International Journal of Biological Macromolecules 53 (2013) 114–121

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Evaluation of carboxymethyl gellan gum as a mucoadhesive polymer

Munish Ahuja*, Seema Singh, Ashok Kumar

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125 001, India

ARTICLE INFO

Article history:
Received 18 August 2012
Received in revised form 22 October 2012
Accepted 28 October 2012
Available online 21 November 2012

Keywords:
Carboxymethyl gellan
Mucoadhesion
Cytotoxicity
HET-CAM

ABSTRACT

The study was conducted to evaluate carboxymethyl gellan gum as bioadhesive polymer for drug delivery applications. Gellan gum was carboxymethylated by reacting it with monochloroacetic acid. Degree of carboxymethyl substitution was found to be 1.18. Further, carboxymethylation of gellan gum was found to increase its degree of crystallinity, surface roughness and diminish the cation-induced gelation. On comparative evaluation carboxymethyl gellan gum showed 2.71-fold higher mucoadhesive strength than gellan gum. Evaluation of *ex vivo* ocular tolerance using chorioallantoic membrane of hen's egg and cytotoxicity screening on Vero cells using resazurin assay revealed that caroboxymethyl gellan gum is non-irritant and biocompatible. Ionotiropically gelled beads of carboxymethyl gellan gum formulated using metformin as the model drug and calcium chloride as the cross-linking agent showed *ex vivo* bioadhesion of 100% over 24 h. Further, it was observed that carboxymethyl gellan gum beads released metformin at a rate faster than gellan gum.

1. Introduction

Natural polymers find extensive applications in food and pharmaceutical industry because of their easy availability, biocompatibility, biodegradability and cost effectiveness. To improve their functional properties a number of physical modification approaches such as microfluidization [1], extrusion [2], freeze–thaw cycling [3] and chemical modification approaches such as graft co-polymerization [4,5], oxidation [6], thiolation [7], and carboxymethylation [8], have been employed. Among the various chemical modification approaches, carboxymethylation is widely used because of its ease of processing, lower cost and versatility. Carboxymethylation of polysaccharides have earlier been done to improve their aqueous solubility and gelling behaviour.

Gellan gum (GG) is an anionic exopolysaccharide secreted by the microorganism, *Pseudomonas elodea*. It is a linear polysaccharide comprising of repeating tetrasaccharide unit of glucuronic acid, rhamnose and glucose residues in a molar ratio of 1:1:2. It possesses the characteristic property of undergoing ionic gelation in the presence of mono- and divalent-cations [9]. It has been employed in pharmaceutical applications as *in situ* gelling agent in ophthalmic formulations [10] and as a sustained release matrix in bead formulation [11]. Gellan gum has earlier been chemically modified by thiolation [12], carbamoylation [13] and carboxymethylation [14]. Carboxymethylation of gellan gum was reported to improve its aqueous solubility and gelling behaviour. It was observed that carboxymethylated gellan gum does not gel at 0 °C even at the concentration of 10% (w/v). However, there are no literature reports on further use of carboxymethyl gellan gum (CMGG) in pharmaceutical applications. Recently it was reported that carboxymethylation improves the bioadhesive properties of natural polymers [8].

Thus considering the same, the present study was designed with the objective of evaluating CMGG as a mucoadhesive polymer for pharmaceutical applications. CMGG was synthesized and characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscopy (SEM) studies. Further, the effect of calcium ions on gelling behaviour of CMGG was studied. Mucoadhesive potential of CMGG was evaluated by texture profile analysis. CMGG was comparatively evaluated with GG for *ex vivo* ocular tolerance by Hen's egg test-chorioallantoic membrane (HET-CAM) and for cytotoxicity by resazurin assay. The mucoadhesive applications of CMGG were explored by formulating ionotropically gelled beads employing metformin as a model drug.

2. Materials and methods

2.1. Materials

Gellan gum (Gelrite®, CP Kelcogel, UK) and metformin samples were gifted by Burzin Leons Argenturon (Mumbai, India) and GMH Laboratories Pvt. Ltd. (Baddi, India), respectively. Monochloroacetic acid was procured from Hi-Media Lab. Pvt. Ltd. (Mumbai, India). Sodium hydroxide, methanol and glacial acetic acid were obtained from Sisco Research Laboratory (Mumbai,

* Corresponding author. Tel.: +91 1662 263515; fax: +91 1662 276240.
E-mail address: munishahuja17@yahoo.co.in (M. Ahuja).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.10.033

India). All other chemicals used were of analytical grade. Freshly excised chicken ileum was obtained from local slaughter house (Hisar, India).

2.2. Synthesis of carboxymethyl gellan gum (CMGG)

CMGG was synthesized from gellan gum as per the synthetic procedure reported by Miyamoto et al. [14]. Briefly, an aqueous dispersion of GG (1.25%, w/v) in ice cold sodium hydroxide (45%, w/w) was prepared by stirring for 30 min. To this 25 mL of aqueous solution of monochloroacetic acid (45%, w/v) was added with constant stirring. The reaction mixture was then heated to 70 °C under constant stirring for 30 min, cooled and suspended into (80%, v/v) methanol. Precipitates of CMGG so formed were filtered and neutralized with glacial acetic acid, followed by washing with 3 × 60 mL portions of methanol (80%, v/v), filtration and drying in an oven at 40 °C.

2.3. Characterization of CMGG

2.3.1. Fourier transform infra-red spectroscopy (FT-IR)

GG and CMGG samples were subjected to FT-IR spectroscopy in a Fourier-transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan) in range of 4500–400 cm^{-1} as KBr pellets.

2.3.2. Differential scanning calorimetry (DSC)

DSC thermograms of GG and CMGG samples were recorded using a differential scanning calorimeter (Q_{10} V9.0 Build 275, TA Systems, USA). About 7–8 mg of sample were crimped in a standard aluminium pan and heated in a temperature range of 40–250 °C at a heating rate of 10 °C per min with nitrogen purge of 50 mL/min.

2.3.3. X-ray diffraction

Powder X-ray diffraction pattern of GG and CMGG were recorded employing X-ray diffractometer (Table top XRD, Miniflex 2, Rigaku, Japan). The sample powders were scanned from 0° to 80° diffraction angle (2θ) range under the following measurement conditions: source, nickel filtered Cu-Kα radiation; voltage 30 kV; current 15 mA; scan speed 0.05 min^{-1}; division slit 1.25°; receiving slit 0.3 mm.

2.3.4. Scanning electron microscopy (SEM)

Scanning electron micrographs of GG and CMGG samples were taken using SEM (JEOL, JSM-6100). These were coated with gold and mounted in a sample holder. The photomicrographs of samples were taken at an accelerating voltage at 5 kV at different magnifications.

2.3.5. Determination of degree of substitution

The degree of substitution was determined by classical acid-wash method [15]. In brief, freshly precipitated CMGG (1.5 g) was dispersed in hydrochloric acid reagent (20 mL) for 3–4 h, followed by filtration and washing with 70% methanol to remove the acid followed by drying to constant weight in an oven at 70 °C.

The dried CMGG, so obtained, was well dispersed in 70% methanol followed by addition of excess of sodium hydroxide (0.5 N) with stirring for 3 h to dissolve the sample completely. The excess of sodium hydroxide was back titrated with hydrochloric acid (0.5 N) using phenolphthalein as an indicator. The degree of carboxymethyl substitution (DS) on GG was calculated using the following equation:

$$DS = \frac{0.162A}{1 - 0.058A} \quad (1)$$

where A is the milliequivalents of sodium hydroxide required per g of the CMGG sample.

2.3.6. Effect of cations on gelling behaviour

The effect of cation concentration on gelling behaviour of GG and CMGG was investigated by plotting partial ternary phase diagram. Aqueous solutions of GG (0.2–2.0%, w/v) and CMGG (0.5–3.0%, w/v) in sodium hydroxide (1%, w/v) were prepared and to them appropriate amount of $CaCl_2$ solution (0.2–2.0%, w/v) for GG and (1–3.5%, w/v) for CMGG was added and left overnight to equilibrate. Solutions were assessed visually for their appearance and flow by tilting the test tubes to an angle of 90°, and were categorized as solutions, precipitate, viscous solutions or gels [16].

2.4. Evaluation of mucoadhesive potential of CMGG

Mucoadhesive potential of CMGG was assessed and comparatively evaluated with GG by determining the force of detachment of their polymer compacts with the mucin-coated model membrane using texture analyzer. Polymer compacts were prepared by compressing 200 mg of polymers in IR hydraulic press (KP 795, Kimaya Engineers, Thane, India) using 13 mm die at a pressure of 5 ton for 1 min. The mucoadhesive strength was measured in texture analyzer (TAX_2, Stable Microsystem, UK), equipped with 5 kg load cell. Polymer compacts were attached to the upper probe, while the model membrane comprising of a cellophane membrane hydrated with mucin dispersion (0.3%, w/v) was attached to the lower probe. The upper probe was lowered at a rate of 0.1 mm/s until it contacts the model membrane followed by application of constant force of 0.25 N for 300 s. The upper probe was then withdrawn at a rate of 0.1 mm/s and the force required to detach the polymer compact from the model membrane was taken as the indicator of mucoadhesive performance.

2.5. HET-CAM study

Ex vivo ocular tolerance of gellan gum with CMGG and control solutions of irritant (NaOH) and non-irritant (NaCl) was assessed employing hen's egg test on chorioallantoic membrane (HET-CAM) assay [17]. Ten-day old fertilized hen's eggs were candled with an illuminating lamp and eggs with an air sac and live embryo were used for further testing. Egg shells were then opened and carefully removed the membrane without injuring any blood vessel using tapered forceps. Eggs which got damaged were rejected. GG and CMGG aliquots of 0.5 mL were applied over CAM in triplicates and observed for next 5 min for the signs of irritation such as haemorrhage, vasoconstriction and coagulation [18]. The time of appearance of irritation was recorded and potential irritation (PI) score were calculated using the formula:

$$PI = \frac{(301 - h) \times 5}{300} + \frac{(301 - \nu) \times 7}{300} + \frac{(301 - c) \times 9}{300} \quad (2)$$

where h = appearance time in seconds of haemorrhage, ν = appearance time in seconds of vasoconstriction, c = appearance time in seconds of coagulation [17]. On the basis of PI values, irritation was recorded as 0–0.9, non irritant; 1–4.9, slight irritation; 5–8.9, moderate irritation; 9–21, severe irritation.

2.6. Cytotoxicity screening

CMGG was screened comparatively for cytotoxicity with GG employing resazurin assay method. The resazurin assay is based on measuring the metabolic activity of living cells by determining the concentration of resorufin. Resorufin is a pink fluorescent compound produced by reduction of resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) by viable cells. The Vero cells were seeded in 96 well plate in a density of 10^5 cells in Dulbecco's Modified Eagle's Medium (DMEM) containing 5% Foetal Bovine Serum (FBS) and incubated for 24 h at 37 °C in 5% CO_2 humidified