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Formulation and Evaluation of Pitavastatin Nanoparticles

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A B S T R A C T

Nanoparticles can be used for the targeting of anti low density lipoprotein used in treatment of dyslipidemia, thereby achieving major benefits such as reduction in total dose and avoidance of systemic toxicity and other side effects. Nanoparticles were prepared by solvent evaporation technique and characterized by particle size analysis, drug entrapment efficiency, SEM, *in vitro* evaluation studies. *In vitro* release studies were performed in Franz diffusion cell using dialysis membrane in phosphate buffer solution of pH 6.8. The kinetics of release was determined and fitted to an empirical equation. At highest speed the resultant Nanoparticles were smaller in size and their size increased with increase in lipid concentration. Nanoparticles were obtained with polymers like Eudragit RL-100, Ethylcellulose, PVP-K30 different ratios. Pitavastatin Nanoparticles showed maximum entrapment efficiency and controlled release. Nanoparticles prepared with stearic acid showed maximum release. The *in-vitro* release was found to follow Non- Fickian Diffusion mechanism. The surface characters were found to be better with all the lipid carriers. All the formulations showed significant inhibition and anti-inflammatory properties. Pitavastatin loaded Nanoparticles can be prepared by solvent evaporation method with narrow size range, high entrapment efficiency with better stability. The formulation showed better of anti low density lipoprotein used in treatment of dyslipidemia.

Keywords: Nanoparticles, Pitavastatin, Particle size, SEM, *in vitro* evaluation studies.

A R T I C L E I N F O

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1. Introduction

The primary concept of nanotechnology was presented by Richard Feynman in 1959. The term nanotechnology was introduced by Tokyo Science University Professor Norio Taniguchi1. 'Nano' is a Greek word synonymous to dwarf meaning extremely small. Nanoparticles are usually 0.1 to 1000 nm in each spatial dimension and are commonly synthesized using two strategies: top-down and bottom-up. One involves molding and etching materials into smaller components and the other involves the assembling structures into useful devices [1]. Nanotechnology is currently a frontier of research due to wide applications of nanomaterials in biomedical, agricultural, catalysis, optical and electronic fields. Recently inorganic nanoparticles have invoked a lot of interest owing to their distinct physical, chemical and biological properties as compared to the respective bulk materials. The nanoparticles are synthesized by physical, chemical, biological and other hybrid methods. The physical and chemical methods are very useful for production of mono dispersed nanoparticles. These methods are harmful in one or the other way, as the chemicals used are toxic, flammable and do not dispose-off in the environment easily. These methods lead to presence of toxic chemicals adsorbed on the surface of nanoparticles that may have adverse effect in the medical applications. Therefore, development of clean, biocompatible, non-toxic and eco-friendly methods for nanoparticles synthesis deserves merit. nanoparticles are aqueous colloidal dispersions, the matrix of which comprises of solid biodegradable lipids [2].

Nanoparicles combine the advantages and avoid the drawbacks of several colloidal carriers of its class such as physical stability, protection of incorporated labile drugs from degradation, controlled release, and excellent tolerability. The mechanism of action of ptavastatin statins excert their major effect reduction of low density lipoprotein levels through a mevalonic acid like moiety that competitively inhibitors HMG-CoA to mevalonate, statins inhibits cholesterol synthesis, the statins affect blood cholesterol level by inhibiting hepatic cholesterol synthesis which result in increased expression of LDL receptor. Pitavastatin is a low density lipoprotein used in treatment of dyslipidemia. These drugs are competitive inhibitor of HMG-CoA reductase, which catalyses an early rate limiting step in cholesterol biosynthesis. It was isolated form penicillium citrinum, pitavastatin is a heptanoic acid side chain that forms a structural analogue of the HMG Co A intermediate. It is active with calcium salts of pitavastatin and open ring form [3].

2. Materials and Methods

Material and Methods: Pitavastatin (Gifted by Alembic pvt. Ltd.,) Eudragit RL – 100 & Ethylcellulose (Gifted by Signet Chemical Corp.,) PVP – K 30, Sodium lauryl sulphate, (Indchem international Mumbai) and all other chemicals & Solvents used were of analytical grade.

Methodology:

Solubility studies: The solubility of Pitavastatin in various oils, surfactants was determined by dissolving an excess of drug in 2 ml of each of selected oils, surfactants, co-surfactant in 5 ml stoppered vials. Excess amount of drug was added to each 5 ml stoppered vials and mixed using vortex mixer. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of drug was determined in each solution by UV spectrophotometer at particular λ_{max} [4].

Preparation of nanoparticles:

Formulation Procedure: The technique used for nano suspension preparation is solvent evaporation method the drug Pitavastatin along with Eudragit RL-100 was taken and dissolved in 10 ml Methanol. The dissolved ethyl cellulose solution is mixed with the surfactant. The organic phase was added drop by drop to the aqueous phase placed on a magnetic stirrer and stirred for 15 min. The obtained solution is sonicated for 3 hrs which lead to droplet breakage and subsequent formation of nano suspension [5].

Characterization of Nanoparticles

Size and surface morphology of the Nanoparticles: The surface morphology (roundness, smoothness and formation of aggregates) and the size of nanoparticles formulations were studied by scanning electron microscope. The data obtained after the observation were analyzed accordingly [6].

Zeta sizer:

The size of the nanoparticles were evaluated by Zeta Sizer 3000HS (Malvern Instruments, UK). Diluted aqueous dispersion of the nano particles was measured by the device. Mean and standard deviation of each of the samples were measured and presented for comparison [7].

Lyophilization:

The obtained centrifuged samples were lyophilized and stored at 2-8^{0}C. The samples are lyophilized to attain stability. The obtained lyophilized powder is utilized for determination of entrapment efficiency and *in-vitro* drug release parameters [7].

X-Ray Powder Diffractometry:

Powder X-ray diffraction patterns were taken with a Bruker D8-Advance X-ray diffractometer, by using a Ni-filtered Cu K radiation over the 2^0 range of 2–75 .Samples were finely ground in glass substrate and the experiment all parameters were set as: voltage, 40kV current, 20mA angular speed, 48/min [8].

Percentage Yield: The percentage yield of different formulations was determined by weighing the nanoparticles after freeze drying. The percentage yield was calculated as follows

$$\% \text{ Yield} = \frac{\text{Total weight of nanoparticles}}{\text{Total weight of Drug and Polymer}} \times 100$$

Drug Entrapment Efficiency:

Drug content was determined by centrifugation method. The re-dispersed nanoparticles suspension was centrifuged at 15,000 rpm for 40 min at 25^{o}C to separate the free drug in the supernatant. Concentration of pitavastatin in the supernatant was determined by using UV- Vis

spectrophotometer at 241 nm after suitable dilution**.** The entrapment efficiency was calculated according to the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Drug Total} - \text{Drug Supernatant}}{\text{Drug Total}} \times 100$$

Drug loading efficiency:

The supernatant obtained as in case of entrapment efficiency was removed and the remaining sediments (precipitations) were washed by distilled water and dispersed in a mixture of chloroform: acetone (2.5:2.5, v/v) in a 10 mL volumetric flask, to ensure complete extraction of drug from SLNs, then it was sonicated (Altrasonics, India) for 30 min and volume was made-up to 10 mL with chloroform [9]. The resulting solution was centrifuged at 14,000 rpm at 10°C for 30 min and supernatants were obtained and analyzed in triplicate for the loaded drug by UV spectrophotometer at 241 nm. Drug loading efficiency (%DL) was calculated from the analyzed pitavastatin in the Nanoparticles sediments and the total amount of pitavastatin and the excipients added in the preparation of Nanoparticles according to the following

***In vitro* release drug diffusion studies were performed using modified Franz diffusion cell:**

In vitro release of pitavastatin from the naoparticles was evaluated in water. nanoparticles were added directly into the dissolution medium set 50 rpm with the temperature maintained at 37°C [10]. At specified times, 5ml samples of the release media were withdrawn and analysed by UV spectrophotometry at 241 nm.

Data Analysis: The Colloidal systems were reported to follow the zero order release rate by the diffusion mechanism for the release of the drug. To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Krosmeyer and Peppas model. Comparing the r-values obtained, the best-fit model was selected [11].

Zero order kinetics: Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation:

$$Q_t = Q_o + K_o t$$

Where

Q_t = amount of drug dissolved in time t,

Q_o = initial amount of drug in the solution

K_o = zero order release constant.

First order kinetics: To study the first order release rate kinetics the release rate data were fitted to the following equation:

$\text{Log } Q_t = \log Q_o + K_1 t / 2.303$

Where

Q_t is the amount of drug released in time t,

Q_o is the initial amount of drug in the solution,

K_1 is the first order release constant.

Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is

$Q_t = K_H . t^{1/2}$

Where

Q_t = Amount of drug released in time t,

K_H = Higuchi dissolution constant.

Krosmeyer and Peppas release model: To study this model the release rate data are fitted to the following equation.

$M_t / M_\infty = K.t^n$

Where

M_t / M_∞ is the fraction of drug release,

K is the release constant, t is the release time

n is the Diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.

Hixson-Crowell model: To study the Hixson – Crowell model the release rate data are fitted to the following equation

$W_o^{1/3} - W_t^{1/3} = Kst$

Where

W_o is the amount of drug in the dosage form,

W_t is the remaining amount of drug in the pharmaceutical dosage form,

Ks is a constant incorporating the surface-volume relationship.

3. Results and Discussion

Solubility studies:

The solubility of pitavastatin in various organic solvents, surfactants was determined by dissolving an excess of drug in 2ml of each of selected solvents, surfactants, co-surfactant in 5 ml stoppered vials.

Table 2: Solubility of pitavastatin in different solvents

S. No	Solvents	Solubility (mg/ml)
1	Chloroform	29.92
2	Dilute HCl	32.87
3	Water	15.01
4	Ethanol	

Characterization of nanoparticles

Particle size and shape:

SEM confirmed the presence of the nanoparticles and provided morphological information on the nanosuspension loaded with pitavastatin. Spherical, distinct, regular and smooth shape was seen for the Nanoparticles through the SEM images. All the Nanoparticles prepared were in between 40–250 nm in size except formulation F12, which is size of Nanoparticles below 200 nm.

Zeta sizer:

The size of the nano particles were evaluated by Zeta Sizer 3000HS (Malvern Instruments, UK). Particles had a mean Z-average diameter of 157±1 nm (mean ± S.E.M.) and a number average size of 84 ± 8 nm, with a mean polydispersity index of 0.815 ± 0.02. Pitavastatin nano suspension had a mean Z- average number of 160±2 nm. Zeta potential study proved that the above formulation have

an excellent stability. The positive value (+36.09 ± 0.24 mV) indicates that the nano suspension was stabilized by electrostatic repulsion forces.

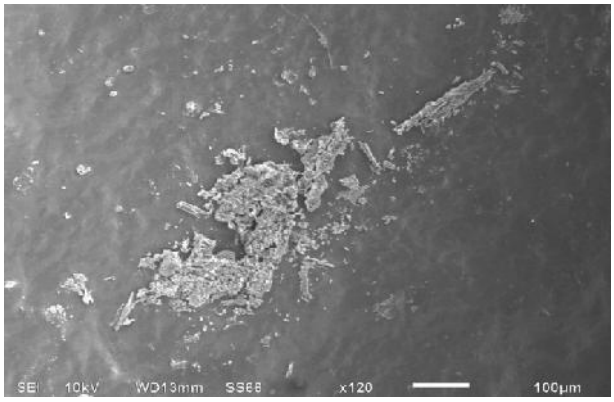

Figure 1: SEM image of F 12 formulation.

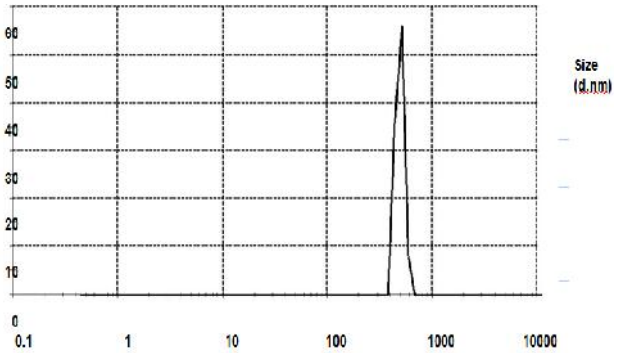

Figure 2: Size distribution by intensity of pitavastatin nanoparticles of F 12

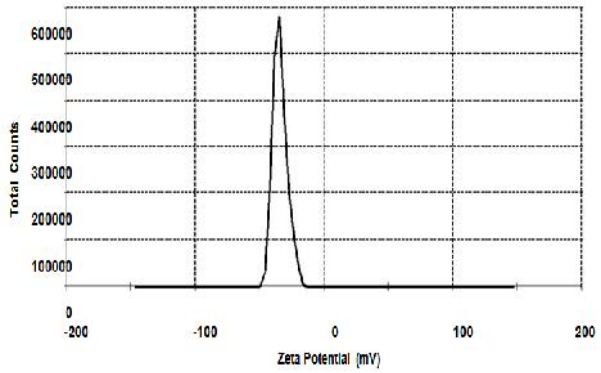

Figure 3: Zeta potential distribution of Formulation F12

X-Ray Powder Diffractometry:

In the X-ray diffract grams of pitavastatinn powder, sharp peaks at a diffraction angle of 2ɵ =11.536, 13.6832, 15.4244, 18.8857, 24.4640, are present which suggest that the pitavastatinn is present as an amorphous material. There are also two strong peaks in the diffract grams of eudragit at 2ɵ= 17.7914, 19.128, indicating the crystallinity. However, the diffraction peaks relevant to pitavastatin and eudragit were no longer detectable in the nano-suspension, thus meaning a greater amorphousness of the compounds when compared to the free molecules. These results, confirmed pitavastatin is no longer present as a crystalline material after encapsulation and exist in the amorphous state.

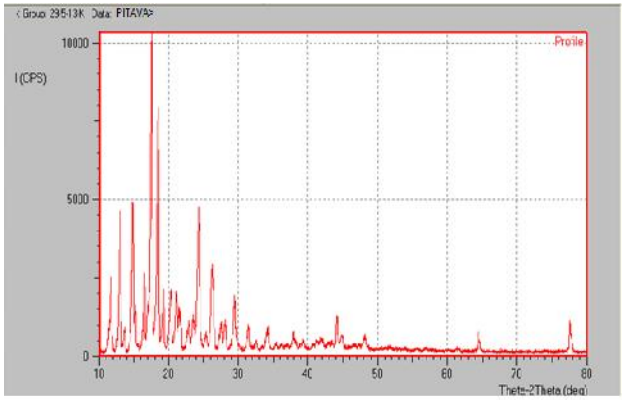

Figure 4: Powder XRPD analysis of pitavastatin Nanoparticles

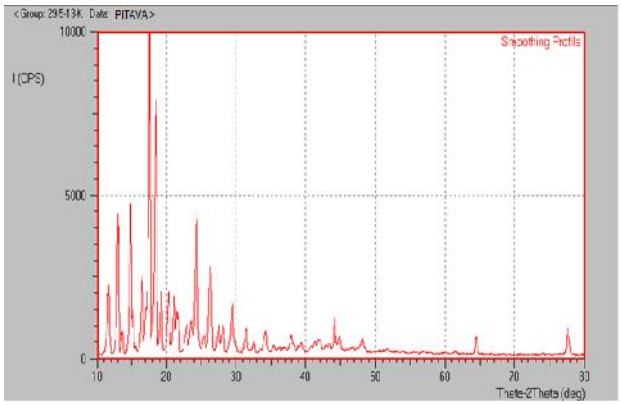

Figure 5: Powder XRPD analysis of Nanosuspension Nanoparticles Formulation 12

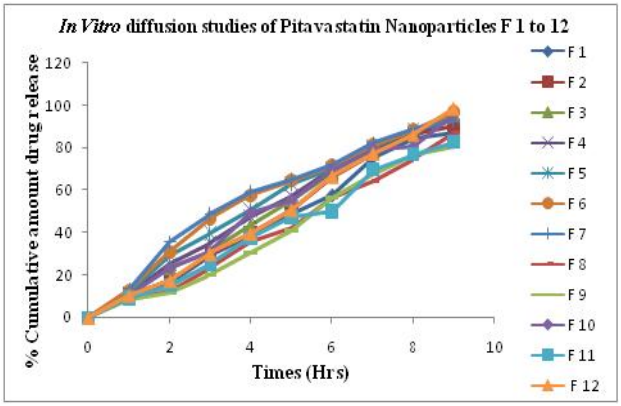

Figure 6: *In-vitro* dissolution studies of Pitavastatin Nanoparticles F1 to F12

Percentage Yield: The percentage yield of different formulations was determined by weighing the nano particles after freeze drying. The *percentage yield* was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Total weight of nanoparticles}}{\text{Total weight of Drug and Polymer}} \times 100$$

The above results revealed that all the formulations showed good % yield while F_{12} formulation showed the highest percentage yield of about 52.60% respectively.

Drug entrapment efficiency (%EE): The Percentage of entrapment efficiency of F 1 to F 12 the values are 56.4 ± 1.15 % to 98.7 ± 0.40 % respectively. The high drug incorporation may be attributed to the fact that rapid quenching of drug occurred in lipid phase due to presence of lipid polymer. As shown in Table 8.

Loading efficiency (%LE):

Drug loading efficiency of F 1 to F 12 was found to be satisfactory, that is, 44.4 ± 0.72 % to 97.1 ± 0.30 %. There was an increase in the entrapment and loading efficiencies in case of F 12 as higher percentage of lipid mixture was used in its preparation. As shown in Table 8.

Table 1: Formulation of Pitavastatin loaded Nanopaticles

Formulation Code	Drug: polymer ratio	Drug (mg)	Eudragit RL-100 (mg)	Ethyl Cellulose (mg)	PVP-K30 (mg)	SLS (mg)	Methanol (ml)	Water (ml)
F1	1:0.5	20	10	-----	20	0.01	10	10
F2	1:1	20	20	-----	20	0.01	10	10
F3	1:1.5	20	30	-----	20	0.01	10	10
F4	1:0.5	20	10	10	10	0.01	10	10
F5	1:1	20	20	20	10	0.01	10	10
F6	1:1.5	20	30	30	10	0.01	10	10
F7	1:0.5	20	10	-----	10	0.01	10	10
F8	1:1	20	10	-----	10	0.01	10	10
F9	1:1.5	20	10	-----	10	0.01	10	10
F10	1:0.5	20	10	10	-----	0.01	10	10
F11	1:1	20	10	20	-----	0.01	10	10
F12	1:1.5	20	10	30	-----	0.01	10	10

Table 2: Characterization of Nanoparticles

Formulation code	%Yield	Particle size (nm)	Ent. Efficiency (%)	Drug loading (%)
F1	39.86	250.1 ±30.2	76.2 ± 0.19	54.4 ± 0.42
F2	41.82	216.2 ±15.7	78.8 ± 0.25	74.4 ± 0.75
F3	48.45	155.2 ±13.7	77.3 ± 1.78	76.6 ± 0.65
F4	50.62	96.2 ± 12.8	75.8 ± 1.90	78.6 ± 0.65
F5	46.90	96.9 ± 12.2	82.6 ± 2.10	80.5 ± 0.50
F6	49.60	98.5 ± 12.8	75.4 ± 2.50	73.1 ± 0.30
F7	43.24	150.1 ± 20.2	74.2 ± 0.12	44.4 ± 0.72
F8	47.56	86.2 ± 15.7	86.8 ± 0.19	64.4 ± 0.95
F9	42.74	85.2 ±12.7	56.4 ± 1.15	96.6 ± 0.55
F10	44.60	98.2 ± 11.8	65.8 ± 1.80	88.6 ± 0.55
F11	45.75	78.9 ± 15.8	72.3 ± 1.20	80.6 ± 0.40
F12	52.60	100.5 ± 00.8	98.7 ± 0.40	97.1 ± 0.30

***In-vitro* Release Studies:** *In vitro* release of Pitavastatin from the Nanoparticles was evaluated with water by using USP-II apparatus.

Table 3: *In-vitro* diffusion profile of Pitavastatin Nanoparticles formulation F 1 to F 6

Time (hrs)	Cumulative % drug release					
	F 1	**F 2**	**F 3**	**F 4**	**F 5**	**F 6**
1	10.2	10.6	11.1	11.3	11.7	12.7
2	16.5	17.4	23.4	25.3	29.1	30.9
3	29.3	30.2	31.1	35.4	39.7	46.8
4	38.1	39.7	43.3	47.5	50.7	57.6
5	48.6	50.9	55.4	57.6	63.1	64.4
6	57.6	66.6	68.4	70.1	70.5	71.6
7	75.6	77.4	79.2	79.9	80.8	81
8	84.9	86.4	86.7	87.1	88.2	88.3
9	87.2	90.6	95.5	93.8	93.6	96.8

Table 4: *In-vitro* diffusion profile of Pitavastatin Nanoparticles formulation F 7 to F12

Time (hrs)	Cumulative % drug release					
	F 7	**F 8**	**F 9**	**F 10**	**F 11**	**F 12**
1	13.1	9.1	8.6	10.1	9.3	10.6

2	35.4	12.5	11.4	23.4	15.3	17.4
3	48.6	23.3	20.2	31.1	25.4	30.2
4	59.2	36.1	30.7	49.3	37.5	39.7
5	64.9	42.6	40.9	55.4	47.6	50.9
6	71.8	56.6	56.6	69.4	50.1	66.6
7	82.4	64.6	67.4	79.2	69.9	77.4
8	88.9	74.9	76.4	80.7	77.1	86.4
9	92.6	87.2	80.6	95.5	82.8	98.6

4. Conclusion

All the Nanoparticles prepared were in between 40–250 nm in size except formulation F12, which is size of Nanoparticles below 200 nm. The size of the nano-particles were evaluated by Zeta Sizer 3000HS (Malvern Instruments, UK). Particles had a mean Z-average diameter of 157±1 nm (mean ± S.E.M.) and a number average size of 84 ± 8 nm, with a mean polydispersity index of 0.815 ± 0.02. In the X-ray diffractograms of pitavastatinn powder, sharp peaks at a diffraction angle of 2ɵ =11.536, 13.6832, 15.4244, 18.8857, 24.4640, are present which suggest that the pitavastatinn is present as an amorphous material. There are also two strong peaks in the diffractograms of eudragit at 2ɵ= 17.7914, 19.128, indicating the crystallinity. The Percentage of entrapment efficiency of F 1 to F 12 the values are 56.4 ± 1.15 % to 98.7 ± 0.40 % respectively. Drug loading efficiency of F 1 to F 12 was found to be satisfactory, that is, 44.4 ± 0.72 % to 97.1 ± 0.30 %. The prepared Nanoparticles were found *in-vitro* diffusion studies the best formulation F12 drug release 98.6%.

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