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Calcium alginate-carboxymethyl cellulose beads for colon-targeted drug delivery

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ABSTRACT

The present study delineates preparation, characterization and application of calcium alginate (CA)-carboxymethyl cellulose (CMC) beads for colon-specific oral drug delivery. Here, we exploited pH responsive swelling, mucoadhesivity and colonic microflora-catered biodegradability of the formulations for colon-specific drug delivery. The CA-CMC beads were prepared by ionic gelation method and its physicochemical characterization was done by SEM, XRD, EDAX, DSC and texture analyzer. The swelling and mucoadhesivity of the beads was found higher at the simulated colonic environment. Variation was more prominent in compositions with lower CMC concentrations. CA-CMC formulations degraded slowly in simulated colonic fluid, however the degradation rate increased drastically in the presence of colonic microflora. In vitro release study of anticancer drug 5-fluorouracil (5-FU) showed a release (>90%) in the presence of colonic enzymes. A critical analysis of drug release profile along with FRAP (fluorescence recovery after photobleaching) study revealed that the presence of CMC in the formulation retarded the release rate of 5-FU. 5-FU-loaded formulations were tested against colon adenocarcinoma cells (HT-29). Cytotoxicity data, nuclear condensation-fragmentation and apoptosis analysis (by flow cytometry) together confirmed the therapeutic potential of the CA-CMC formulations. In conclusion, CA-CMC beads can be used for colon-specific drug delivery.

1. Introduction

The colon-targeted oral drug delivery is desirable in order to treat a variety of colon diseases, such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, etc. [1–6]. In recent years, there have been a number of developments for the improvement of target specificity of colon-targeted delivery systems [7]. The primary approaches pertaining to the colon-specific delivery include: (i) covalent linkage of a drug with polymers as a prodrug, (ii) coating of the delivery system with the pH-sensitive polymers (e.g. Eudragit polymers), bioadhesive polymers (e.g. polycarbophil-based polymers) or biodegradable polymers and (iii) microbially triggered release of the drug. In addition, some of the novel drug-delivery approaches have also been introduced such as: (i) pressure-controlled drug delivery, (ii) CODESTM (combined approach of pH-dependent and microbially triggered drug delivery), (iii) osmotic pressure-controlled drug delivery through a semipermeable membrane and (iv) multiparticulate systems like microspheres and nanoparticles [1]. However, in a recent review, Talaei et al. [8] highlighted that although the novel drug-delivery systems have shown good potential, yet further improvements are needed before their full translation into clinical use.

Calcium alginate (CA) and carboxymethyl cellulose (CMC) are two biopolymers that can be used for developing oral drug-delivery systems. Alginate (salts of alginic acid) is a linear polysaccharide composed of alternating blocks of β (1→4) linked d-mannuronic acid and α (1→4) linked L-guluronic acid residues [9–14], whereas CMC consists of linear chains containing β (1→4)-linked glucopyranose residues [15]. These biopolymers have been reported to show a pH-dependent swelling behavior [13,16–18]. Both the polymers are anionic in nature due to the presence of negatively charged

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Table 1
Composition of the CA-CMC beads.

S. No.	Samples[a]	Polymer concentrations (wt%)		Glutaraldehyde concentration (%)
		Sodium Alginate	Carboxymethyl Cellulose	
1	T1G	1.8	–	0.25
2	T3G	1.8	0.5	0.25
3	T5G	1.8	1	0.25

[a] G stands for glutaraldehyde crosslinked beads.

carboxyl groups at pH > 5. These negative charges allow the polymer to shrink in the acidic pH and to swell when they are exposed to neutral or basic pH. These properties make these polymers suitable for applications in the design of oral drug-delivery systems. Apart from the pH sensitivity, SA and CMC have also been known to possess an excellent mucoadhesive property [19].

Keeping the aforesaid perspective in mind, here we have explored the potential of calcium alginate (CA)-carboxymethyl cellulose (CMC) bead as a colon-specific drug-delivery system. We hypothesize that an appropriate composition of CA-CMC will ensure: (1) least amount of drug release at non-specific sites (stomach and small intestine) during its transit through the GI tract, (2) higher adhesion to the colonic mucosa in comparison to other parts of the GI tract and (3) controlled degradation of the formulations by the colonic microflora. These factors are expected to promote sustained release of the drug in the colon. The rationale behind such ventures is: (i) to ensure the appropriate therapeutic dose at colon for effective treatment, (ii) to avoid dose and activity loss of the therapeutics during the GI transit and (iii) to minimize the adverse side effects of the therapeutics caused from absorption at non-specific tissue locations. 5-Fluorouracil (5-FU) was taken as the reference drug in this study.

2. Materials and Methods

2.1. Materials

Sodium alginate (SA) (molecular weight: 7.72×10^4 g/mol, degree of polymerization: 476, M/G ratio 1.08) was bought from SDFCL, Mumbai, India. Calcium chloride ($CaCl_2$, fused) was purchased from MERCK, Mumbai, India. Glutaraldehyde (25% aqueous solution) was procured from LOBA Chemie, Mumbai, India. Carboxymethyl cellulose sodium (CMC) salt (molecular weight: 6.62×10^5 g/mol, degree of polymerization: 3062, degree of substitution: 0.68), MaCoy's 5A media, Dulbecco's phosphate-buffered saline (DPBS), trypsin-EDTA solution, fetal bovine serum, antibiotic-antimycotic solution, MTT assay kit and nutrient broth were purchased from Himedia, Mumbai, India. HT29 adenocarcinoma cell line was procured from NCCS, Pune. 5-FU and FITC-Dextran (molecular weight: 10 kDa) were obtained from Sigma-Aldrich, Mumbai.

2.2. Methods

2.2.1. Preparation of CA-CMC beads

Preparation of the CA-CMC beads was done by ionic gelation method as described by Girhepunje et al. [20]. Both SA and CMC were dissolved in deionized water at a specific concentration (Table 1). Thereafter, the prepared polymeric solution was extruded as droplets using a syringe (28G) and poured into 2% calcium chloride (w/v) solution under constant stirring at 80 rpm and 37 °C and cured for 10 min. Then, 1.1 ml of glutaraldehyde reagent [glutaraldehyde (25%, 0.5 ml) + ethanol (0.5 ml) + HCl (0.1 N, 100 μl)] was added to 50 ml of calcium chloride solution and bead curing was done for another 10 min. The beads were washed with deionized water, neutralized with glycine and dried overnight at 40 °C. To determine the average size of the swollen and dried beads, images were taken using the camera (Canon A2400 IS) and the images were analyzed by NIH ImageJ software.

2.2.2. Physicochemical characterization of the beads

Morphological characterization of the dried beads was carried out using scanning electron microscopy (JOEL India JSM-6480Lv) at 15 kV after platinum sputter coating. The calcium content of the beads was analyzed by energy-dispersive X-ray spectroscopy. Variation in percentage crystallinity of CA-CMC beads were recorded using X-ray diffraction (Philips XRD-PW1700 diffractometer). Scanning was done in the range of 5–60° 2θ with a step size of 0.02°/s using monochromatic Cu Kα radiation of wavelength (λ = 1.514 Å). Differential scanning calorimetry analysis was carried out by heating 20 mg of CA-CMC beads from 35 to 250 °C, at a rate of 5 °C/min using DSC-200-F3 MAIA instrument (Netzsch, Germany). Bulk compressive strength of the CA-CMC beads was analyzed by TA.XT2i Texture analyzer (Stable Micro Systems Ltd, Surrey, UK). The analysis was done using 30 mm probe, 1 mm/s test speed and auto (force) mode (5 g, 5 mm).

2.2.3. Swelling analysis

Swelling of the beads in simulated GI fluids was studied following the protocol described by Pasparakis et al. [21]. For this, accurately weighed, dried CA-CMC beads were immersed in phosphate-buffered saline (PBS; pH 7.4 and 6.8) and in 0.1 N HCl (pH 1.2) at 37 °C. At defined time intervals, the beads were withdrawn from the solution and increase in the weight of the beads was measured as a function of time. Swelling ratio (SR) was expressed as

$$\mathrm{SR} = \frac{W_2 - W_1}{W_1}$$

where W_1 and W_2 represent the dry and wet weight of the beads, respectively.

2.2.4. Mucoadhesivity

Mucoadhesivity testing was carried out following in vitro wash-off protocol as reported by Lehr et al. [22]. In brief, fresh tissue portions from the goat stomach and colon were obtained from a commercial slaughter house and cleaned with cold normal saline. The tissues (1.5 cm × 1.5 cm) were fixed on a glass slide using adhesive glue keeping the mucosal surface upward. Fifty milligrams of the beads were placed on the mucosa and a 5 g load was applied onto it for 15 min to ensure uniform adhesion of the bead on the mucosa. Thereafter, bead-loaded stomach and colon mucosa were placed in 0.1 N HCl (pH adjusted to 1.2, specific to stomach) and PBS (pH 6.8, specific to colon), respectively, onto the groves of USP24 tablet disintegration apparatus. The disintegration apparatus was then operated in a way that ensured up and down movement of tissue specimen in 1 l of buffer at 37 °C. The experiment was run for 24 h and the time corresponding to the complete wash off of the beads was noted.