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Formulation and characterization of mucoadhesive microspheres of atorvastatin calcium

Arpana Sharma*[1], P K Singour[1], Ankit Mishra[1], Prabhat Sharma[2]

[1] Department of pharmacy, VNS Group of Institutions, Bhopal, Madhya Pradesh, India
[2] Technocrats Institute of Technology , Bhopal, Madhya Pradesh, India

Corresponding author: Arpana Sharma, ✉ aadhya.16@gmail.com,
Department of pharmacy, VNS Group of Institutions, Bhopal, Madhya Pradesh, India

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ABSTRACT

Oral bioavailability of atorvastatin calcium (ATC) is very low (only 14%) due to instability and incomplete intestinal absorption or extensive gut wall extraction. Atorvastatin belongs to BCS class II. Atorvastatin, as a synthetic lipid-lowering agent (for hypercholesterolemia). The Mucoadheive microsphere of Atorvastatin calcium (ATC) were developed to expand the gastric residence time of the drug, as ATC has maximum rate of absorption in the upper GI tract. Mucoadhesive microspheres were prepared by emulsification technique followed by cross-linking in mucoadhsive polymers carbopol 934P and sodium alginate used. The microspheres were assessed for percentage yield, average particle size, zeta potential, micromeritic properties, swelling properties, FTIR spectroscopy of microspheres, percentage drug entrapment, mucoadhesion testing by *in-vitro* wash-off test, *in-vitro* drug release, drug release kinetic & pharmacokinetic studies. Pharmacokinetic study performed on albino rabbits illustrated that the bioavailability of atorvastatin microspheres significantly increased. This research indicated that mucoadhesive microspheres for delivery of ATC can improve its bioavailability.

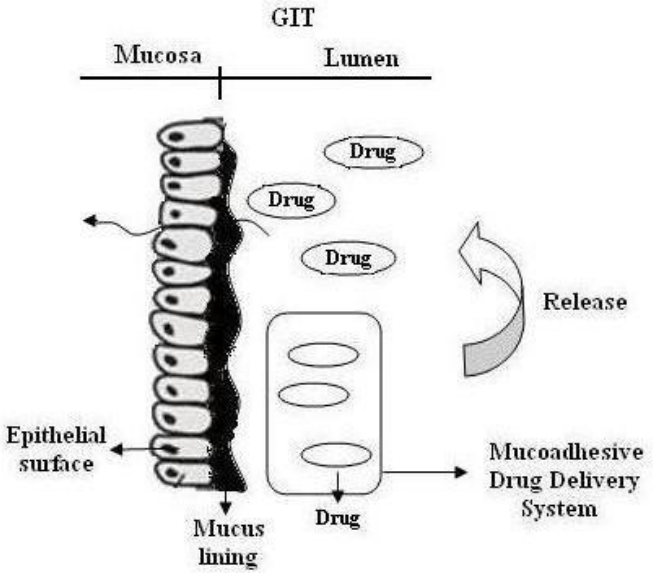
Keywords: Atorvastatin calcium, Bio-availability, Muco-adhesive microspheres, Crosslinking, Emulsification

INTRODUCTION

Atorvastatin calcium (ATC) is is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG - CoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Atovastatin the treatment of choice in moderate to severe familiar or non-familiar hypercholesterolemia. ATC has a maximum rate of absorption in the upper GI tract, but because of poor solubility in this region, it is less bioavailable. Unpredictable and short gastric emptying time can result in incomplete drug release from the drug delivery system above the absorption region, which may be the stomach or upper component of the small intestine, leading to a reduced systemic availability of the administered dose. A previous study investigating improved solubility, stability and bioavailability of ATC includes a tablet formulation containing a complexing agent (cyclodextrins) and a surfactant (d-alpha tocopherol polyethylene glycol 1000 succinate. A self-micro emulsifying drug delivery system (SMEDDS) of ATC consisting of Labrafil, propylene glycol and Cremophor RH40 has been developed. Recently, another self-emulsifying drug delivery system (SEDDS) of ATC in various vehicles such as Captex 355, Captex 355 EP/NF, Ethyl oleate, Capmul MCM, Capmul PG-8, Gelucire 44/14, Tween 80, Tween 20, and PEG 400 has been reported. All these drug delivery systems have the disadvantage of complex manufacturing procedure and use of expensive additives with additional equipment and apparatus. The negative aspect of formerly reported solo unit systems such as tablets and capsules are the high inconsistency of their GI transfer moment because of their all-or-nothing emptying course of action. To overcome these limitations, mucoadhesive microspheres have been developed to form cost-effective stable ATC formulation with enhanced bioavailability. Mucoadhesive microspheres of ATC will ensure a complete and constant drug release, particularly for drugs absorbed in the specific gastric area. Such a dosage form can be

circulated extensively throughout the gastrointestinal tract (GIT), providing a longer and more dependable release of the drug from the dosage form. Therefore, the focus of this research was to develop solubilized gastro-retentive mucoadhesive microspheres of ATC to achieve better bioavailability [1].

Figure 1: Mechanism of mucoadhesion



MATERIAL AND METHOD

ATC was obtained as a gift sample from Cipla Ltd. Indore. Carbopol 934P & Sodium alginate were purchased from oxford chemicals, Mumbai. Light Liquid Paraffin, Span80 & Calcium chloride were purchased from Loba chemie, Mumbai. All other chemicals were of analytical grade [2].

Method of formulation

The emulsification method was used for the preparation of mucoadhesive microspheres followed by cross-linking with calcium chloride. Atorvastatin calcium was dispersed in aqueous solution of sodium alginate and carbopol 934P, this aqueous phase was emulsified in light liquid paraffin containing 0.2% (v/v) span 80 using a homogenizer (IKA, Japan) at 2000 rpm for 120 min. Five ml of 5% calcium chloride was added to the emulsion. The solidified microspheres were recovered by centrifugation, washed with petroleum ether and dried in vacuum desiccators.

Characterization of formulation

Microspheres were characterized by determination of percentage yield, average particle size, zeta potential, micromeritic properties, swelling properties, FTIR spectroscopy of microspheres, percentage drug entrapment, mucoadhesion testing by in-vitro wash-off test, in-vitro drug release, and drug release kinetic & pharmacokinetic studies.

Determination of percentage yield

The dried microspheres were weighed and their percentage yield (w/w) was determined by using following formula.

Determination of average particle size

Average particle size distribution of each formulation was measured by a Malvern Zetasizer, Nano Z (Malvern Instrument). The measurements were performed at scattering angle of 90° and at a temperature of 25±1°C.

Determination of zeta potential

The interaction of the particles with charged drug and also on the adhesion of drug delivery system on surface. The particle charge was quantified, measuring the zeta potential by Laser Doppler Anemometry using a Malvern Zetasizer, Nano Z (Malvern Instruments).

Determination of micromeritic properties

Angle of repose

Weighed quantities of microspheres were passed through a fixed funnel on a specific height upon graph paper. The height of the heap (h) and the radius (r) of lower part of cone were measured. The angle of repose was calculated using formula

$\tan \theta = h/r$

Therefore $\theta = \tan^{-1} h/r$

Where θ = Angle of repose, h = height of cone and r = radius of cone base [3].

Carr's Index

This test was conducted for the flowability of microspheres by comparing the poured density and tapped density. It was determined by 1 gm of microspheres samples in 10ml measuring cylinder. The height of the sample was measured before and after tapping which indicates the poured and tapped density respectively.

Carr's Index was calculated as:

$$I = \left(\frac{V_b - V_t}{V_b}\right) \times 100$$

Where V_b= bulk volume and V_t= tapped volume

Determination of swelling properties

Swelling index was determined by measuring the extent of swelling of microspheres in phosphate buffer pH 6.8. Weighed 100mg of microspheres were allowed to swell in simulated intestinal fluid pH 6.8 for 24 hrs. Degree of swelling was then calculated by the following formula

$$\text{Degree of Swelling} = \left(\frac{M_0 - M_t}{M_t}\right) \times 100$$

Where

M_t = initial weight of microspheres and M_o= weight of microspheres at equilibrium swelling in the media [4].

FTIR spectroscopy of microspheres

Drug excipients compatibility study was performed by Alpha Bruker FTIR spectrophotometer. The crushed microspheres were mixed with potassium bromide and dried at 40^0C. The mixture was compressed to a disk by applying pressure for 2 min. The FTIR spectra were recorded.

.**Determination of entrapment drug percentage**

Microsphere (25 mg) was crushed and extracted with methanol and after appropriate dilution with PBS (pH 6.8), it was subjected to drug content analysis UV-visible spectrophotometer at a wavelength of 247nm. Drug concentration was determined with respect to absorbance and draw calibration curve for linear drug release. The range of drug concentration in the calibration curve was 5-25 µg/ml. The entrapment efficiency was determined using the following formula [5].

$$\%EE = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Where

%EE = Percent Entrapment Efficiency

Mucoadhesion testing by *in-vitro* wash-off test

The mucoadhesive property of microspheres was evaluated by *in-vitro* adhesion testing method called as wash-off method. A 2×1 cm piece of eggshell membrane was tied onto a glass slide using thread. About 100 mg microspheres were spread on wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating height of the sample was measured before and after tapping which indicates the poured and tapped density respectively [6].

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AUC (Area under the plasma level-time curve) calculated by trapezoidal rule, the method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the each trapezoid and adding them together. Pharmacokinetic result of animal.

The extent of bioavailability can be determined by following equation [10].

$$Fr = \frac{[\text{AUC}]\text{test Dstd}}{[\text{AUC}]\text{std Dtest}}$$

RESULTS AND DISCUSSION

Atorvastatin calcium is a major therapeutic for hypercholesterolemia. To achieve the objective of formulation development. Preformulation studies were performed by organoleptic properties, melting point determination, solubility studies, determination of absorption maxima (λ_{max}) of drug by UV-visible spectrophotometer and compatibility studies of drug and excipients and excipients-excipients were performed by FTIR spectroscopy and DSC analysis.

Mucoadhesive microspheres prepared by emulsification method followed by cross-linking with calcium chloride. Average

particle size range was found in micrometer range and it was observed spherical in shape. Among all the formulations A4 formulation was optimized with desired percentage drug entrapment and *in-vitro* drug release. All formulations were characterized for determination of percentage yield, average particle size**,** zeta potential, micromeritic properties**,** swelling properties**,** FTIR spectroscopy of microspheres**,** percentage drug entrapment**,** mucoadhesion testing by *in-vitro* wash-off test, *in-vitro* drug release, and drug release kinetic & pharmacokinetic studies.

The optimized formulation A4 was found to be desired percentage yield 60.2±0.51 particle size i.e. 3.82±0.07 µm, zeta potential -30 mV, free flow with angle of repose 25 ± 0.41^0, Carr's index 15.2±0.23%, swelling index percentage 18±0.12%, *in-vitro* mucoadhesion time 6±0.11 hrs, percentage drug entrapment 64.1±0.01%, *in-vitro* drug release 73.62±0.23%. The data obtained were also fitted to Korsemeyer Peppas model in order to find out the n value. The n value obtained was 0.673 > 0.48 which indicate non-fickian transport, the main mechanism for drug release from microspheres. In pharmacokinetics of A4 formulation and pure drug, A4 formulation was significantly higher than that of drug ($p < 0.05$). The oral bioavailability of drug was increased as the relative availability values were 1.43 times for A4 formulation compared with pure drug [11].

CONCLUSION

Mucoadhesive microspheres composed of carbopol 934P and sodium alginate, prepared by emulsification cross linking could be a quite good approach to improve the gastric residence time and bioavailability of drug due to mucoadhesive nature of carbopol 934P and sodium alginate. Sodium alginate was added in microspheres with mucoadhesive polymers carbopol 934P, in an attempt to retard the drug release. After oral administration of mucoadhesive microspheres of Atorvastatin calcium to rabbits, the bioavailability of drug increased as compared with that of pure drug. The pharmacokinetic study in rabbits showed the oral bioavailability of Atorvastatin calcium was enhanced due to prolonged gastric residence time.

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