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Formulation and Evaluation of Microspheres of Atorvastatin Calcium by Particle Engineering Through Spherical Crystalisation

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ABSTRACT

The aim of the present experiment was to prepare and evaluate spherical crystals of Atorvastatin calcium using methanol, water and chloroform and then microspheres were prepared by solvent evaporation method using Eudragit S -100 polymer,polyvinyl alcohol used as the droplet stabilizer. The prepared spherical crystals were characterized for their micromeretic properties as well by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) revealed the crystalline nature of drug in a final stage. The *in vitro* release studies were performed in pH 1.2 (0.1 N HCl) for 60 min. The prepared microspheres were characterized for their micromeretic properties and entrapment efficiency; as well by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) revealed the crystalline nature of drug in a final stage. The *in vitro* release studies were performed in pH 1.2 (0.1 N HCl) for 2 h followed by 6.8 pH phosphate buffer for 8 h. The best fit release kinetics was achieved with a Zero order. The yield of preparation and entrapment efficiencies were very high with a largerparticle size for all the formulation. Mean particle size, entrapment efficiency and production yield were highly influenced by the type of polymer and polymer concentration. It is concluded from the present investigation that Eudragit S-100 are promising controlled release carrier for atorvastatin calcium.

Keywords: Atorvastatin Calcium; Eudragit S100; Microspheres; Spherical Crystals.

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INTRODUCTION

Atorvastatin calcium is a HMG-CoA reductase inhibitor used in the treatment of hyperlipidemia. It has an oral bioavailability of less than 12% after a 40mg oral dose. It also undergoes high first pass metabolism. It is highly soluble in acidic pH and absorbed more in the upper part of the GIT. The major hurdle of atorvastatin is rate limited bioavailability. The main objective of the present work is to formulate microspheres of atorvastatin. The microspheres of atorvastatin may improve solubility and higher dissolution rate by decreasing particle size and increasing surface area. They may increase the patient compliance by significantly enhancement in oral bioavailability of the drug.

Spherical agglomeration is the novel technique of particle engineering that can directly transfer the fine crystals produced in the crystallization or in the reaction process into a spherical shape. It is the versatile process that enables to control the type and the size of the crystals. Spherical crystallization was defined by Kawashima as an agglomeration process that transfers crystals directly to compact spherical forms during the crystallization process."

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. Eudragit polymers are a series of acrylate and methacrylate polymers available in different ionic forms. Eudragit S100 is insoluble in aqueous media, but they are permeable and have pH dependent release profile. The aim of the study was to prepare Eudragit microspheres containing Atorvastatin calcium to achieve a prolonged release and specific site targeting drug delivery system profile suitable for oral administration. The microspheres were prepared by a solvent evaporation technique using Eudragit as a matrix polymer. Dichloromethaneand water systemwere used for the preparation of microspheres. Polyvinyl alcohol wasused as a droplet stabilizer to prevent droplet coalescence in the oil medium [4]. Firstly, we investigated formulation variables (polymer type and drug:polymer ratio) to obtain spherical particles. The effects of various Eudragit on the yield of production, particle size distribution,encapsulation efficiency, surface properties and Atorvastatin calcium release rate from microspheres were investigated. The influences of formulation variables on the microspheres properties were examined. The prepared spherical microspheres were evaluated for micromeretic properties and drug content, and also by FTIR and SEM, as well as for *in vitro* drug release studies.

EXPERIMENTAL WORK

Materials

Atorvastatin calcium candida health pharmaceutical;Eudragit S100 Evonik Industries; chloroform, methanol S.D Fine Chemicals; Other substances used were all of analytical grade.

Preparation of Spherical crystals

Atorvastastin is to be dissolved in methanol at 40°C to prepare an under-saturated solution for ensuring that everything is dissolved. The obtained solution to be cooled to 20°C and the required amount of bridging liquid is added. This mixture is fed by a syringe pump , at the selected feeding rate, onto the surface (of 76 ml) of water agitated with a three-blade marine propeller (2.5 cm in diameter) at certain stirring speed in a 250 ml jacketed crystallizer (6 cm in diameter). The agitator is to be centrally located at fixed distance (1 cm) from the bottom of the crystallizer. The temperature should be adjusted via a heat and refrigeration circulation unit to a required temperature. After some time the experiment is to be terminated and the filter the agglomerates, wash with water and dry at room temperature

Characterization of Spherical Crystals of Atorvastatin Calcium

Percentage Yield

The yields of production ofspherical crystals of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of spherical crystals and percent production yields were calculated.

$$\% \text{ yield} = \text{Practical mass} \,/\, \text{Theoretical mass} \times 100$$

Drug Content Determination:

ATR spherical agglomerates equivalent to 40 mg of ATR were accurately weighed, crushed and transferred to a 10 mL volumetric flask. To this, 50 mL of methanol was added and sample was sonicated for 20 min so as todissolve the drug and the polymer. The volume was made up to 100 mL withmethanol and filtered through a 0.45 µm filters. The filtrate was diluted withmethanol and analyzed at 246.5 nm by uv-spectrophotometer.

Solubility Study:

The apparent solubility of Spherical crystals of Atorvastatin calcium determined In water.Each Spherical crystals in excess of drug equivalent to (40 mg) was added to 10 ml of solvent in glass vials with rubber closers. Then the vials were kept on a shaker incubator maintained at 37 ± 0.5 °C for 24 h. After shaking, the vials were kept in an incubator at 37 ± 0.5 °C for equilibrium for 10h. The solution was then filtered through 0.45 µm Millipore, filtered and the filtrate was assayed spectrophotometrically at 246.5 nm.

Dissolution Study of Spherical Agglomerates:

In-vitro dissolution studies were carried out with spherical agglomerates. Each test was carried out in United States Pharmacopoeia dissolution apparatus II (Paddle) consisted of 900 ml , 0.1 N HCl maintained at 37.0 ± 0.5°C and stirring at 75 rpm. An accurately weighed quantity of each sample equivalent to 40 mg of Atorvastatin Calcium was subjected to the test. Samples 5 ml were withdrawn at predetermined time interval (5, 10, 15, 20 & 30 minutes) and

immediately replace with the equal volumes of dissolution medium.. Diluted samples were analyzed at 246.5 nm by uv-spectrophotometer.

Flow Property

Flowability of ATR and its spherical agglomerates were determined in terms of the following parameters,Bulk density,Tapped density, Hausner ratio,Carr's index and Angle of repose.

Evaluations of Spherical Crystals of Atorvastatin Calcium:

Fourier Transforms IR Spectroscopy:

Fourier-transform infrared (FT-IR) spectra were obtained by using shimadzu FTIR- 8400 Spectrophotometer. The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample/KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press.

Differential Scanning Calorimetry (DSC):

The DSC measurements were performed on a differential scanning calorimeter with a thermal analyzer. All accurately weighed samples were placed in sealed aluminum pans, before heating under nitrogen flow (20ml/min) at a scanning rate of 10 °C min−1 from 25 to 250 °C. An empty aluminum pan was used as reference.

X-ray Diffraction Studies (XRD) [21]:

X-ray diffraction analysis was performed using Bruker axs diffractometer D 8Advanced model (high beam monochromatic) using Cu radiation which was generated at 40 Kv and 40 mA at 1.540600A. The rate of the scanning was 0.30°C /min.

RESULTS AND DISCUSSION

1.	Production yield	91.28%
2.	Drug content	97.82%
3.	Aqueous solubility	0.24mg/ml
4.	Dissolution	76.59%
5.	Bulk density	0.45
6.	Tap density	0.54
7.	Carr's index	16.66
8.	Hausner ratio	1.2
9.	Angle of repose	22.5

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FTIR spectroscopy

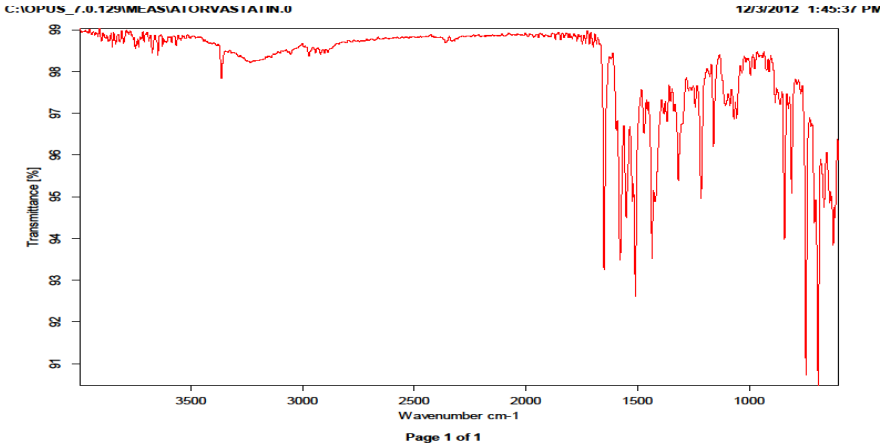

Fig.1. FTIR Spectra of Pure Atorvastatin Calcium

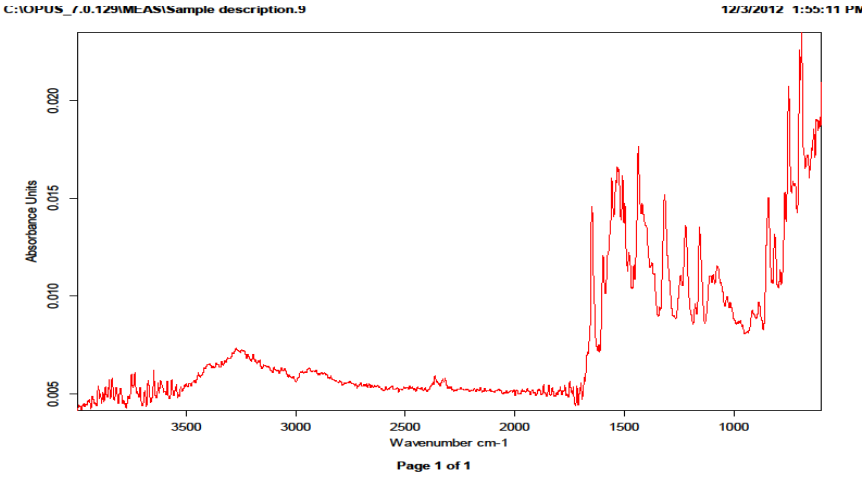

Fig. 2. FTIR Spectra of Formulation Spherical Crystals

Scanning Electron Microscopy

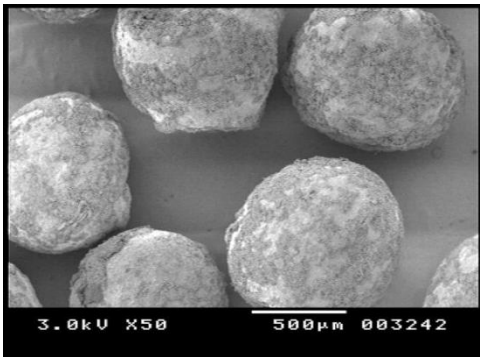

Fig. 3. Scanning electron microscopy of spherical crystals

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Preparation of microspheres

The technique used in preparation of microspheres is a double emulsion solvent evaporation technique.Desired amount of drug was dissolved in distilled water. Polymer(eudragit) was dissolved in dichloromethane.Then the aqueous drug solution was gradually added to above prepared polymeric solution with constant stirring at 600 rpm,stirring was continued for few minutes. Then the primary emulsion was added to PVA solution containing 2% span 80 stirring was continued upto 2 hrs at a temperature of 60^{o}C in a 250 ml glass beaker. After 2 hours of stirring, hard, spherical microspheres were obtained. Microspheres were then washed three times with petroleum ether and vacuum-dried to obtain free flowing microspheres.

Table 1. Formulae for Atorvastatin Calcium Loaded Eudragit Microspheres

Batch	Amount of drug	Amount of polymer	Drug:Polymer ratio	Quantity of dichloromethane	Quantity of PVA
F1	250	250	1:1	10	50
F2	250	500	1:2	10	50
F3	250	750	1:3	10	50
F4	250	1000	1:4	10	50

Production yield

The yield was calculated by dividing the weight of the collected microspheres by the weight of all the non-volatile components used for the preparation of microspheres and expressed in the terms of percentage.

Percent Yield = (the amount of microspheres obtained/the theoretical amount) × 100

Particle Size Distribution Analysis:

Formulations of the microspheres were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1 unit of eyepiece micrometer was equal to 13.33 µm. 100 microspheres sizes were calculated under 10x magnification.

Drug Entrapment Efficiency (DEE):

About 10 mg equivalent Atorvastastin calcium loaded microspheres were dissolved in 100 ml of Phosphate buffer (pH 6.8). by shaking on bottle shaker for 10 h. The solution was filtered through Whatman no. 41 filter paper. An aliquot was assayed spectrophotometrically (UV-1701 Schimadzu corporation, Japan) for Atorvastatin calcium at 245 nm. Drug entrapment efficiency was determined by using the following relationship.

% Entrapment = (Actual drug content/Theoretical drug content) × 100

***In vitro* Drug Release Study:**

The dissolution rate of Atorvastatin calcium from the microspheres was studied at pH 1.2 for 2 h followed by pH 6.8 for 8 h using the TYPE- II (Paddle Apparatus) method. Accurately weighed microspheres (equivalent to 20 mg of Atorvastatin calcium) were taken for dissolution studies. The dissolution medium was kept at 37 ± 0.5°C. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 246.5 nm. The volume withdrawn at each time intervals was replaced with the same amount of fresh dissolution medium.

Release Kinetics:

Data obtained from *in vitro* release studies were fitted to various kinetic equations to discover the mechanism of drug release from microspheres. The kinetic models used were Zero order, Korsemeyer-Peppas, and Higuchi. The rate constants were also calculated for the respective models.

FTIR study

Drug polymer interactions were studied by FTIR spectroscopy. IR spectra for drug and drug loaded Eudragit microspheres were recorded in a Fourier transform infrared (FTIR) spectrophotometer (Bruker, Tensor-27, Germany). The Scanning range was 400-3500 cm^{-1}.

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine the surface morphology of microspheres. Dried microspheres were mounted on to stubs by using double-sided adhesive tape. The microspheres were coated with gold and observed under a scanning electron microscope (Joel, JSM-5600 LV, Japan) for surface characteristics.

Mean Particle Size:

The mean size of the formulation of Eudragit S100 (F1-F4) found in the range of 150 to 170 µm

Production Yield:

The production yields obtained were very high for all the formulations. As the % yield of the formulations of Eudragit S100 (F1-F4) found in the range of 80%-90%.

Entrapment efficiency

As shown in table 2 and figure 1, high entrapment efficiency of the drug was obtained for all Eudragit formulations. The % entrapment efficiency of the formulations of Eudragit S100

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(F1-F4) found in the range of 80%-95%

In vitro Release Study

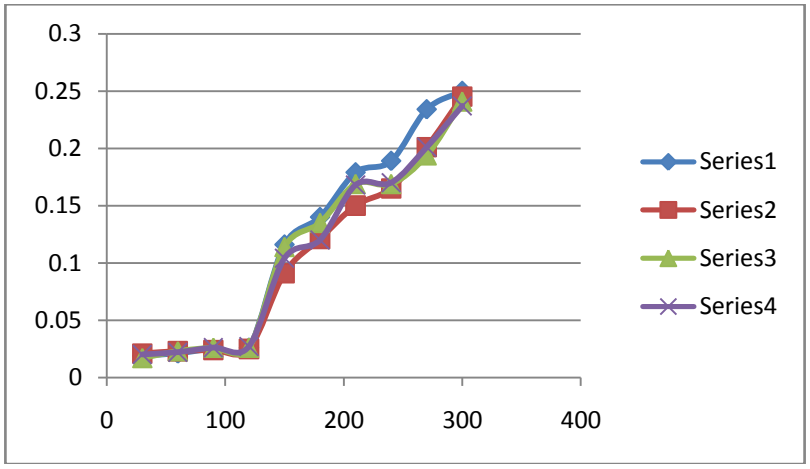

Fig. 4. Cumulative Drug release versus time profile

In vitro release studies of the formulation of Eudragit were carried out in the pH 1.2 (0.1 N HCl) at 37 ± 0.5°C for 2 h followed by in phosphate buffer (pH 6.8) at 37 ± 0.5°C for 8 h. As shown in figure(4), the initial release of Atorvastatin calcium from all the formulation might have resulted from the dissolution of the drug presented on the surface of the microspheres.The formulations of Eudragit S-100, F4 showed the complete drug release after 8 h. Formulation F4 showed the complete release in 8h.

FTIR spectroscopy

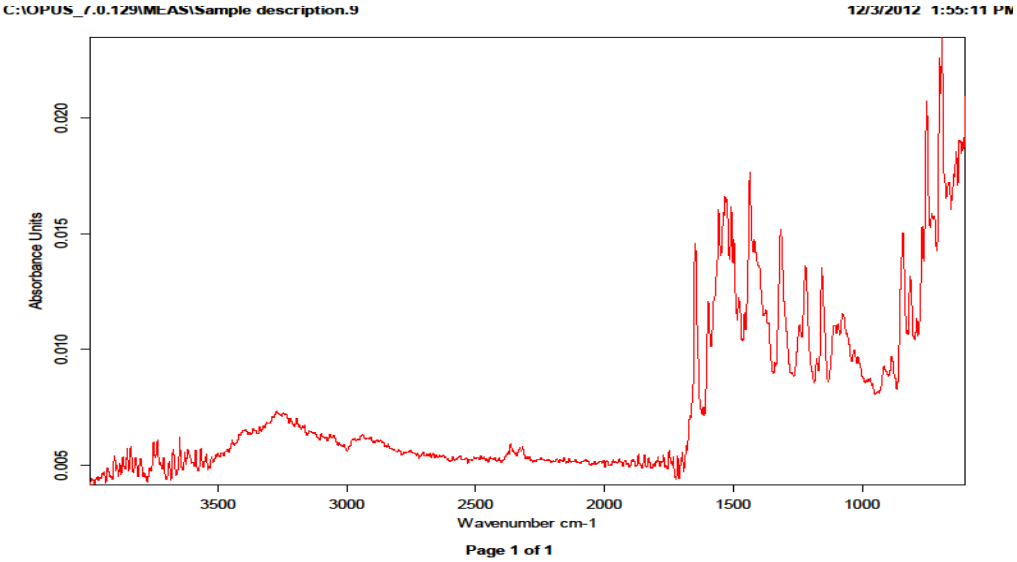

Fig.5. FTIR spectra of microspheres

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Scanning Electron Microscopy

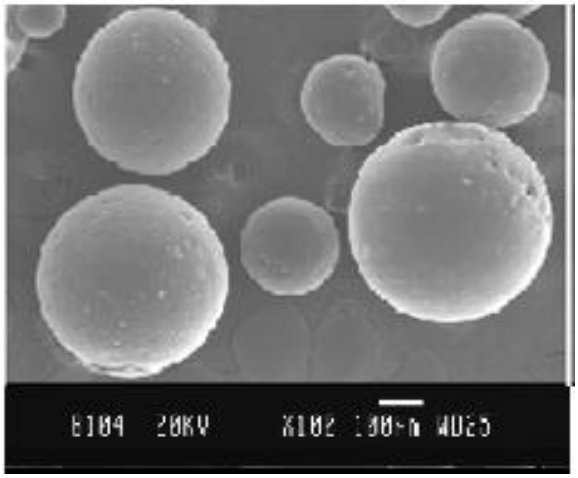

Fig. 6. Scanning electron microscopy of F4

CONCLUSION

Atorvastatin calcium were prepared easily and successfully using the solvent evaporation method. The yield and entrapment efficiency was high for all the formulation prepared. Particle size, entrapment efficiency and production yield were highly influenced by the type of polymer and polymer concentration. *In vitro* dissolution of optimized formulations F4 of Eudragit S100 in PBS (pH 6.8) has the potential to target Atorvastatin calcium in the intestine. According to the results of FTIR, no drug interaction occurred with polymer and Atorvastatin calcium.

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