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FORMULATION AND *IN VITRO* EVALUATION OF ATORVASTATIN CALCIUM CONTAINING MICROSPHERES BY SOLVENT EVAPORATION METHOD

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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. The prepared spherical crystals were characterized for their micromeritic properties. The in vitro release studies were performed in 0.1 N HCl solutions (1.2 pH) for one hour. The prepared microspheres were characterized for their micromeritic properties and entrapment efficiency; as well by Fourier transform infrared spectroscopy, scanning electron microscopy revealed the crystalline nature of drug in a final stage. The in vitro release studies were performed in 0.1 N HCl solutions (1.2 pH) for 2 hour followed by 6.8 pH phosphate buffer for 8 hour. 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 research that Eudragitare promising controlled release carrier for atorvastatin calcium.

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 multi particulate delivery systems and are prepared to obtain prolonged or controlled drug delivery to improve bioavailability or stability and to target drug to specific sites[1]. 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[2-3]. 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. Dichloromethane and water system were used for the preparation of microspheres. Polyvinyl alcohol was used as a droplet stabilizer to prevent droplet coalescence in the oil medium [4-5]. 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 infrared spectroscopy and Scanning electron microscopy, as well as for in vitro drug release studies.

Experimental work

Materials: Atorvastatin calcium obtained from RochemPharma, Gangtok as gift sample; Eudragit S100 was purchased from Evonik Industries. Other substances used were all of analytical grade.

Preparation of microspherical crystals

Microspheres of atorvastatin calcium are prepared by solvent evaporation technique. Here, required amount of drug (atorvastatin calcium) and polymer (eudragit S-100) were dissolved in a mixture of methanol and dichloromethane in 1:1 ratio. The resultant solution was added drop wise in 100ml of water containing 0.4% Poly Vinyl pyrollidone, with continuous stirring at 800rpm. Stirring was continued till all organic solvent gets evaporated. The resulting microspheres were then filtered through whatman filter paper. Microspheres were then dried at room temperature for 24 h[6].

Characterization of Microspherical Crystals of Atorvastatin Calcium

Percentage Yield: The yields of production of microspherical 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.

% yield = Practical mass Theoretical mass × 100

Drug Content Determination: Atorvastatin microspherical agglomerates equivalent to 40 mg of Atorvastatin calcium 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 to dissolve the drug and the polymer. The volume was made up to 100 ml with methanol and filtered through a 0.45 μm filters. The filtrate was diluted with methanol and analyzed at 246.5 nm by uv-spectrophotometer[7-8].

Solubility Study: Solubility of atorvastatin in water was determined by Shake flask method. Here anexcess of drug was shaken with water in conical flask on the shaker, overnight till the equilibrium is achieved. The supernatant was taken and analyzed for drug content, using UV-Spectrophotometer at 242 nm.

Flow Property: Flow ability of Atorvastatin calcium and its microspherical agglomerates were determined in terms of the following parameters, Bulk density, Tapped density, Hausner ratio, Carr's index and Angle of repose.

Evaluations of microspherical 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[9].

Differential Scanning Calorimetry (DSC): The DSC measurements were performed on a differential scanning calorimeter with a thermal analyzer. About 5 mg of sample was weighed and capped in aluminum crucible. The crucible wasthen put in DSC instrument (NETZSCH, DSC-204 F1 PHOENIX) and the spectra depending on thermal transitions were taken.

Distribution Analysis: The particle size analysis of microspheres was performed by optical microscope. The microspheres were dispersed in double distilled water (DDW) and the system was set at25^{o}C.

Drug Entrapment Efficiency (DEE): Weighed quantity of microspheres was powdered & added to 100ml of phosphate buffer (pH 7.4). Resulting mixture was centrifuged at 4^{O}C and at 10,000 rpm. The supernatant was taken and filtered through whatman filter paper. It was then analyzed by UV spectrophotometer at 242 nm. The pellet settled at bottom was taken and 1ml of Triton X-100 was added to it. It was then suitably diluted and analyzed.

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

***In vitro* Drug Release Study:** *In vitro* drug release study was carried out in USP XXIII basket type dissolution test apparatususing 900 ml of Phosphate buffer pH7.4 as dissolution medium. The temperature was maintained at 37±1°C and stirring speed at 100 rpm. 5ml samples were withdrawn at every one hour and were replaced with the same amount of fresh buffer. The atorvastatin content was determined by UV-Visible spectrophotometer at 242 nm[10].

Scanning electron microscopy (SEM): The shape and surface characteristics of microspheres were evaluated by scanning electron microscopy (SEM). The samples were mounted directly on to the SEM sample holder using double-sided sticking tape and images were recorded at the required magnification.

RESULTS AND DISCUSSION

Characterization of Spherical Crystals of Atorvastatin Calcium

Characterization of Spherical Crystals of Atorvastatin Calcium is shown in table 1 by the characteristics flow study, percentage yield, dissolution and bulk density

Percentage Yield	89.46%
Drug content	94.23%
Aqueous solubility	0.12mg/ml
Dissolution	74.61%
Bulk density	0.43
Tap density	0.51
Carr's index	17.21
Hausner ratio	1.3
Angle of repose	23.6

FTIR: The FTIR Spectra for atorvastatin calcium was shown in **Figure 1**. The IR spectra of pure ATC showed characteristic peaks at 2955.15 cm-1 (C-H – stretching), 1313.56 cm-1 (C-N – stretching), 3059.15 cm-1 (C-HO - stretching alcoholic group), 1564.97 cm-1 (C=O – stretching amidic group), 3403.27 cm-1 (N-H - stretching), 1656.97 cm-1 (C=C - bending), 751.62 cm-1 , 696.95 cm1 (C-F- stretching), 1104.39 cm-1 (O-Hbending). It might be the possibility of intermolecular hydrogen bonding between adjunct Atorvastatin calcium molecules.

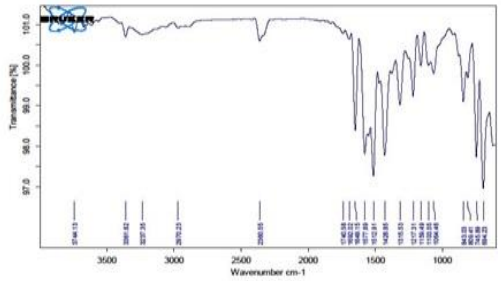


Figure 1: FTIR Spectra for atorvastatin calcium

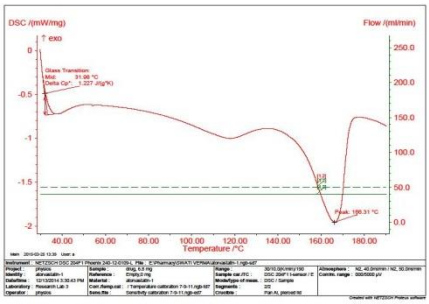


Figure 2: DSC curve for atorvastatin calcium

Differential Scanning Calorimetry (DSC): The DSC curve for atorvastatin calcium was shown in **Figure 2**. It shows an endothermic peak at 166.3ºC, corresponding to the melting point of the drug.

Preparation of microspheres

Batches F1 to F4 were prepared by keeping PVA concentration at 0.4%, stirring speed 800rpm, temperature of aqueous PVA solution at 25ºC and D/P ratio 1:40 to optimize the amount of drug loaded in final formulation, shown in **Table 2**.

The entrapment efficiency was found to be highest when 5mg of drug was loaded. The size of microspheres increases as the amount of drug loaded was increased. Microspheres were found to be spherical in all the formulations from F1 to F4. So 5mg of drug will be loaded in final formulations.

Distribution Analysis:

The average particle size for optimized formulation was found to be 75nm and polydispersity index was 0.3.

Drug Entrapment Efficiency (DEE):

The entrapment efficiency of the optimized formulation was foundto be **94.37%,** determined by centrifugation method.

Scanning electron microscopy (SEM)

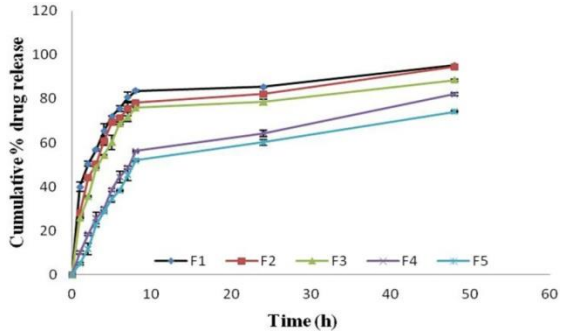
Scanning electron microscopy was used to examine the surface morphology of microspheres.

Shape and surface morphology of microspheres were analyzed by Scanning Electron Microscopy (SEM), as shown in **Figure 4**.The microspheres were found to be spherical in shape and the particle size was found to be approximately 1119 nm.

Table 2: Selection of Amount of Drug Loaded

Formulation	Amount of drug loaded	Eudragit S-100(mg)	Mean	Entrapment	Shape
F1	5	200	318.7	74.37	Spherical
F2	10	200	449.3	71.00	Spherical
F3	20	200	528.1	68.32	Spherical
F4	30	200	530.4	67.99	Spherical

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



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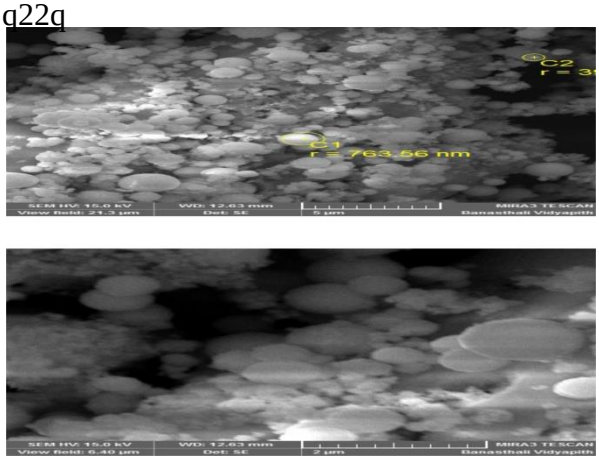

Figure 4: SEM photograph of atorvastatin microspheres

CONCLUSION

Atorvastatin calcium microspheres 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 F2 of Eudragit S100 in PBS (pH 6.8) has the potential to target Atorvastatin calcium in the intestine.

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