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Simvastatin loaded composite polyspheres of gellan gum and carrageenan: *In vitro* and *in vivo* evaluation

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ABSTRACT

We investigated the lipid lowering ability of simvastatin loaded gellan gum–carrageenan composite polyspheres, which were prepared by ionotropic gelation/covalent crosslinking method. The surface morphology revealed that the polyspheres have rough and dense surface. The drug entrapment efficiency of the polyspheres prepared by ionic crosslinking was higher than those prepared by dual crosslinking. The *in vitro* drug release study indicated that the ionically crosslinked polyspheres discharged the drug quickly whereas, dual crosslinked polyspheres extended the drug release for longer period. The hypolipidemic activity performed on Wistar rats indicated that the polyspheres have effectively reduced the elevated total serum cholesterol and triglycerides.

1. Introduction

Multi-particulate matrices (designated as "polyspheres" herein) are oral dosage forms containing a number of smaller matrices with diameter ranging from 0.05 to 2 mm, which exhibit desired properties for drug delivery applications [1]. For administration of the recommended dose, these polyspheres may be filled into a sachet and encapsulated or compressed into a tablet [2]. Polyspheres are the discrete particles that make up a multiple unit system and provide many advantages over single-unit systems because of their small size. Polyspheres are less dependent on gastric emptying, thus resulting in less inter and intra-subject variability in gastrointestinal transit time [3]. Recently, much importance is being laid on the development of multiparticulate dosage forms than the single unit dosage form, because of their benefits such as increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying [4]. The drug safety can also be increased using multiparticulate dosage forms, particularly for modified release systems [5,6]. Several natural and synthetic polymers are being used for development of polyspheres, but use of natural polysaccharides is the focused area of research in multiparticulate drug delivery.

Gellan gum (GG) is an exocellular natural anionic heteropolysaccharide produced by aerobic fermentation of the bacterium *Sphingomonas elodea* [7]. The gelation of gellan gum can be induced by cations as well as temperature [8,9]. GG solution becomes gel in the presence of mono and divalent cations, but, its affinity for divalent cations such as Ca^{2+} and Mg^{2+} is stronger than the monovalent ions such as Na^{+} and K^{+} [10]. In the literature, GG has been reported in the development of ophthalmic drug delivery [11], oral sustained/controlled delivery systems [12–15] and floating *in situ* gelling system [16]. Carrageenan (CRG) is a high molecular weight anionic heteropolysaccharide obtained from marine algae, *Rhodophyceae*. It is a sulfate ester of galactose and 3,6-anhydrogalactose copolymers, linked by alternating α-1,3 and β-1,4 glycosidic linkages [17]. CRG has been used in the development of drug delivery systems [18–20].

Simvastatin (SMT) is a lipid-lowering drug, used in the treatment of hypercholesterolemia. It decreases cholesterol levels by inhibiting the HMG-CoA reductase, the enzyme that reduces the biosynthesis of cholesterol. After oral administration, it shows less than 5% bioavailability and plasma half life of about 1.9 h [21]. Hence, there is a need for the development of controlled release dosage form, which could avoid the repeated dosing.

Therefore, the objective of the present work was to develop the composite polyspheres of GG-CRG for controlled release of SMT and to evaluate the lipid lowering ability of the prepared polyspheres in Wistar rats. The prepared polyspheres were characterized by

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Table 1
The formulation details of simvastatin loaded polyspheres.

Formulation codes	Gellan gum (%, w/v)	Carrageenan (%, w/v)	Simvastatin (%, w/w of polymer)	Zinc sulfate (%, w/v)	Glutaraldehyde (%, w/w of polymer)
GC1	0.50	1.00	20	5	–
GC2	0.75	0.75	20	5	–
GC3	1.00	0.50	20	5	–
GC4	1.00	0.50	20	10	–
GC5	1.00	0.50	20	15	–
GC6	1.00	0.50	40	15	–
GC7	0.50	1.00	20	5	5
GC8	0.75	0.75	20	5	5
GC9	1.00	0.50	20	5	5
GC10	1.00	0.50	20	5	10
GC11	1.00	0.50	40	5	10

Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray diffraction (X-RD) studies and scanning electron microscopy (SEM).

2. Materials and methods

Simvastatin (SMT) was obtained as gift sample from Strides Arco lab Ltd. (Bangalore, India). κ-Carrageenan (CRG) was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Gellan gum (GG) was purchased from Ozone International (Mumbai, India). Glutaraldehyde (GA; 25%, v/v) was procured from S.D Fine Chemicals (Mumbai, India). Zinc sulfate ($ZnSO_4$) was procured from New Modern Chemical Corporation (Mumbai, India). Cholesterol and triglyceride kits were purchased from Transasia Bio-Medicals Ltd. (Baddi, India). Double distilled water was used throughout the study. All other chemicals were used without further purification.

Male Wistar rats weighing between 150 and 250 g were used for the study. The animals were acclimatized to laboratory conditions for five days before the experiments, with adequate food and water *ad libitum*. Animal experimental protocols were approved by our institutional ethics committee which follows the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

2.1. Preparation of polyspheres

Drug–polymer dispersion was prepared by mixing the accurately weighed quantities of GG, CRG and SMT with double distilled water using a magnetic stirrer. Twenty milliliters of the dispersion was taken into 25 cc disposable syringe fixed with # 23 needle and added dropwise into an aqueous solution of $ZnSO_4$ under constant stirring. For hardening, the polyspheres were left in the in the $ZnSO_4$ solution for additional 15 min and then separated from $ZnSO_4$ solution; dried at 40 °C for 12 h. Further, the polyspheres were dual crosslinked by placing in a solution containing different concentrations of glutaraldehyde (GA) and 1 N HCl for 30 min at 50 °C. After which they were washed with distilled water repeatedly to remove the unreacted GA. The polyspheres were dried at 50 °C for 2 h and stored in a closed container fro further evaluation. The ingredients used for the preparation of polyspheres are summarized in Table 1.

2.2. Scanning electron microscopic (SEM) analysis

The shape and surface morphology of the polyspheres was examined by using SEM analysis. The photographs were taken by placing the polyspheres on stub of the instrument using double sided adhesive tape; further polyspheres were sputter coated with platinum with the help of sputter coater (Edward S 150, UK). The coated polyspheres were observed under SEM (JEOL, JSM-6360, Kyoto, Japan) at the required magnification at room temperature with the secondary electron image as a detector and 20 kV acceleration voltage.

2.3. Measurement of size

The average diameter of the polyspheres of was determined with the help of a digital micrometer (MDC-25S Mitutoyo, Tokyo, Japan) with an accuracy of 0.001 mm. A total of 100 polyspheres per batch were measured and average size was calculated.

2.4. Estimation of drug entrapment efficiency (DEE)

The DEE of the polyspheres was determined by swelling method. Accurately weighed quantities of polyspheres were soaked in 100 ml of phosphate buffer (pH 7.4) for complete swelling at 37 °C. Then the polyspheres were crushed and the solution was gently heated for 2 h and centrifuged to separate the polymeric debris. The supernatant solution was analyzed for the drug content using UV-vis spectrophotometer (Model Pharmaspec UV-1700, Shimadzu, Japan) at 238 nm. The DEE was calculated using the following equation:

$$\text{Drug entrapment efficiency} = \frac{\text{experimental drug content}}{\text{theoretical drug content}} \times 100 \quad (1)$$

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the samples were recorded using FTIR spectrophotometer (8400S, Shimadzu, Japan). The samples were crushed with potassium bromide to get thin pellets under a pressure of 600 kg and were scanned between 450 and 4000 cm^{-1}.

2.6. Differential scanning calorimetric (DSC) analysis

The DSC analysis of SMT, drug-free GC6 polyspheres and drug-loaded GC6 polyspheres was carried out using a microcalorimeter (DSC Q20 V24.4 Build 116, TA Instruments, USA) and thermograms were obtained. The samples were heated in the temperature of 0–300 °C at a heating rate of 10 °C/min under argon atmosphere.

2.7. Thermogravimetric analysis (TGA)

TGA was performed on GC3 and GC9 polyspheres using a microcalorimeter (DSC Q20 V24.4 Build 116, TA Instruments, USA) under a dynamic argon atmosphere flowing at a rate of 50 ml/min and at a heating rate of 10 °C/min. in the temperature range 0–600 °C.