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Physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs

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Abstract

In the study, a complex composed of alginate blended with a water-soluble chitosan (N,O-carboxymethyl chitosan, NOCC) was prepared to form microencapsulated beads by dropping aqueous alginate–NOCC into a Ca^{2+} solution. These microencapsulated beads were evaluated as a pH-sensitive system for delivery of a model protein drug (bovine serum albumin, BSA). The main advantage of this system is that all procedures used were performed in aqueous medium at neutral environment, which may preserve the bioactivity of protein drugs. The swelling characteristics of these hydrogel beads at distinct compositions as a function of pH values were investigated. It was found that the test beads with an alginate-to-NOCC weight ratio of 1:1 had a better swelling characteristic among all studied groups. With increasing the total concentration of alginate–NOCC, the effective crosslinking density of test beads increased significantly and a greater amount of drug was entrapped in the polymer chains (up to 77%). The swelling ratios of all test groups were approximately the same ($\sim$3.0) at pH 1.2. At pH 7.4, with increasing the total concentration of alginate–NOCC, the swelling ratios of test beads increased significantly (20.0–40.0), due to a larger swelling force created by the electrostatic repulsion between the ionized acid groups ($-COO^-$). It was shown that BSA was uniformly distributed in all test beads. At pH 1.2, retention of BSA in hydrogels may be improved by rinsing test beads with acetone (the amount of BSA released was below 15%). At pH 7.4, the amounts of BSA released increased significantly ($\sim$80%) as compared to those released at pH 1.2. With increasing the total concentration of alginate–NOCC, the release of encapsulated proteins was slower. Thus, the calcium–alginate–NOCC beads with distinct total concentrations developed in the study may be used as a potential system for oral delivery of protein drugs to different regions of the intestinal tract.
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Keywords: Alginate; N,O-carboxymethyl chitosan; Hydrogel; pH sensitivity; Oral drug delivery

1. Introduction

Production of pharmaceutically active peptides and proteins in large quantities has become feasible [1]. It is known that the oral route is the most convenient and comfortable way of administering drugs. However, peptide and protein drugs are readily degraded by the low pH of gastric medium in the stomach. Therefore, these peptide and protein drugs need to be protected from the harsh environment in the stomach, if given orally [2].

For designing oral dosage forms, the formulator must consider that the natural pH environment of gastrointestinal tract varies from acidic in the stomach to slightly alkaline in the intestine [3]. In the design of oral delivery of peptide or protein drugs, pH-sensitive hydrogels have attracted increasing attention. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. The extent of swelling increases as hydrogels pass down the intestinal tract due to increase in pH. A variety of synthetic or natural polymers with acidic or basic pendent groups have been employed to fabricate pH-sensitive hydrogels [4,5]. Among them, alginate is one of the commonly used. Alginate, a polyanionic copolymer of mannuronic and guluronic sugar residues, has been widely used in biomedical applications [6,7]. It was

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reported that alginate is non-toxic and biodegradable when given orally [8,9].

Hydrogels are crosslinked polymers, which can swell considerably in aqueous medium without dissolution. Crosslinks within polymeric hydrogels can be created either chemically or physically. The use of crosslinking agents to chemically form polymeric hydrogels may lead to toxic side effects (owing to residual crosslinking agents) or to unwanted reactions with drugs [10]. Therefore, drug encapsulation using alginate is often carried out physically by dispersion of the alginate/drug solution into a gelation medium in the presence of divalent cations such as Ca^{2+} [11,12].

It was reported that the biological activity of drugs can be retained in the calcium-crosslinked alginate encapsulation process [13]. This is because the encapsulation process is performed under mild aqueous-based conditions, thus allowing the retention of the biological activity of encapsulated drugs. Gel formation of calcium-crosslinked alginate in the presence of Ca^{2+} has been used to immobilize cells and drug delivery [13,14]. Nevertheless, it was found in the present study that swelling of the calcium-crosslinked alginate beads at pH 7.4 was minimal (data presented in the Results and Discussion section), due to the relatively strong ionic interaction between the carboxylic groups on alginate and Ca^{2+}. This may limit the drug release at the intestinal tract.

To overcome this problem, a complex composed of alginate blended with a water-soluble chitosan (N,O-carboxymethyl chitosan, NOCC) was prepared to form microencapsulated beads by dropping aqueous alginate–NOCC into a Ca^{2+} solution (calcium–alginate–NOCC beads). These microencapsulated beads were used as a pH-sensitive-based controlled release system for protein drug delivery. The process in the preparation of such a system is simple and in an all-aqueous environment. It is known that organic solvents may cause degradation of peptide or protein drugs that are unstable and sensitive to their environments [15,16]. NOCC is a chitosan derivative having carboxymethyl substituents on some of both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure [17]. It was reported that NOCC is non-toxic, either in vitro in fibroblast culture assays or in vivo in testing with intraperitoneal, oral, or subcutaneous treatments [18]. Additionally, NOCC is suitable as an excipient in ophthalmic formations to improve the retention and bioavailability of drugs [20].

In the study, preparation of calcium–alginate–NOCC beads was reported. Swelling characteristics of these hydrogel beads as a function of pH values were investigated. Additionally, release profiles of a model protein drug (bovine serum albumin, BSA) from test hydrogel beads were studied in simulated gastric and intestinal media.

2. Materials and methods

2.1. Materials

Chitosan (M.W. $\sim 2.5 \times 10^5$) with a degree of deacetylation of approximately 85% was acquired from Challenge Bioproducts Co. (Taichung, Taiwan). Sodium alginate of low viscosity (250 cps for a 2% solution at 25°C), calcium chloride, monochloroacetic acid, isopropyl alcohol, phosphate buffered saline (PBS), bovine serum albumin (BSA), and Bradford reagent (BSA protein assay reagent) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). All other chemicals and reagents used were of analytical grade.

2.2. Synthesis of N,O-carboxymethyl chitosan (NOCC)

NOCC was synthesized as per a procedure described in the literature with some modifications [17,18]. Detailed procedures were previously reported by our group [19].

2.3. Preparation of calcium–alginate–NOCC beads

The calcium–alginate–NOCC beads tested in the study were prepared by dropping aqueous alginate–NOCC into a calcium chloride solution. Aqueous alginate–NOCC solutions at distinct compositions (alginate:NOCC = 0%:6%, 2%:4%, 1%:1%, 1.5%:1.5%, 3%:3%, 4.5%:4.5%, 4%:2%, and 6%:0% by w/v) were prepared. The prepared aqueous alginate–NOCC solutions were then dropped into a gently stirred calcium chloride solution (0.1–0.3 M) through a pipette tip (1000 μl). Gel formation in the shape of beads was formed instantaneously. The beads were allowed to crosslink with Ca^{2+} in solution for distinct durations. The calcium-crosslinked beads (calcium–alginate–NOCC beads) were rinsed with distilled water several times to remove unreacted calcium chloride on surface and subsequently dried at 37°C.

The morphology of prepared beads was examined using an optical microscope (Zoom Stereo SZX12, Olympus Optical Co. Ltd., Tokyo, Japan). The crosslinking densities of calcium–alginate–NOCC beads were evaluated by determining the modulus of elasticity in compression as described elsewhere [21,22].

2.4. Swelling characteristics of calcium–alginate–NOCC beads

The swelling characteristics of calcium–alginate–NOCC beads were determined by immersing dried test samples to swell in 5 ml of a solution at pH 1.2 and 37°C for 2 h and subsequently transferred into a pH 7.4 medium, simulating gastrointestinal tract conditions [23,24]. At specific time intervals, samples were removed

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