Formulation of Modified Release Atorvastatin Nanoparticles by Emulsion Interfacial Reaction Method

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ABSTRACT

The aim of the study is to formulate a modified release chitosan nanoparticles for the oral delivery of atorvastatin and to study the in vitro release of atorvastatin from chitosan nanoparticles. Atorvastatin-loaded chitosan nanoparticles were prepared with different concentration of cross-linking agent (glutaraldehyde) by emulsion interfacial reaction method. The formed nanoparticles were characterized in terms of size and morphological characteristics by scanning electron microscopy (SEM) and transmission electron microscope (TEM). Spherical and regular nanoparticles with the size range of 100-250nm were formed. Atorvastatin encapsulation efficiency of nanoparticles was found to be highest in ANP3, followed by ANP2 and ANP1. The in vitro release of atorvastatin was studied by membrane diffusion technique. The resulted cumulative percentage of drug released for ANP1, ANP2 and ANP3 were 60.08%, 34.81% and 20.39% respectively. Through this study, the nanoparticles preparation technique has shown to be a promising approach for enhancing the dissolution of hydrophobic drugs like atorvastatin calcium. The application of this novel delivery system offers good therapeutic potential in the management of hypercholesterolemia and dyslipidemia.

KEYWORDS: Hydrochlorothiazide; Metoprolol; Mannitol; Drug-drug dispersion; solubility.

Introduction

Nanoparticles are solid colloidal particles with diameters ranging from 1 to 1000nm. They are used therapeutically as drug matrix, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or attached (Mohanraj et al., 2006). Two types of nanoparticles can be obtained based on the preparation process: nanospheres and nanocapsules (Allemann et al., 1993). Chitosan nanoparticles have been extensively studied and widely used as drug delivery carriers owing to their better stability, low toxicity, mild preparation methods and providing versatile routes of administration.

Chitosan is a natural polymer composed of β-(1-4)-2-amino-2-deoxy-D-glucopyranose units. It is a deacetylation derivative of chitin, a widely distributed polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp. Chitosan possesses a number of favorable properties like biodegradability, biocompatibility, abundant availability, unique mucoadhesivity, non-toxicity and low immunogenicity (Manish P. Patel et al., 2010). The polymer is insoluble at neutral pH yet is soluble and positively charged at acidic pH, with a pKa of approximately 6.5.

Atorvastatin ((R-(R,R,R))-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-phenylamino carboxyl)-1H-pyrrole-1-heptanoic acid, calcium salt (2:1 trihydrate), is a member of the drug class of statins, a main drug class used for lowering blood cholesterol levels. Atorvastatin is an orally administered drug used for the treatment of elevated total cholesterol, low density lipoprotein and triglycerides and to elevate high density lipoprotein cholesterol (Law et al., 2003). It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanisms (Mohammed Anwar et al., 2011). Like other statins, atorvastatin works by selectively inhibiting HMG-CoA reductase, an enzyme that is involved in the biosynthesis of cholesterol (Law et al., 2003). Atorvastatin is a BCS class II drug, insoluble in aqueous solutions of pH 4, very slightly soluble in distilled water and pH 7.4 phosphate buffer, and has high intestinal permeability (Wu et al., 2000). Poor oral bioavailability of atorvastatin results in administration of higher doses of drug and causes patients to discover dose related adverse effects such as liver abnormalities, rhabdomyolysis, arthralgia and kidney failure.

Chitosan nanoparticles preparation technique has been evolved from the chitosan microparticles technology over the past 3 decades. The nanoparticles preparation methods are classified into two main categories (Catarina et al., 2006). One is formulated by polymerization reaction and another formulation is achieved directly from a macromolecule or preformed polymer. The polymerization methods can be further classified into emulsion and interfacial polymerization.
Direct production of nanoparticles can be obtained from synthetic preformed polymers, natural macromolecules or by desolvation of macromolecules. The synthetic preformed polymers are mainly synthesized by four methods: emulsification-solvent evaporation, solvent displacement and interfacial deposition, emulsification-solvent diffusion and salting out with synthetic polymers. The present study is to formulate and evaluate modified-release chitosan nanoparticles loaded with atorvastatin which is capable to release the drug for a long period of time. Nanoparticles will be synthesized by emulsion interfacial reaction method. The study is expected to open up the future application of developing controlled-release atorvastatin drug delivery system in order to enhance therapeutic efficiency of the drug.

Materials and Methods

Materials

Atorvastatin calcium was obtained as a gift sample from Ranbaxy (M) Sdn. Bhd. Malaysia, chitosan and isooctane were purchased from R and M Chemicals, Malaysia; Glutaraldehyde was procured from AP Laboratory, Malaysia. All the other reagents were commercially available and of analytical grade.

Methods

Formulation of atorvastatin-loaded chitosan nanoparticles: Atorvastatin-loaded chitosan nanoparticles were formulated by adapting emulsion interfacial reaction method with the aid of the emulsifying agent. The process for formulating the nanoparticles was initiated by the preparation of two emulsions (Given name as Emulsion A and Emulsion B respectively). Emulsion A was prepared by using 1.0 % chitosan polymer solution containing and 1.5 % of acetic acid, 5 % SPAN 80 and isooctane. Firstly, 10 mL of aqueous polymer solution was prepared by adding 0.1g of chitosan into 1.5 % acetic acid solution. 20 mg of atorvastatin calcium was dissolved with 1 mL of methanol. The atorvastatin solution was added into 4 mL of polymer solution under stirring with overhead mechanical stirrer (HS 50 A, Wisestir) at 1000 rpm. To this, 0.5 mL of SPAN 80 was added into the mixture, followed by 45 mL of isooctane. Emulsion A was continued mixing with mechanical stirrer under agitation of 2000 rpm for 5 minutes. Emulsion B was prepared by inserting 0.25 mL of 25% glutaraldehyde into 50 mL of 2% sulfuric acid. Then 0.5 mL of SPAN 80 was added followed by 50 mL of isooctane under agitation of 3000 rpm for 5 minutes by mechanical stirrer. Emulsion A and Emulsion B were mixed 1:1 under agitation of 5000 rpm for 10 minutes. After nanoparticles were formed, they were separated with centrifuge (Hettich Zentrifüger, Germany) at 5000 rpm for 5 minutes and washed with petroleum ether for 4 to 5 times. Nanoparticles were then lyophilized in a freeze drier after washing. The first formulation was named as ANP1. The second and third formulations, given names of ANP2 and ANP3 were formulated by the same method, using the same ingredients with different concentrations in cross linking agent viz 1.0 mL of 25 % glutaraldehyde and 2.5 mL of 25 % glutaraldehyde respectively.

Morphology and structure characterization of nanoparticles: The morphology and particles size measurements of the chitosan nanoparticles were examined using scanning electron microscopy (SEM) and transition electron microscopy (TEM) under various magnifications and different accelerated potential.

Determination of atorvastatin entrapment efficiency of chitosan nanoparticles: After the nanoparticles were centrifuged, the free amount of atorvastatin was determined in clear supernatant by UV double beam spectrophotometer (V630, Jasco Corporation) by using placebo chitosan nanoparticles as control. Atorvastatin encapsulation efficiency (AE) was calculated according to the equation (1) indicated below:

$$AE = \frac{T - F}{T}$$

Where T is the total amount of atorvastatin used and F is the amount of free atorvastatin present in supernatant liquid.

In-Vitro drug release study: Atorvastatin release from chitosan nanoparticles was determined by membrane diffusion method. The dissolution medium consists of 500 ml of phosphate buffer pH 7.4 at 37 °C, stirred at 50 rpm with magnetic stirrer (LMS-1003, Daihan Lab Tech. Co. Ltd) for 5 hr. Atorvastatin loaded chitosan nanoparticle holders were prepared by covering one end of an opened-end cylinder with cellophane paper which is presoaked in phosphate buffer for 12 hr. 100 mg of freeze-dried nanoparticles were transferred into the holders. The temperature of the medium was maintained at 37 °C ± 0.5 °C. At regular intervals of 30 minutes 3 mL of sample was removed and tested by UV spectrophotometry at the wavelength of 240 nm. 3 mL of fresh medium was added to maintain sink condition.

Results and Discussion

Formulation of atorvastatin-loaded chitosan nanoparticles: The atorvastatin-loaded chitosan nanoparticles were successfully formed with the adaptation of emulsion interfacial reaction method. Three sets of samples, namely ANP1, ANP2 and ANP3 were formulated by the association of different cross-linking agent concentrations.

Morphology and structure characteristic of nanoparticles: Morphological information of the atorvastatin-loaded chitosan nanoparticles was provided by the examination under the SEM and TEM. SEM image is obtained under magnification 500x (Fig. 1). TEM images were captured and magnifications 66000X and 88000X with accelerated potential 120 kv, shown the particle sizes were in the range of 100-250 nm (Fig. 2 and 3). Also the particles were seen to be spherical and smooth. The transition electron microscopy analysis confirmed the presence of nanoparticles which comprised of chitosan layer surrounding the core (Fig. 3). The particle shape and size may be depends upon the stirrer speed, so that in the lower speed macro particles were obtained.
Encapsulation efficiency of chitosan nanoparticles:

Atorvastatin encapsulation efficiency integrated with different concentrations of cross linking agent of the formulated nanoparticles was determined and given in Table 1. ANP3 has the highest encapsulation efficiency, which is 84.72%, followed by ANP2 and ANP1 batches respectively. The encapsulation study result reveals that nanoparticles cross-linked with higher concentrations of glutaraldehyde establish higher encapsulation efficiency. This may be due to the affinity between the cross linking agent and the polymer which surrounds the drug particles.

**TABLE 1**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Encapsulation efficiency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP1</td>
<td>78.40 ± 0.018</td>
</tr>
<tr>
<td>ANP2</td>
<td>81.36 ± 0.012</td>
</tr>
<tr>
<td>ANP3</td>
<td>84.72 ± 0.001</td>
</tr>
</tbody>
</table>

(*Values are given in mean± SD: n=6*)

**In-vitro drug release study:** The release profile of atorvastatin from the formulated nanoparticles in phosphate buffer pH 7.4 shows a gradually increased order with time. The cumulative drug release of atorvastatin from ANP1, ANP2 and ANP3 after 5 hours was 60.08%, 34.81% and 20.39% respectively. From Figure 4, it can be seen that the release pattern of atorvastatin from nanoparticles was lower corresponding to the higher concentrations of cross-linking agent used. In conclusion, cross linking agent binds strongly between the core and polymer which resists the release of drug from nanoparticles.

**Conclusions**

Atorvastatin-loaded chitosan nanoparticles were successfully prepared by emulsion interfacial reaction method. This study shows the effectiveness of the method in novel delivery of hydrophobic drugs. This technique can be a promising approach for enhancing the dissolution of poorly soluble drugs like atorvastatin calcium with modified release. In conclusion, application of this modified release delivery system offers good therapeutic potential and better patient compliance in the management of hypercholesterolemia and dyslipidemia.

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**References**


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