Formulation and evaluation of Pitavastatin nanosuspension

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ABSTRACT

The main aim of the study is to prepare oral Nanosuspension of Pitavastatin is a lipid-lowering agent in order to overcome bioavailability problems, to reduce dose dependent side effects and frequency of administration. Nanosuspension containing the drug were prepared by precipitation method using combinations of polymers (such as PVP K-90, Tween80, LutrolF127, urea and methanol). Estimation of Pitavastatin was carried out spectrophotometrically at 250 nm. The Oral Nanosuspension were evaluated for various physical and biological parameters, drug content uniformity, *in-vitro* drug release, short-term stability, drug- excipient interactions (FTIR). Short-term stability studies (40 ± 2^0C/ 75±5% RH for three months) indicated that the oral nanosuspension is stable with respect to drug content and dissolution. IR spectroscopic studies indicated that there are no drug-excipient interactions. The formulations F1 to F13 (Containing PVPK-30, Tween 80, Lutrol F127 urea and methanol used different ratio) and F4 (Containing PVP K-90, LutrolF127 Methanol and Water) were found to be promising, which showed values of 5,10 & 25 min respectively and released 98.2% drug within 25 min. These formulations have displayed good Nanosuspension strength.

Key-Words: Pitavastatin, oral Nanosuspension, PVP K-90, Tween80, LutrolF127, Urea Methanol and Water.

INTRODUCTION

Poor solubility of drug substance has always been a challenging problem faced by pharmaceutical scientists and it is increased now because more than 40% of new chemical entities are poorly water soluble. One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implication of low bioavailability and lack of dose proportionality. There are number of technologies like solid dispersion**1-2**, complexation, co-solvency, use of surfactants, etc., but they lack universal applicability to all drugs. A novel technology that can used to overcome problems associated with this method is nanosuspension, which is based on size reduction mechanism.

In the present research work an attempt was made to improve the solubility and dissolution rate of model drug pitavastatin. Pitavastatin as a synthetic lipid-lowering agent is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Pitavastatin is a hyperlipidemic drug whose bioavailability is reported has less than 5%. Nanosuspension of pitavastatin is prepared by precipitation method using Urea and PVP K90 as carriers and lutrolF127and Tween 80 as surfactants.

MATERIAL AND METHODS

Pitavastatin, methanol and Urea are obtained as a gift sample from spectrum labs, Hyderabad. All other chemicals and solvents used are from SD Fine Chemicals, Mumbai.

Preparation of pitavastatin nanosuspension by nanoprecipitation: Nanosuspensions were prepared by the precipitation technique. Pitavastatin was dissolved in a methanol at room temperature. This was poured into water containing different combinations of Urea and tween 80; and PVP K90 and lutrolF127 maintained at room temperature and subsequently stirred on magnetic stirrer (Remi, India.) to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour followed by sonication for 1 hour.

Evaluation parameters of Nanosuspension Pitavastatin: The nano suspension was evaluated for various parameters

1. Content uniformity
2. Entrapment efficiency
3. % transmittance

4. pH
5. Particles size and shape
6. FTIR
7. zetapotential
8. *In-vitro* drug release studies.

Drug content uniformity: 10ml of each formulation was taken and dissolved in 10ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to

10µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at 250 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

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 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.

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

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

%Transmittance: % Transmittance was measured by U.V spectroscopy at wavelength of 400 to 500nm. A graph for %particle range vs. formulations was plotted.

pH measurement: The pH values were measured at 25 ◦C using a pH digital meter at 20 ± 1 ◦C. The formulation was brought in contact with the electrode of pH meter and equilibrated for 1 min. This method was done in triplicate and mean was calculated along with standard deviation.

Particle size and shape: Particle size and shape of the formulated microcapsules was determined by using Optical Microscope.

Fourier transform infra-red spectroscopy (FTIR): Spectral analysis of Pitavastatin, Urea (or) PVP K90 and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients.

***In vitro* drug release study:** This is carried out in USP XXIII dissolution test apparatus-II (Electrolab TDT-06N), employing paddle stirrer at 50 rpm and 200 ml of pH 6.8 phosphate buffer as dissolution medium. The release study is performed at 37 ± 0.5^{o}C. The disk is placed at the bottom of the dissolution vessel. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for Pitavastatin after appropriate dilution by measuring the absorbance at 250 nm.

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.

As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions. The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The most widely-used theory

for calculating zeta potential was developed by Smoluchowski in 1903. 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.

Table.1.Composition of Nano suspension of Pitavastatin

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Pitavastatin	10	10	10	10	10	10	10	10	10	10	10	10	10
PVP K90	2.5	2.5	5	7.5	5	7.5	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	2.5	2.5	5	7.5	2.5	5	7.5
Lutrol f127	-	1	1	1	2	2	-	-	-	-	-	-	-
Tween 80	-	-	-	-	-	-	-	1	1	1	2	2	2
Methanol	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Water up to	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml

RESULTS AND DISCUSSION

Pitavastatin is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of pitavastatin particles in an aqueous solution. Solvent evaporation with precipitation has been employed to produce nanosuspension of pitavastatin. The different formulative variables (1) amount of Urea or PVP K90 (2) amount of Tween 80 or lutrolF127 and organic to aqueous solvent ratio were contributed much towards the change in particle size in nanosuspension preparation. Determination of Pitavastatin λ-max was done in 0.1N Hcl buffer medium for accurate quantitative assessment of drug dissolution rate. The λ-max was found to be 250 nm, i.e., at its absorption maxima. Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The characteristic absorption peaks of were obtained as above table no. and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients. The drug content of the formulated Nanosuspension was found in the range of 93.74to 99.91 respectively. The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 56.4%-96.9% respectively. UV-Visible spectrum of pure Nanosuspension was recorded in range of 200-400 nm.

The measurement of Zeta potential itself 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. The optimized batch (F4) had an average particle size of 300.3nm with 0.218 poly-dispersivity index which indicate the particles are in uniform distribution. The particle size distribution pattern of the optimized nanosuspension formulation is given in figure. The invitro drug release studies were compared for F_1 to F_{13} formulations. Urea, PVP K90 used as carriers and tween 80, Lutrol F127 used as surfactants in these formulations. When compared to the urea and tween 80, the PVP K90 and Lutrol F127 drug release was more 98.21% drug was released within 25 minutes. On comparing the best optimized formula i.e., F_4 with conventional formulation, it was clearly observed that the %drug release was more i.e 98.21% within 30 mins by best formulation, whereas it is 95.88% for the conventional formulation. So, the % of drug release was more in F_4 Nanosuspension than the conventional tablet.

Table.2.*In vitro* drug release data of formulation F1 to F13

Time (mins)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}
0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	15.19	20.36	29.68	35.87	39.3	38.92	14.7	15.64	19.84	21.64	21.68	31.54	39.8
10	24.23	29.68	36.49	47.64	71.26	71.24	21.64	28.33	31.23	33.76	31.39	4957	58.74
15	38.85	46.47	48.93	78.93	86.24	84.33	36.64	38.94	44.57	48.34	46.37	58.74	78.59
20	50.44	68.34	76.44	87.34	97.36	97.36	48.94	52.36	57.08	68.94	68.94	71.68	85.84
25	57.26	89.41	87.23	98.21	-	-	56.88	63.94	73.41	83.37	78.69	83.61	96.4
30	68.83	99.73	100.2	-	-	-	71.92	77.44	89.64	99.24	85.61	93.64	99.41
35	80.54	-	-	-	-	-	77.96	83.29	98.6	-	98.43	97.88	-
40	84.12	-	-	-	-	-	83.94	97.37	-	-	-	-	-
45	98.46	-	-	-	-	-	89.83	-	-	-	-	-	-
50		-	-	-	-	-	100.6	-	-	-	-	-	-

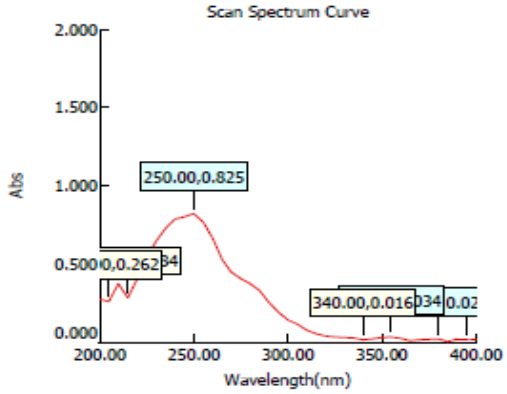

Figure.1.UV spectrum of Pitavastatin 250 nm.

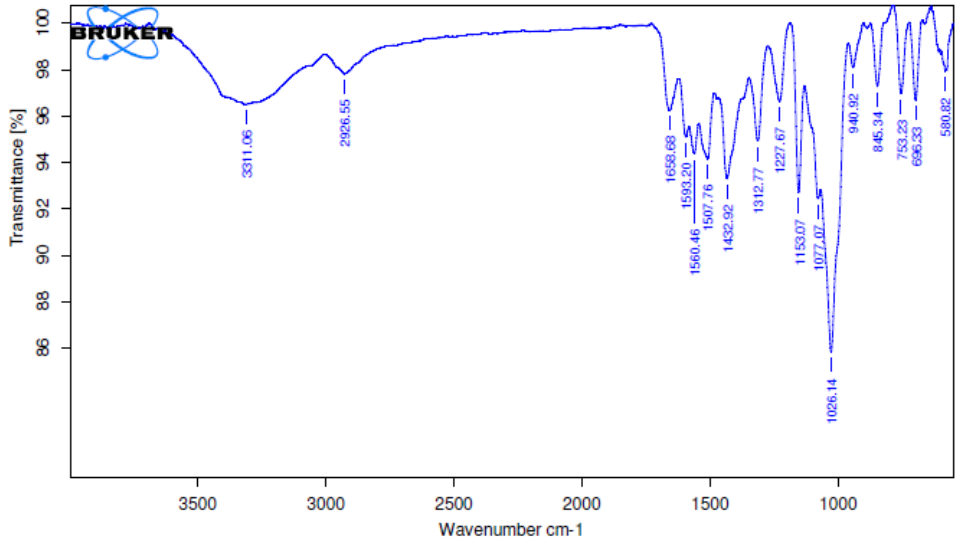

Figure.2.FTIR spectra of optimized formulation

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	134.6	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	5

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	12.5	Peak 1:	12.5	100.0	4.11
Zeta Deviation (mV):	4.11	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.141	Peak 3:	0.00	0.0	0.00

Result quality : Good

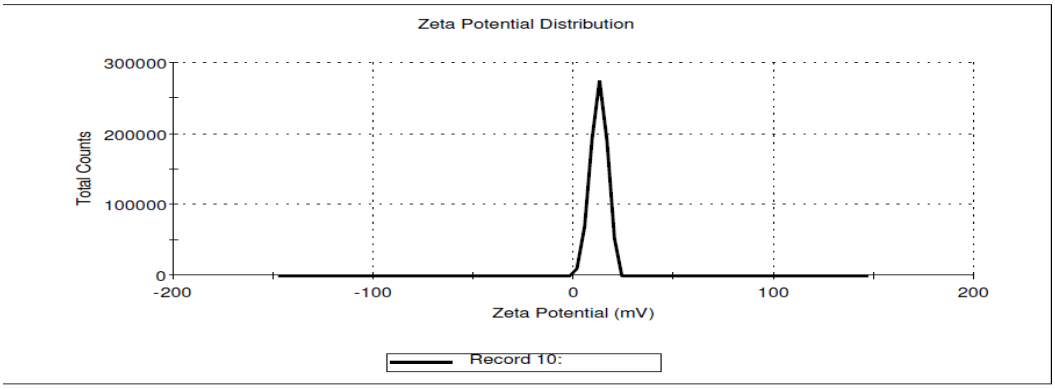

Figure.3.Zeta Potential Analysis

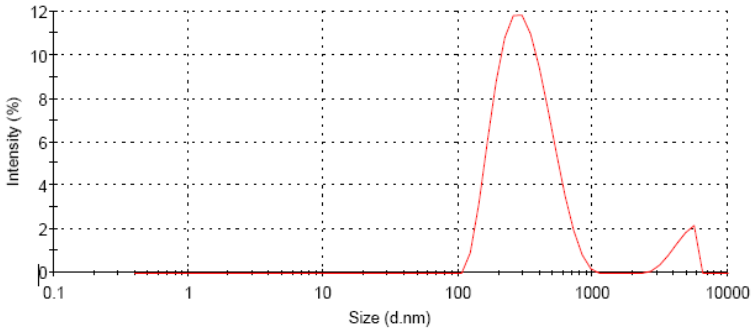

Figure.4.Particle size graph for optimized formulation F4

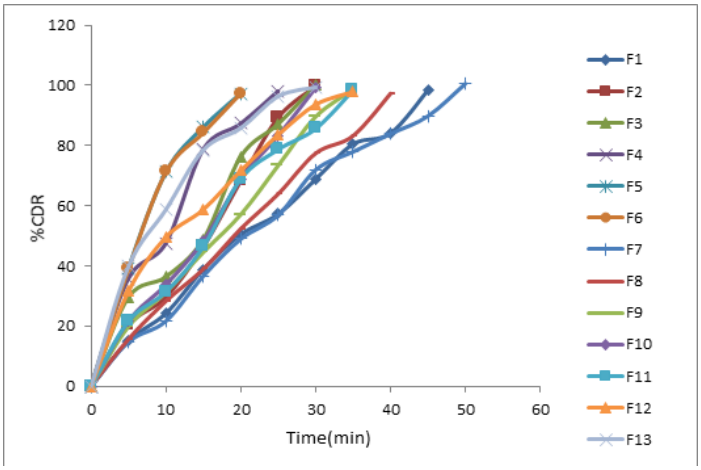

Figure.5.Dissolution parameters for the formulations F7 to F13

CONCLUSION

Oral Nanosuspension of Pitavastatin can be prepared by precipitation method using polyvinyl PVP K90, Tween 80, lutrol F127, urea and methanol. As the amount of polymer, the drug release rate decreases, whereas Nanosuspension strength increases. The optimized batch (F4) had a Zeta Potential and average particle size with in the acceptable range. Short-term stability studies of the promising formulations indicated that there are no significant changes in drug content and dissolution parameter values after 3 months at 40 ± 2° C / 75 ±5% RH. IR spectroscopic studies indicated that there are no drug-excipient interactions. The formulations F_1 (containing polyvinyl PVP K90, methanol and water) and F_2 to F6 (containing PVP K-90, lutrol F127, methanol and water) and F7 to F13(containing urea, Tween 80, methanol and water) were found to be promising, which showed formulation F4 is 98.5 of drug released respectively within 25 min.The formulation F4 is compared to other formulations the F4 is the best formulation of the released the percentage drug of Nanosuspension. Remaining formulation are drug releasing percentage showing respectively of Nanosuspension of Pitavastatin.

BIBILIOGRAGHY

Abraham MA, Shirwaikar A, Formulation of multilayered sustain release tablets using insoluble matrix system, Indian Journal of Pharmaceutical Science, 59(6), 1997, 312-315.

Charles Slchioo, Joseph R. Robinson, Remington‘s Pharmaceutical science 1985, 17th edition, 1644-1653.

Gilbert S Banker, Neil R Anderson, Tablets. In Leon Lachman, Herberta Liebermann, Joseph L Kanig. (Edition), the theory and practice of Industrial pharmacy, 3rd ed. Lea and Febiger, Philadelphia, 1987, 293-294, 330-331, 430-431.

Harkrishan Sinsh & Kapoor. V.K. Medicinal and pharmaceutical chemistry, second edition- 2005, reprint- 2007, 377.

Raja Ponnambalam, Pavithra VS, Review article of Diabetes Mellitus in The AsianJournal of Diabetology, 9(2), 2007, 17-21.