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The multifunctional synergistic effect of chitosan on simvastatin loaded nanoparticulate drug delivery system

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ABSTRACT

The present study reported design and evaluation of nanoformulations of Simvastatin using different polymers. The study emphasizes upon the synergistic activity of the drug and the polymers owing to the reported anti- hyperlipidemic activity of the selected polymers. Preliminary studies advocated for chitosan formulations due to the expected particle size (543 ± 26 nm) among the three polymer formulations. Four formulations (F1–F4) were prepared varying chitosan ratio. F4 demonstrated optimal characteristics (particle size – 549 ± 23.43 nm, narrow size distribution – 0.515 ± 0.06) and qualified for further investigation. The formulation parameters modified the intrinsic properties of chitosan. Formation of low M. wt chitosan (70,000 ± 10,000 Da) enhanced swelling & mucoadhesive properties. These influenced the drug properties in which amorphization of drug increased solubility and decreased partition coefficient. This led to better absorption at intestine sustaining the drug release up to 66.18 ± 1.26% in SIF during the *in vitro* study. Better absorption was confirmed by reduction in lipid profile with several fold reduced dose in mouse model. The *in vitro* bile binding property of chitosan in formulation demonstrated the enhancement of hypolipidemic activity of Simvastatin. The outcomes of the study showed a successful preparation of optimum nanoformulations and also revealed possible synergistic functionalities of chitosan and the Simvastatin as potential hypolipidaemic modality without any toxic manifestations.

1. Introduction

One of the indentified self-governing risk factor for ischemic heart disease is elevated plasma cholesterol concentration. A good health status can be achieved by maintaining adequate levels of circulating blood fats, cholesterol, and triglycerides. Naturally, body can control the production and removal of lipoproteins in our system so as to keep them at healthy levels. It is also considered recently that risk of myocardial infarctions diminishes on reducing plasma cholesterol concentration (Nelson, 2013). On the other hand if the level exceeds to a high level and natural depletion by body do not occur, both dietary cholesterol and cholesterol produced by the liver should be reduced by application of external mode of drug delivery system.

The level of cholesterol from body especially dietary cholesterol can be reduced by variety of agents like Chitosan while balancing the circulatory fats (Kerch, 2015). Chitosan is an amino polysaccharide, polymer of glucosamine, found in the exoskeleton and the fungal cell wall of various arthropods, including insects, crabs, and shrimps. In pharmaceutical applications it has pharmaceutical applications due to its non-toxicity, biocompatibility, high charge density, and mucoadhesion. Besides, capable of enhancing the dissolution of poorly soluble drugs, Chitosan exerts favorable biological activities, notably hypolipidemic activity, bioadhesion, and permeability-enhancing properties. In addition, it has significant physicochemical characteristics, which makes it a distinctive material for the design of drug delivery mediums (Najafi, Pazhouhnia, Ahmadi, Berenjian, & Jafarizadeh-Malmiri, 2014; Rajan and Raj, 2013).

Another distinct characteristic property of chitosan is its capability of preventing the normal emulsification of neutral lipids, *i.e.* cholesterol and other sterols, by binding them with hydrophobic bonds. Presence of electrostatic and hydrophobic bonding formed by this process facilitates the formation of larger polymer complexes, which are weak and easily breakable by the digestive process in human body (Walsh, Sweeney, Bahar, & O'Doherty, 2013). Moreover, Chitosan can inhibit the absorption and enterohepatic circulation of bile acids, leading to the reduction of plasma cholesterol levels, together with an increase in oxidative production of bile acid from hepatic cholesterol. The physicochemical property of an agent is associated to its biological activity. To exemplify, the hypocholesterolemic activity of chitosan is better when deacetylated degree is high (>90%); when it is of lower molecular

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weight and there is an increase in the viscosity of the intestinal contents due to absorption of greater water content (Foster, Ho, Hook, Basuki, & Marcal, 2015).

Recently, there is an increase in interest in the area of research which employs combination of polymers and biopolymers with biologically active small molecule compounds to develop delivery system. Low molecular weight active material when conjugated with polymer has been found to produce a modified action (Abozaid, Abd El-hamid, & Atwa, 2015). Chitosan, due to its capacity of binding lipids, cholesterol, fatty acids, triglycerides and bile acids and having biocompatibility, greatly synergizes the activity of drugs. A molecule of this cationic polymer is capable of binding acid drugs like statin.

Therefore, in the present investigation, chitosan is utilized as delivery carrier for a poorly soluble drug (BCS-CLASS II – Simvastatin). The intrinsic properties of chitosan gives a more powerful effect than when used alone. Many properties of active drug Simvastatin can be enhanced like increase in solubility of drug in gastric environment thereby increasing its bioavailability (Zhong, Yizhu, Luo, & Weikesu, 2013). Increase in solubility thereby causes decrease in partition coefficient making drug appropriate for absorption. It can prolong the release of the drug by improving residence time by binding with mucus, swelling itself and forming gel (Mazzarino et al., 2012). The pka of Chitosan is 6.3 (weak base), due to which it exists in unionized form in an alkaline environment making the drug stable which is unstable at that pH (Karolewicz, 2015). Moreover, it has the ability to bind bile acid and fat like bile acid sequesterants, hence when delivered along with statins it might enhance hypolipidemic activity and synergize its action. Statins like Simvastatin which is a HMG-CoA reductase inhibitor decreases the production of cholesterol in liver and lower the risk of coronary diseases. However, they are also known to have side effects such as myopathy with or without rhabdomyolysis (Bhardwai, Shalini, & Schneider, 2013; Bitzur, Kamari, Cohen, & Harats, 2013) and cannot control dietary cholesterol. On the other hand, chitosan can effectively control dietary cholesterol. Owing to the poor solubility of statins, a high dose of the drug is usually prescribed for a better therapeutic outcome.

In this study, with the aid of nanotechnology an approach has been made to reduce the conventional dose of a statin while capable of producing the same pharmacological action by the synergic addition effect of chitosan (Najafi et al., 2014).

2. Materials and methods

2.1. Materials

Simvastatin was received as gift sample from Biocon Pvt. Ltd. All other chemicals and polymers, namely, Chitosan, Guar gum, almond gum, polyvinyl alcohol (PVA), Tween 80 and solvents utilized in the present work were of analytical grade and obtained from Sigma Aldrich (USA).

2.2. Drug–polymer interaction studies

The possible interactions between the drug and the polymer/excipients were ruled out using various sophisticated analytical techniques including FTIR (Fourier transform infrared), DSC (Differential scanning calorimetry) and XRD (X-ray diffractometry). The crystal structure of the physical mixture was determined using an X-ray diffractometer (Rigaku model D/Max-2BX X-ray diffractometer) with CuKα radiation. The thermal stability of the physical mixture was measured on a Differential scanning calorimetry (Du Pont thermal analyzer with 2010 DSC module) with a heating rate of 10 °C/min under nitrogen flow. The structural features of physical mixture were estimated by FTIR (Fourier transform infrared) (FTIR- 410® Jasco Colchester, United Kingdom), using KBr pellets.

2.3. Preparation of drug-loaded polymeric nanoparticles

Nanoparticles are prepared by solvent evaporation method. Drug, Simvastatin was dissolved in methanol which forms the organic phase. The aqueous phase was comprised by dissolving polymer (chitosan in 2% acetic acid, Guar gum in water, Almond gum in water) in 0.5% polyvinyl alcohol and 0.2% tween 80. Polyvinyl alcohol and Tween 80 were used as stabilizer and surfactant respectively. The organic phase consisting of simvastatin (SS) solution was added drop-wise to the aqueous solution containing polymer, polyvinyl alcohol, and tween 80 under magnetic stirring at 400 rpm for 3 h. Then it was homogenized at 10,000 rpm using Remi overhead stirrer. The formation of nanoparticles was confirmed when a rich emulsion was formed post homogenization. The formed nanoparticles were separated by centrifugation (REMI cooling centrifuge) at 20,000 rpm for 30 min followed by freeze drying. Using above method three different formulation keeping drug as constant and varying polymer (CS, GG, AG) were prepared.

2.4. Selection of best formulation

Using the above method, three different formulations (CS-SS, GG-SS, AG-SS) keeping drug as constant and varying polymer [chitosan (CS), guar gum (GG), almond gum (AG)] were prepared. The polymers are known to have their own hypolipidemic activity which is evident and well-established from trustworthy literatures published worldwide. Based on the particle size, entrapment efficiency, drug loading, surface charge characterization, the best polymer was selected. The process of selection of best polymer was optimized previously in our lab by Design of Experiments. However, that part of the work is not included and presented in this manuscript. Using selected best polymer and the above preparation method, four formulations (F1, F2, F3, F4) were prepared keeping drug amount (mass) as constant and varying polymer (mass) ratio alone (1:2, 1:3, 1:4, 1:5) and the best formulation was selected by physicochemical characterizations mentioned in 2.5.1. Each sample was measured in triplicate wherever necessary.

2.5. Characterization of nanoparticles

2.5.1. Physicochemical characterization of chitosan coated simvastatin nanoparticles

The morphology of the samples was determined using scanning electron microscope (JE0L JSM 7600f). The particle size, size distribution [polydispersity index (PDI)] and zeta potential of particles were measured by Zetasizer (Malvern Instruments, UK), based on the dynamic light scattering (DLS) technique. The percentage of yield, drug loading and entrapment efficiency were measured by centrifugation method using the following formulae.

$$\%\ Nanoparticle\ yield = \frac{Mass\ of\ nanoparticle\ recovered}{Mass\ of\ polymer,\ drug\ and\ Excipients} \times 100 \quad (1)$$

$$\%\ Drug\ loading = \frac{Mass\ of\ drug\ in\ nanoparticle\ preparation}{Mass\ of\ nanoparticle\ Recovered} \times 100 \quad (2)$$

$$\%\ Entrapment\ efficiency = \frac{Mass\ of\ drug\ in\ nanoparticle\ preparation}{Mass\ of\ drug\ added\ initially} \times 100 \quad (3)$$