Chitosan-sodium Alginate Nanoparticle as a Promising Approach for Oral Delivery of Rosuvastatin Calcium: Formulation, Optimization and In vitro Characterization

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Authors’ contributions
This work was carried out in collaboration between both authors. Author MAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NMA managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

ABSTRACT
Rosuvastatin calcium is the most effective antilipidemic drug and is called "super-statin" but it exhibits low aqueous solubility and poor oral bioavailability of about 20%. The present work aimed to develop and optimize chitosan-alginate nanoparticulate formulation of rosuvastatin which can improve its solubility, dissolution and therapeutic efficacy. Chitosan-alginate nanoparticles were prepared by ionotropic pre-gelation of an alginate core followed by chitosan polyelectrolyte complexation and optimization was done in terms of two biopolymers, crosslinker concentrations. The chitosan-alginate nanoparticles were characterized by various techniques such as particle properties such as size; size distribution (polydispersity index); Zeta-potential measurements and Fourier transform infrared spectra respectively. The designed rosuvastatin loaded chitosan-alginate nanoparticle had the average particle size of 349.3 nm with the zeta potential of +29.1 mV, and had
1. INTRODUCTION

The prevalence of dyslipidemia is high and increasing in many developing countries, including Saudi Arabia because of the westernization of diet and other lifestyle changes [1]. Rosuvastatin calcium (ROC) is a widely used antihyperlipidemic drug use for the treatment of atherosclerosis and is called "superstatin" [2]. It competitively inhibits the enzyme HMG CoA reductase, prevents the conversion of 3-hydroxy-3-methylglutaryl CoA to mevalonate and the production of cholesterol and its circulating blood derivatives, including LDL [3]. ROC is a poorly water-soluble drug with only 20% oral bioavailability. It is classified by the biopharmaceutical classification system as a class II drug [4]. The poor solubility of ROC affects its dissolution rate which in turn, its bioavailability. Thus, enhancing the dissolution of ROC can lead to improving its oral bioavailability.

Drugs with poor solubility possess difficulty in the formulation by applying conventional approaches as they present problems such as the slow onset of action, poor oral bioavailability, lack of dose proportionality, failure to achieve steady-state plasma concentration, and undesirable side effects. Nanotechnology is a promising strategy in the development of drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility, low permeability, inadequate bioavailability, and other poor biopharmaceutical properties [5,6]. Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids, and polysaccharides have been developed and tested over the past decades. Nowadays, polymeric nanoparticles prepared by natural biopolymers such as chitosan and alginate are of interest in the drug delivery system due to their biodegradability, biocompatibility, and safe [7,8]. Alginate is a natural anionic polymer derived from brown algae and its chemical structure is composed of α-L-guluronic acid and β-D-mannuronic acid. It can form nanoparticles by ionotropic gelation with divalent cations or cationic polymers [9], but these nanoparticles are not stable at room temperature [10] and encapsulated active compounds easily leak from the nanoparticles [11]. Mujtaba et al. [8] and Lertsutthiwong et al. [12] overcame these limitations by coating the alginate nanoparticles with chitosan, a cationic polysaccharide produced by deacetylation of chitin. Additionally, chitosan has been reported to possess the property of reducing cholesterol levels, which may make chitosan as an attractive polymer in the development of drug delivery systems for the treatment of cardiac diseases [13,14].

Recently, nanoparticle-based drug delivery systems have emerged as a solution for increasing the bioavailability of potential therapeutic agents. Therefore, the aim of present work is to develop and optimize the chitosan-alginate nanoparticulate formulation of ROC which can improve its solubility, dissolution and therapeutic efficacy.

2. MATERIALS AND METHODS

2.1 Materials

Rosuvastatin calcium (ROC) was obtained from Jamjoom Pharmaceutical Co. Ltd. (Jeddah, Kingdom of Saudi Arabia) as a gift sample. Chitosan with medium molecular weight and degree of deacetylation about 85% were purchased from Sigma–Aldrich, St. Louis, MO, USA. The medium viscosity sodium alginate isolated from Macrocystis pyrifera, having a molecular weight between 75 and 100 kDa, and mannuronic to guluronic acid ratio of 1.5 (60:40), was purchased from CDH Labs., India. All other reagents and chemicals used were of analytical reagent grade.
2.2 Preparation of Blank Chitosan-alginate Nanoparticles (CANPs)

Accurately weight 20 mg sodium alginate and dissolved in 10 mL distilled water to form a 0.2% (w/v) sodium alginate solution. This solution was adjusted to a pH of 5, and then it was added dropwise to 10 mL of 0.2% (w/v) calcium chloride solution under magnetic stirring at room temperature. 50 mg of chitosan was dissolved in 50 mL of 1% (v/v) acetic acid solution to obtain a 0.1% (w/v) chitosan solution. After the pH of the chitosan solution was adjusted to 5, it was incorporated into the resultant calcium alginate pre-gel and the mixture solution was further stirred for 1 h. The mixture was then subjected to probe sonication (Vibra Cell, Sonics & Materials, Inc., USA) for 15 min at 20°C of probe temperature in a pulsatile manner (50 s sonication with 10 s pause) with 30% amplitude. After probe sonication, the resulting opalescent suspension was equilibrated overnight to allow nanoparticles to form uniform particle size.

2.3 Preparation of ROC-loaded CANPs

Accurately, 2 mL of 1 mg/mL ROC solution was added dropwise to 10 mL of 2 mg/mL alginate and stir for 30 min. The above solution was then added dropwise to 10 mL of 2 mg/mL calcium chloride solution followed by a continuous stirring of 30 min. Afterward 10 mL of 1 mg/mL chitosan stock solution was added to the resultant alginate solution and stir for another 60 min. The mixture was then subjected to probe sonication for 15 min at 20°C of probe temperature in a pulsatile manner (50 s sonication with 10 s pause) with 30% amplitude. After probe sonication, the resulting opalescent suspension was equilibrated overnight to allow nanoparticles to form uniform particle size.

2.4 Characterization of CANPs

2.4.1 Nanoparticles size and surface charge

The diameter of the nanoparticles (z-average), polydispersity index (PDI) and zeta potential was measured for the formulation using dynamic light scattering zetasizer (PSS NICOMP Z3000, Port Richey, FL). The nanoparticle dispersions were diluted with distilled water before the measurement to avoid multi scattering phenomenon. All the measurements were performed in triplicates. Dynamic light scattering (DLS) is used to measure the average nanoparticle size and size distribution. This technique measures time-dependent fluctuations in the intensity of scattered light as a result of Brownian motion exhibited by the particles. The zeta potential is considered as one of the highest measured parameters which denotes the overall charges acquired by the particles in a particular medium and it is considered as one of the important factors for the stability of the nanoparticles [15].

2.4.2 Fourier transform infrared spectroscopy (FT-IR)

CANPs separated from nanoparticulate suspensions were dried by a freeze dryer, and their FT-IR transmission spectra was obtained using a FT-IR spectrophotometer (FTIR-7600, Angstrom Advanced Inc. USA). A total of 2% (w/w) of the sample, with respect to the KBr disc, were mixed with dry KBr. The mixture was ground into a fine powder using an agate mortar before compressing into KBr disc under a hydraulic press at 10,000 psi. Each KBr disc was scanned at 4 mm/s at a resolution of 2 cm over a wavenumber region of 400–4000 cm\(^{-1}\) using IR solution software. The characteristic peaks were recorded for different samples. The interaction between ROC and formulation excipients was investigated by FT-IR spectrometry.

2.4.3 Determination of encapsulation efficiency of CANPs

The encapsulation efficiency (EE) of nanoparticles was determined by the separation of drug-loaded nanoparticles from the aqueous medium containing non-associated ROC by ultracentrifugation at 18,000 rpm at 4°C for 30 min. The amount of ROC loaded into the nanoparticles was calculated as the difference between the total amount used to prepare the nanoparticles and the amount that was found in the supernatant. The amount of free ROC in the supernatant was measured by a validated UV-vis spectrophotometric method. The ROC encapsulation efficiency (EE) of the nanoparticles was determined in triplicate and calculated as follows [16]:

\[
\text{Encapsulation efficiency} = \frac{\text{Total amount of initial ROC} - \text{Total amount of ROC in the supernatant}}{\text{Total amount of initial ROC}} \tag{1}
\]
**2.4.4 In vitro drug release characteristics**

The release profiles of ROC from the ROC-loaded CANPs were determined by dialysis bag diffusion techniques [17]. The release media were taken as simulated body fluid (SBF, pH 7.4) was taken as release media under sink conditions for ROC. ROC-loaded CANPs suspension (20 mL) prepared under optimal conditions and pure drug ROC at a concentration equivalent to that in the nanoparticles will be loaded into dialysis bags with a molecular weight of 35 kDa (Fisher Scientific, USA) and suspended in 150 mL of release medium at 37°C with a stirring rate of 150 rpm. At time intervals, 0.2 mL of the release medium was taken and immediately replaced by a fresh release medium. The amount of drug in the samples was determined using a UV-VIS spectrophotometer. All experiments were performed in triplicate.

**3. RESULTS AND DISCUSSION**

The main effort of this work was to develop a nano delivery system for the drug ROC using natural, biodegradable, biocompatible polysaccharides especially chitosan and sodium alginate. These nanoparticles are obtained spontaneously under very mild conditions. The preparation of CANPs systems, based on an ionotropic gelation process, involves mixing the two aqueous phases at room temperature. The ionotropic gelation process did not include the emulsification step and stage of organic solvents, thereby minimizing the inactivation of encapsulated drugs [7]. In aqueous solutions, at pH around 4 to 5.5 the amine groups on chitosan became easily positively charged. On the other hand, alginate dissolved in a neutral pH solution where the carboxylate groups were negatively charged. In aqueous solutions with pH around 5.2 chitosan amino groups interacted with alginate carboxylate groups to form the hydrogel. The formulation, which gave an opalescent suspension with a positive zeta potential, was selected as the optimum ratio for the ROC encapsulation.

The DLS analysis revealed that the blank CANPs and ROC loaded CANPs have a mean hydrodynamic diameter of 299.1 nm and 349.3 nm as shown in Fig. 1(A) and (B), respectively. DLS measures the apparent size (hydrodynamic radius) of a particle, including hydrodynamic layers that are formed around hydrophilic particles, leading to an overestimation of nanoparticles size. Hence, the discrepancy in the size of nanoparticles may be due to the fact that the DLS method gives the hydrodynamic diameter rather than the actual diameter of hydrophilic nanoparticles. The PDI value of blank CANPs was 0.493 while that of ROC loaded CANPs was 0.446, thus indicating a narrow and favorable particle size distribution [18]. Zeta potential is quite important for colloids and nanoparticles in suspension. Its value is closely related to suspension stability and particle surface morphology. Zeta potential of the optimized ROC loaded CANPs was found to be +29.1 mV. The zeta potentials of ROC loaded CANPs show large positive values reflecting the stability of the colloidal suspensions. Having positively charged surfaces is an added advantage when using nanoparticles in drug delivery since they are able to transfer easily through negative channels in the cell membrane [19].

FT-IR was adopted to characterize the potential interactions in the NPs and successful loading of ROC into the NPs. FT-IR spectra of alginate, chitosan, ROC and ROC loaded CANPs are shown in Fig. 2. In the spectra of pure chitosan, characteristic peaks at 3449 cm⁻¹ for −NH₂ and −OH stretching, 1659 cm⁻¹ for −CO stretching, and 1596 cm⁻¹ for −NH₂ bending vibrations were observed [20]. The bands around 1079 cm⁻¹ (C-O-C stretching) presenting in the FT-IR spectrum of sodium alginate are attributed to its saccharide structure. In addition, the bands at 1662 and 1400 cm⁻¹ are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups [21]. The characteristic IR absorption peaks of free ROC were present at 3341.5 cm⁻¹, 2972.2 cm⁻¹ and 1425.8 cm⁻¹ correspondings to cyclic amines, CH stretching, C=O stretching, O-H bending. These were similar to what has been previously reported in the monograph for rosuvastatin [22]. The characteristic absorption band of ROC appeared in the ROC loaded CANPs, which probably indicate that the ROC molecule was filled in the polymeric network. These results indicate that the carboxylic groups of alginate associated with ammonium groups of chitosan through electrostatic interactions to form the polyelectrolyte complex. In other words, the characteristic peaks of ROC were not altered in their position after successful encapsulation in the CANPs. Thus, it can be concluded that there is no chemical interaction between the ROC and CANPs upon the encapsulation of ROC within the NPs. The % EE of CANPs was 83.65% ± 1.53 (n = 3). The % EE acts as an important factor influencing the drug release, as well as the
overall efficacy of the production process. The CANPs protect the encapsulant, have biocompatible and biodegradable characteristics, and limit the release of encapsulated materials more effectively than either alginate or chitosan alone [8].

![Fig. 1. Particle size distribution of (A) blank chitosan-alginate nanoparticles (B) ROC loaded chitosan-alginate nanoparticles](image1)

![Fig. 2. FTIR spectra of chitosan, alginate, drug loaded chitosan-alginate nanoparticles and rosvastatin](image2)
The in vitro release profiles of optimized ROC loaded CANPs formulations in phosphate-buffered saline (PBS) solution (pH 7.4) are shown in Fig. 3. It shows the initial burst release of ROC from NPs in PBS solution which can be observed up to 2 h. This accounts for about 45% of ROC from the total encapsulated amount. Then, it followed a more gradual and sustained release phase for the next 24 h. Drug release from CANPs was significantly rapid as well as complete than the pure ROC drug. The initial fast release of ROC may be due to the rapid hydration of nanoparticles because of the hydrophilic nature of chitosan and alginate. The release medium penetrates into the particles and dissolves the entrapped drug; therefore, it can be proposed that the major factor determining the drug release from nanoparticle is its solubilization or dissolution rate in the release medium.

4. CONCLUSION

Preparation of ROC loaded CANPs was successfully carried out through the ionotropic gelation and polyelectrolyte complexation method to obtain particles with the highest payload potential (83.65%) at the smallest size (349.3 nm). The dispersion of ROC in aqueous alginate with calcium chloride as a crosslinking agent created hydrogel nanoparticles with chitosan added for structural support. This occurs due to electrostatic interactions among carboxylate groups on alginate and positively charged calcium ions and protonated amine groups on chitosan. Finally, the in vitro release profile observed for these nanoparticles was characterized by an initial fast release followed by a controlled release phase. In conclusion, this new nanosystem also offers an interesting potential for the delivery of hydrophobic compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the approval and the support of this research study by the grant no. 7681-PHM-2018-3-9-F from the Deanship of Scientific Research at Northern Border University, Arar, K.S.A.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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