E1201; Kollidon; Plasdone; poly[1-(2-oxo-1-pyrrolidinyl)ethylene]; polyvidone; polyvinylpyrrolidone; PVP; 1-vinyl-2-pyrrolidinone polymer.
Chemical Name and CAS Registry Number: 1-Ethenyl-2-pyrrolidinone homopolymer (9003-39-8)
Empirical Formula and Molecular Weight: (C6H9NO)n 2500–3000000
PVP K 30: 50000

Structural Formula:



Functional Category: Disintegrant; dissolution aid; suspending agent; tablet binder.
Typical Properties:
Acidity/alkalinity: pH = 3.0–7.0 (5% w/v aqueous solution).
Density (bulk): 0.29–0.39 g/cm3 for Plasdone. **Density (tapped):** 0.39–0.54 g/cm3 for Plasdone. **Density (true):** 1.180 g/cm3
Flowability: 20 g/s for povidone K-15; 16 g/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 K-value.
Viscosity (dynamic): The viscosity of aqueous povidone solutions depends on both the concentration and the molecular weight of the polymer employed. PVP K 30
Viscosity (dynamic) is 5.5–8.5

Poloxamer



Poloxamers are non-ionic poly (ethylene oxide) (PEO)– poly (propylene oxide) (PPO) copolymers. They are used in pharmaceutical formulations as surfactants, emulsifying agents, solubilizing agent, dispersing agents, and in vivo absorbance enhancer Poloxamers are often considered as "functional excipients" because they are essential components, and play an important role in the formulation.

Poloxamers as Pharmaceutical excipients

Poloxamers possesses properties which appear to make it suitable for use in the formulation of topical dosage forms. Poloxamer 407 had been used in vehicles for fluorinated dentifrices, eye applications and contraceptive gels. A poloxamer based dental gel product has been in use several years for treating patients with sensitive gums and teeth. Moreover, P-407 gel has been shown to possess many favorable characteristics for use as a burn dressing. Not only does the gel provide a non-toxic detergent covering to the wound, but specific studies suggest that the pluronic gel itself may have a beneficial action, accelerating wound healing over controls. This makes P-407 a very suitable vehicle for gels intended to be applied for ulcers and traumatic lesions.

Pre-formulation studies

Prior to the development of dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced. The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics
- To establish its compatibility with different excipients.

Spectroscopic study

Identification of pure drug

Melting Point

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point.

Solubility studies

Solubility of Pitvastatin was carried out in different solvents –like 0.1N HCL, 6.8pH buffer, 7.4pH buffer, ethanol, and methanol. Saturated solutions were prepared by adding excess drug to the vehicles and shaking on the shaker for 48 hr. at 25°C under constant vibration. Filtered samples (1ml) were determined spectrophotometrically at 243 nm.

Drug-Excipient Interactions Studies

There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy. IR spectroscopy is one of the most powerful analytical technique, which offers possibility of chemical identification. The IR spectra was obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).

Preparation of Calibration Curve of Pitvastatin

Procedure for standard curve in pH 6.8

10 mg of Pitvastatin was dissolved in 10 ml of pH 6.8 by slight shaking (1000 µg/ml). 1 ml of this solution was taken and made up to 10 ml with pH 6.8, which gives 100 µg/ml concentration (stock solution). From the stock solution, concentrations of 1, 2, 3, 4, 5 and 6 µg/ml in pH 6.8 were prepared. The absorbance of diluted solutions was measured at 243nm and a standard plot was drawn using the data obtained.

Method of Preparation of Nanosuspension

Precipitation method

Nanosuspensions was prepared by the Precipitation technique. Pitvastatin was dissolved in methanol at room temperature (organic phase). Methanol containing pitvastatin was rapidly poured into water containing different stabilizers of PVP K30, Poloxamer 407and SLS maintained at room temperature under magnetic stirrer. Precipitation occurs under the condition of drug concentration supersaturation. Organic solvents were left to evaporate off under a slow magnetic stirring of the Nanosuspensions at room temperature for 1 hour.

Evaluation parameters of Nanosuspension Pitvastatin

The Nanosuspension was evaluated for various parameters:-

1. Entrapment efficiency
2. Particles size analysis
3. Zeta potential
4. In-vitro drug release studies

5. Scanning electron microscopy

Entrapment efficacy

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of un incorporated drug was measured by taking the absorbance of the appropriately diluted 5 ml of supernatant solution at 243 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 entrapment efficiency (EE %) could be achieved by the following equation

%Entrapment efficiency= Drug content *100/Drug added in each formulation

Scanning electron microscopy

The morphological features of Pitvastatin nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle size and shape

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size [6].

Zeta potential

There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle's surface. Third, due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases [7].

As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions (Figure). The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The theory is based on electrophoresis and can be expressed as:

$$\mu = \zeta\varepsilon/\eta$$

Where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η)
Is the viscosity and (ζ) us the zeta potential.

***In vitro* drug release study**

In vitro dissolution study was performed by USP dissolution apparatus-type II using 900 ml of 6.8pH buffer as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (50 rpm). The freshly prepared nanosuspensions of drug: stabilizer ratios were added to the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time, then filtered through a 0.45 μm filter paper and analyzed for their drug concentrations by measuring at 243nm wavelength.

The results of in vitro release profiles obtained for the NDDS formulations were fitted into

Four models of data treatment as follows:

1. Cumulative percent drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining versus time (first- order kinetic model).

Zero Order Kinetics: A zero-order release would be predicted by the following equation.

$$A_t = A_0 - K_0 t$$

Where:

A_t = Drug release at time 't'

A_0 = Initial drug concentration.

K_0 = Zero-order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

2. First Order Kinetics: A first-order release would be predicted by the following equation [8]

$$\text{Log } C = \text{Log } C0 - \frac{Kt}{2.303}$$

Where:

C = Amount of drug remained at time't'

C_0 = Initial amount of drug

K = First-order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line [9], indicating that the release follows First-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Mechanism of Drug Release

To find out the drug release mechanism due to swelling (upon hydration) along with gradual erosion of

the matrix, first 60% drug release data can be fitted in Krosmeyers–Peppas model which is often used to describe the drug release behaviour from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

$$\mathbf{Log\ (M_t / M_\infty) = Log\ K_{KP} + n\ Log\ t}$$

Where,

$\mathbf{M_t}$ = is the amount of drug release at time t,

$\mathbf{M_\infty}$ = is the amount of drug release after infinite time,

$\mathbf{K_{KP}}$ = is a release rate constant incorporating structural and geometrical characteristics of Tablet

n = is the release exponent indicative of the mechanism of drug release.

RESULTS AND DISCUSSION

Saturation Solubility

Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and other solvents. From this solubility studies in various buffers we can say that pH 6.8 phosphate buffer has more solubility when compared to other buffer solutions. So pH 6.8 buffer is used as dissolution medium, based upon the solubility studies on organic solvents methanol has more solubility than others so methanol was used in the nanosuspension formulation.

Determination of absorption maximum (λmax)

Determination of Pitavstatin λ-max was done in pH 6.8 buffer medium for accurate quantitative assessment of drug dissolution rate. The linearity was found to be in the range of 1-6 µg/ml in acetone, pH 6.8 buffer. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Drug excipient compatibility

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation. Observed that there are no interactions between the pure drug (Pitavstatin) and optimized formulation (Pitavstatin+ excipients) which indicates there are no physical changes.

Zeta Potential

The measurement itself [10] is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25 °C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV. zeta potential value for the optimized formulation (F6) was found to be within the acceptable limits.

Average particle size of nanosuspension of optimized formulations (F9) was found to be having maximum particles at a range of 489.7 nm.

Dissolution

The in vitro dissolution data of all the designed formulations are shown and dissolution profiles depicted in figures. In vitro drug release data of all the Nanosuspension formulations of Pitavstatin was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to equations of drug release. The results of linear regression analysis including regression coefficients from the above data it is evident that the optimized formulation (F9) follows zero-order release kinetics. Among F1-F9 formulations, F1-F3 formualtions were formulated by using SLS in three different ratios. From the above invitro studies we can say that 30mg of SLS shows maximum drug release at the end of 60mins. So further trails were formulated to decrease the drug release time. F4-F6 formualtions were formulated by using PVPK-30 in three different ratios. From the above invitro studies we can say that 30mg of PVPK-30 shows maximum drug release at the end of 60mins than F4 & F5 formulatoions. So further trails were formulated to decrease the drug release time.

F7-F9 formualtions were formulated by using Poloxamer 407 in three different ratios. From the above invitro studies we can say that 30mg of Poloxamer 407 shows maximum drug release at the end of 45mins, where as remaining F7 & F8 formulations at 60mints. From the above invitro studies we can say that increase in the polymer concentration decrease in the dissolution time of all the formulations. So F9 is considered as optimized formulation as it shows drug release with in 45mins.

The drug release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order. Based on the regression values it was concluded that the optimized formulation F9 follows First order kinetics.

Table 1. Solubility data

Media	Solubility(mg/ml)
0.1N HCL	12.64
Ethanol	30.59
Methanol	39.65
pH 6.8 phosphate buffer	22.46
pH 7.4 phosphate buffer	23.97

Table 2. Standard graph of Pitavstatin in pH 6.8 (λmax 243nm)

Concentration (µg/ml)	Absorbance
0	0
1	0.159
2	0.275
3	0.412
4	0.539
5	0.662
6	0.799

Table 3. Entrapment efficiency of formulated Nanosuspensions

Formulation code	Mean % entrapment efficiency
F1	95.64
F2	96.76
F3	99.35
F4	92.64
F5	96.79
F6	101.59
F7	100.69
F8	95.37
F9	97.16

Table 4. *In-vitro* drug release data of formulation F1to F9

Time (Min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	25.64	29.64	32.1	23.95	40.65	45.36	40.69	49.61	53.69
10	30.69	39.76	52.65	32.49	49.61	50.79	46.72	59.75	60.49
15	36.75	53.94	64.21	39.76	56.37	56.21	59.63	63.97	67.51
20	49.05	64.19	78.28	46.19	62.97	68.28	66.94	69.35	79.46
30	56.37	70.53	86.25	53.95	66.29	76.25	70.36	73.05	90.56
45	66.92	79.65	92.51	62.79	73.64	88.94	79.46	86.49	97.52
60	79.64	86.39	96.54	72.45	79.62	98.61	83.94	92.46	

Table 5. Kinetic data of the formulation F9

Order of Kinetics	Zero Order	First Order
Regression	0.720	0.979

Fig. 1. Solubility studies of Pitvastatin

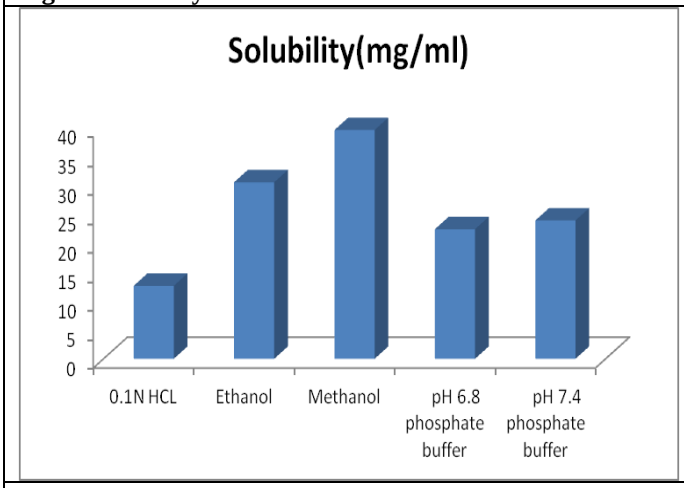


Fig. 2. Uv Spectrum of Pitavstatin

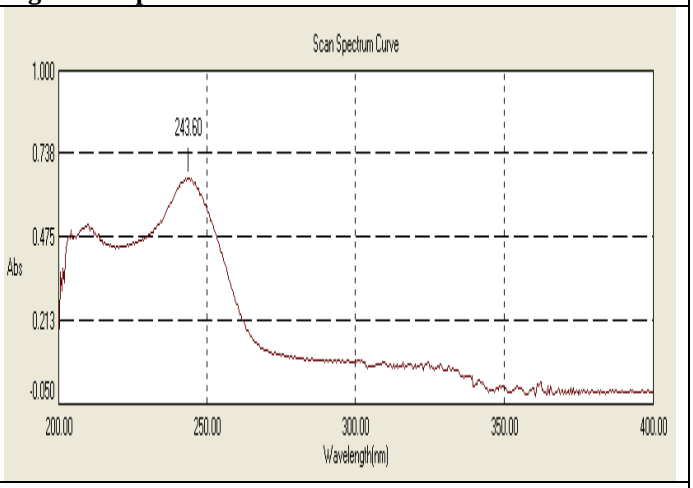


Fig. 3. Standard calibration curve of Pitavstatin in pH 6.8

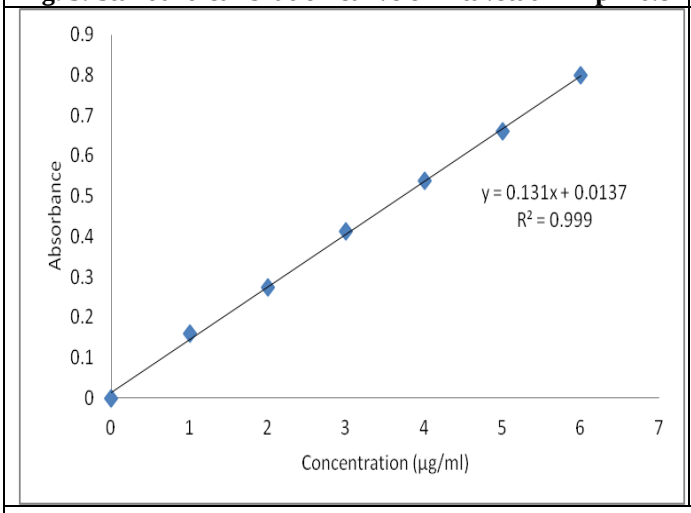


Fig. 4. Zero order release profile of formulation F9

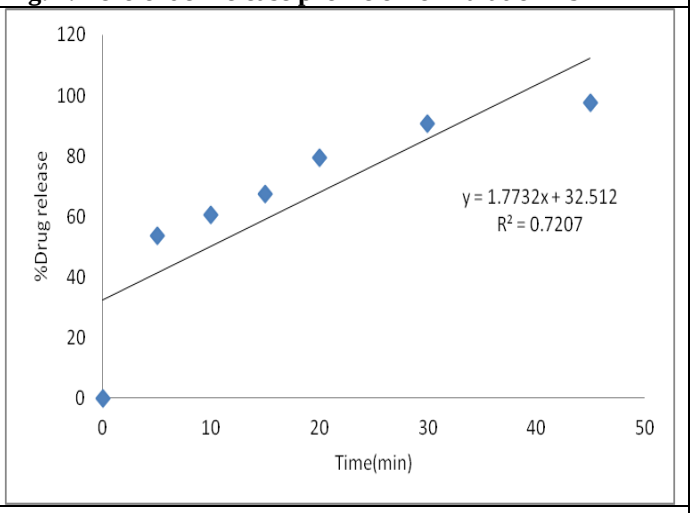


Fig. 5. IR spectrum of Pitavstatin

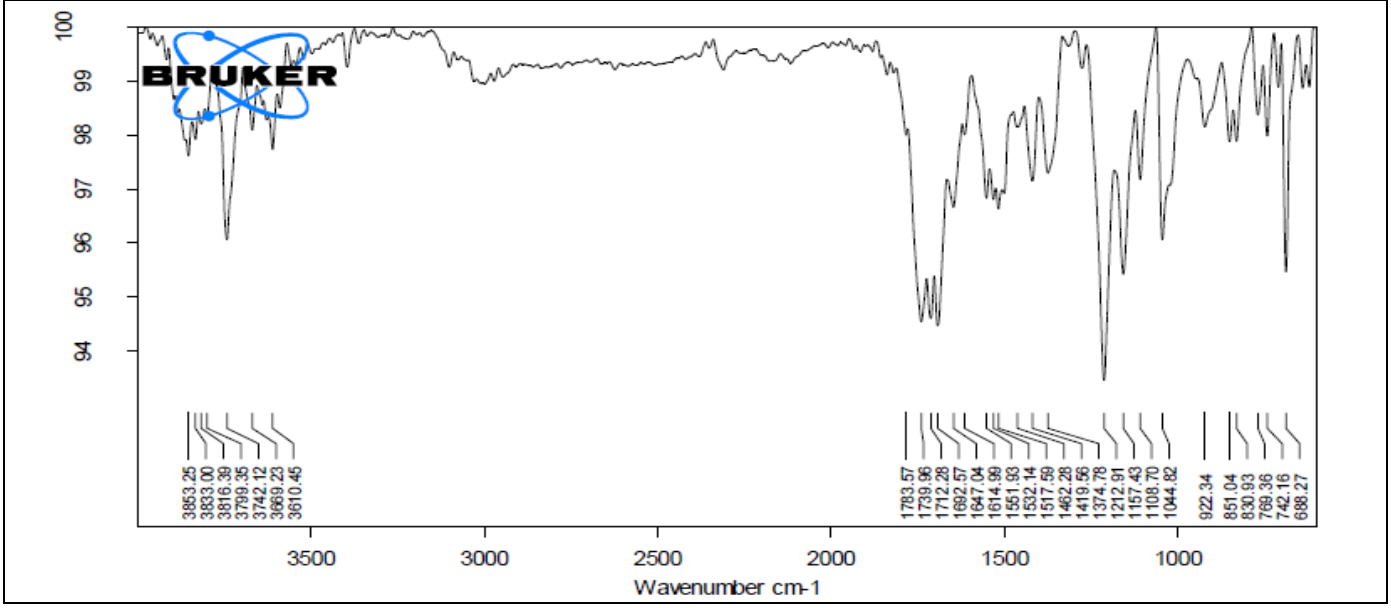


Fig. 6. IR spectrum of Pitavstatin Optimised Formulation

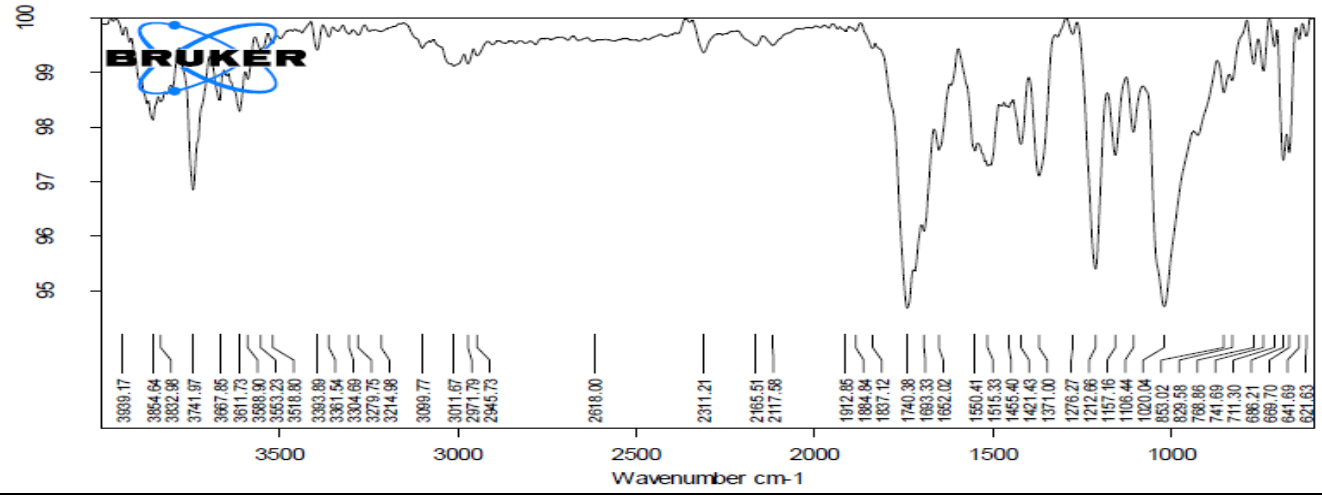


Fig. 7. SEM image of Pitavstatin Nanosuspension

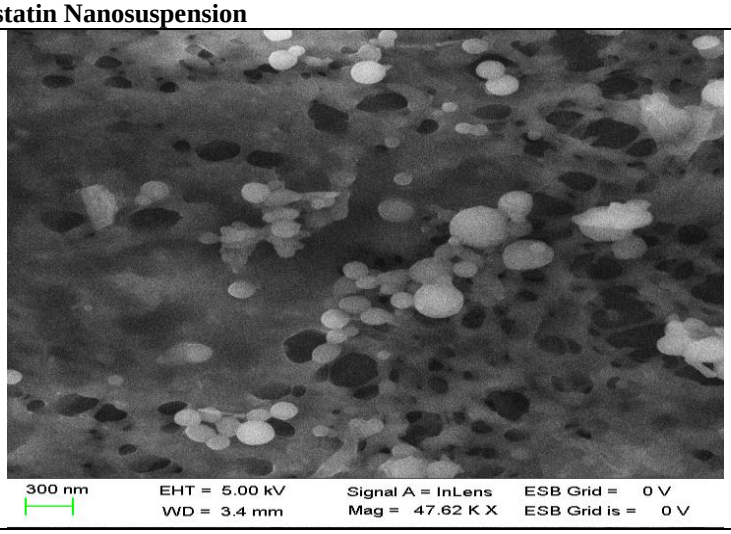
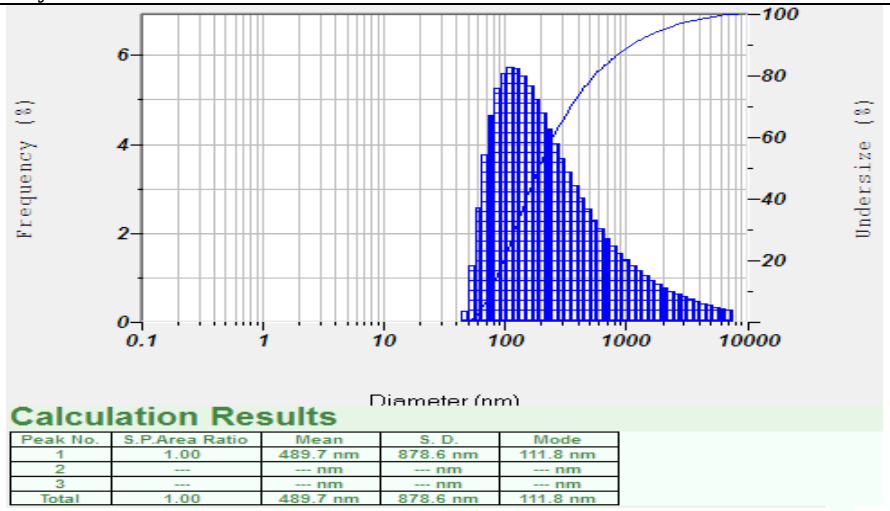


Fig. 8. Particle size analysis



CONCLUSION

Oral Nanosuspension of Pitavastatin by precipitation method using various polymers such as SLS, Polaxomer 407, PVP-K30 and Methanol. The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 92-101% respectively. As the polymer concentration increases, the drug release time decreases, whereas Nanosuspension strength increases. Optimized formulations of Nanosuspension displayed first order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excepient interactions. When compared to other all the formulations F9 is the best formulation which showed 97.52% of drug released respectively within 45 min and follows first order release kinetics. Hence from the study it was concluded that the solubility of Pitavastatin drug was successfully enhanced by using Nanosuspension prepared by precipitation method using Poloxamer 407 (30mg).

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