Swelling Behaviour of Alginate-N,O-
Carboxymethyl Chitosan Gel Beads Coated by Chitosan

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Swell behaviour is one of the important properties of hydrogel-based drug delivery systems, which always affects the diffusion of solvent into and release of drugs from drug loaded microcapsules. In this study, the swelling behaviour of alginate-chitosan beads at acidic and basic conditions, to simulate gastric and intestinal media, were investigated. Spherical hydrogel beads were prepared by addition of aqueous sodium alginate and alginate-N,O-carboxymethyl chitosan (NOCC) solutions into CaCl2 solution. These hydrogel beads were then transferred into a chitosan solution to obtain chitosan coated beads. The effect of concentration of calcium chloride, residence time for ionic cross-linking, concentration of chitosan, addition of NOCC into alginate solution, coating of alginate-NOCC by chitosan as well as drying method on the swelling behaviour of the alginate-chitosan beads was studied. It was found that swelling degree of the air-dried and chitosan coated beads was lower than that for freeze-dried and uncoated beads, respectively. In addition, the presence of NOCC in the network resulted in reducing swelling of hydrogel beads. Swelling degree of hydrogels in basic media (pH 7.4) was also much higher than that in acidic media (pH 1.2). Thus, alginate-NOCC-chitosan beads are good candidate to be studied as colon-specific drug delivery systems.

INTRODUCTION

Chitosan is a natural polysaccharide obtained via deacetylation of chitin that is found particularly in the shells of crustaceans such as crab and shrimp, the cuticles of insects, and the cell walls of fungi [1,3]. Chitosan is a biocompatible, non-toxic, biodegradable and mucoadhesive polymer, with a gel-forming ability at low pH [3-5]. Chitosan is widely used in the pharmaceutical field and has been formulated as films, beads, tablets and microparticles [6-11].
In a recent review by Hejazi and Amiji [12], it was suggested that the degradation of chitosan occurs by the microflora that are available in the colon. These properties could provide a basis for preparation of controlled release formulations, particularly for colon-specific drug delivery systems [12-13].

N, O-Carboxymethyl chitosan (NOCC) is a chitosan derivative having carboxymethyl substituents on some of the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure [3,14]. Carboxymethylated chitosan has been paid more and more attention because of its good water solubility, and it is more convenient to be applied in medicine because it fits the neutral environment of the human body [15-17].

Alginate is a natural polyacid and has a unique property of gel-formation in the presence of multivalent cations such as calcium ions in aqueous medium. This occurs via binding of calcium ions within the cavities of the guluronic acid residues, forming a polyanion microcapsule [18]. A complex composed of alginate blended with NOCC was prepared to form microcapsules by dropping it into a Ca^{2+} solution. The resulting microcapsules showed good mechanical properties because of the strong electrostatic interaction between NH_{2} groups of NOCC with -COOH groups of alginate [14-15]. This may limit the swelling degree and drug release from the network at the intestinal tract and reduce the burst effect. Also, addition of a polycation such as chitosan with unique polycation characteristics, leads to a strong interaction with negatively charged alginate. As shown in Figure 1, when calcium-alginate beads are added into chitosan solution, the electrostatic interaction of carboxylic groups of alginate with the amine groups of chitosan results in the formation of a membrane. This process has been widely used in the preparation of alginate-chitosan membrane with a solid calcium-alginate gel core. There are many advantages of the chitosan coating, such as the improvement of drug payload and bioadhesive property, as well as the prolonged drug release properties [19-21].

The main objective of the present study was to improve the swelling properties of previously studied alginate-chitosan and alginate-NOCC hydrogel beads [19-27]. For this purpose, a complex aqueous solution composed of alginate and NOCC was prepared and then added dropwise into a Ca^{2+} solution. Alginate-NOCC beads obtained in the first step were transferred into chitosan solution for coating and then their swelling behaviour in acidic and basic media was studied. In addition, the effects of calcium chloride concentration, residence time for ionic cross-linking, concentration of chitosan and addition of NOCC to alginate solution as well as drying method of resulting beads on the swelling behaviour of alginate-NOCC-chitosan beads were investigated.

**EXPERIMENTAL**

**Materials and Instruments**

Chitosan (M_{w}~2×10^{5} Dalton) with an 85% degree of deacetylation was provided from Sigma (USA). Sodium alginate was obtained from BDH Laboratory (England). Calcium chloride, monochloroacetic acid and isopropyl alcohol were purchased from Merck (German). All other chemicals and reagents used were of analytical grade.

**Synthesis**

_*Synthesis of N,O-Carboxymethyl Chitosan (NOCC)_*

NOCC was synthesized according to the literature [27]. Chitosan (5 g) was suspended in 50 mL of isopropyl alcohol and resulting slurry was stirred in a 200 mL flask at room temperature. A volume of 13 mL of 10 N aqueous NaOH solutions, divided into five equal portions, was then added to the stirred slurry over a period of 25 min. The alkaline slurry was stirred for additional 30 min. Subsequently, monochloroacetic acid (30 g) was added, in five equal por-
tions, at 1 min intervals. Heat was then applied to bring the reaction mixture to a temperature of 60°C and stirring at this temperature was continued for 3 h. Finally, the reaction mixture was filtered and the solid product (NOCC) was washed with methanol. The NOCC was dried in oven at 60°C.

Characteristics of N,O-Carboxymethyl Chitosan (NOCC)
Proton nuclear magnetic resonance spectroscopy ($^1$H NMR) was used to confirm substitutions of carboxymethyl groups on the amino and primary hydroxyl sites of the modified chitosan (NOCC). $^1$H NMR Studies were carried out with deuterium oxide. Analyses of the proton spectra were conducted on an NMR spectrometer (Bruker DRX 500 Avance NMR spectrometer, German). The degrees of substitution of carboxymethyl groups on amino and primary hydroxyl sites of the modified chitosan (NOCC) were estimated by the relative peak intensities between the H on the carboxymethyl groups and the H at C$_2$ of monosaccharide residue in the $^1$H NMR spectrum of NOCC, as per a method reported in the literature [28].

Preparation of Coated Beads, Calcium-alginate and Calcium-Alginate-NOCC Beads
Aqueous sodium alginate solutions with a concentration of 1% (w/v) and sodium alginate-NOCC solutions with a distinct composition of alginate/NOCC [1%:1% (w/v)] were prepared. These aqueous solutions were then dripped into a gently stirred calcium chloride solution (0.05-0.1 M) through a Pyrex glass tube with a diameter of 0.5 cm, connected to feeding solution. The distance of tube tip from the gelling solution was 10 cm. The beads were allowed to harden in the calcium chloride solution for at least 10-30 min. These beads were filtered and washed with distilled water, and subsequently dried at room temperature for 24 h or freeze-dried. The freeze-dried beads were obtained through rapid freezing at -50°C followed by drying in a freeze-drier (Zirbus, Denmark).

Scanning Electron Microscopy
The shape and surface characteristics of the air and freeze-dried alginate-NOCC-chitosan beads were observed by SEM. The beads were sputter-coated with Au using a vacuum evaporator and examined using a scanning electron microscope (Philips, Netherlands).

Swelling Studies
The swelling characteristics of beads were determined by immersing them at dry state into a buffer solution at pH 1.2 (HCl/KCl) and 7.4 (Tris-HCl) with a temperature of 37°C. At specific time intervals, the samples were removed from the swelling medium and were blotted with a piece of paper towel to absorb excess water on surface. The degree of swelling, S(t), at anytime was calculated using the following expression:

$$S(t) = \frac{(W_t - W_0)}{W_0}$$ (1)

where $W_t$ and $W_0$ are the sample weights at time t and in the dry state, respectively.

It is worth mentioning that preliminary experiments indicated that possible soluble fraction (sol) did not leach out of gels during swelling. It was confirmed by constant dried weight of samples before and after equilibrium swelling.

RESULTS AND DISCUSSION

Characteristics of N,O-Carboxymethyl Chitosan (NOCC)
NOCC is a water soluble derivative of chitosan and Figure 2 shows its proton NMR spectra. As shown, the chemical shifts at 4.06-4.3 and 4.5 ppm were the protons of -CH$_2$-COO- at N-position on C$_2$ and the O-position on C$_6$ of the chitosan modified (NOCC), respectively. This indicated that carboxymethyl substituents were observed on some of the amino and primary hydroxyl sites of the modified chitosan struc-
ture. The degree of substitution (DS) of carboxymethyl groups on the amino and primary hydroxyl sites can be determined from $^1$H NMR spectra based on a method described in the literature [28]. The calculated equations are as follows:

$$DS \text{ on amino} = \frac{1}{2} \left( \frac{I_b}{I_2} \right) \times 100$$

$$DS \text{ on primary hydroxyl} = \frac{1}{2} \left( \frac{I_c}{I_2} \right) \times 100$$

where $I_b$, $I_2$ and $I_c$ are intensity of 4.06-4.3, 3.1 and 4.5 ppm, respectively. The degree of substitution of carboxymethyl groups on the amino and primary hydroxyl sites were approximately 20.3% and 19.2%, respectively.

**Bead Formation**

As expected, dropwise addition of sodium alginate solution into calcium chloride solution immediately induced ionic cross-linking of alginate chains, thus forming hydrogel beads. Gel formation was also observed upon the addition of aqueous NOCC solution into a calcium chloride solution as a result of ionic cross-linking between carboxylate ions (-COO$^-$) of NOCC and Ca$^{2+}$ in electrolyte solution. Lin et al. reported that in the preparation of calcium-alginate-NOCC beads, alginate entangled through the NOCC network, resulting in the formation of interpenetrating polymeric network (IPN) [14].

Then, Ca-alginate and Ca-alginate-NOCC beads were separately coated by chitosan as a result of ionic
interaction between \(-\text{NH}_3^+\) groups of chitosan and \(-\text{COO}^-\) groups of beads. These swollen beads were spherical in shape with a smooth surface (about 3-3.5 mm). After drying, the spherical shape of beads in wet state was usually lost, especially air-dried ones. After drying at room temperature, the diameter of beads was reduced to 1-1.7 mm. The similar results were obtained by Lin et al. [14] for alginate-NOCC beads.

The scanning electron micrographs in Figure 3 show the cross-sectional morphology of alginate-chitosan and alginate-NOCC-chitosan beads. The thickness of chitosan membrane was 168 µm and 433 µm for alginate-chitosan and alginate-NOCC-chitosan beads, respectively. Therefore, it can be concluded that the extent of reaction between chitosan and alginate-NOCC was higher than that of alginate.

Comparison of Figures 3b and 3c shows that freeze-dried beads are more porous than air-dried ones. This structural difference causes different swelling behaviour, as will be described in the following sections.

**Swelling Studies**

*Effect of Concentration of Calcium Chloride*

Figure 4 shows the swelling behaviour of alginate-chitosan beads, prepared at two different concentrations of calcium chloride solution in acidic (pH 1.2)
and basic (pH 7.4) media. Swelling degree of beads at both pHs decreased by increasing calcium chloride concentration from 0.05 to 0.10 M. Similar behaviour was reported for BSA diffusion from alginate-chitosan microcapsules by Vandenberg et al. [29]. With increase of calcium chloride solution, more Ca$^{2+}$ diffuse into alginate gel beads resulting in increased degree of ionic cross-linking [30-32]. By increasing the degree of cross-linking, the elastic strength of beads against swelling increases and hence swelling degree decreases [33-34]. At pH 1.2, the swelling degree of the bead was limited due to the formation of strong hydrogen bonding between alginate (-COOH and -OH) and chitosan (-NH$_2$). At pH 1.2, the swelling degree of the beads reached their maximum value within 30 min and then gradually decreased towards their equilibrium state due to the reduced chemical potential of the network resulted from protonation of carboxylic acid groups.

The initial increase of swelling degree is mostly driven by counterions which neutralize -NH$_3^+$ groups of polymer chains in the network [34]. As swelling proceeds, the -COO- groups become protonated in acidic media [35] and chemical potential of the network for solvent uptake from surrounding media decreases. As a result, previously absorbed solvent diffuses out of the network until equilibrium state is established. The swelling of the coated beads at pH 7.4 is limited due to deprotonation of -NH$_3$ groups [24], particularly in outer shell which results in decreased chemical potential of the network as a whole [34]. The slope of the curves in Figure 4 shows that the rate of swelling of beads with higher degree of cross-linking is lower than that for less cross-linked ones, particularly at pH 7.4.

**Effect of Cross-linking Time**

Figure 5 shows the swelling behaviour of alginate-chitosan beads affected by variation of cross-linking time. Schematic illustrations of alginate beads cross-linked in the calcium chloride solution for distinct durations are shown in Figure 6 [14]. As shown, Ca$^{2+}$ diffuses from outside into the core of the gelled bead gradually, and cross-linking proceeds from the surface to centre step by step. For a cross-linking time of 1-15 min, a distinct boundary between the cross-linked surface layer and the uncross-linked core was clearly observed. After 30 min of cross-linking, a homogeneously cross-linked bead was formed. This indicated that cross-linking of alginate beads with calcium ions can be completed within 30 min. But, cross-

![Figure 5](image5.png)

**Figure 5.** Variation of swelling degree of alginate-chitosan beads, prepared at two different cross-linking times, with time at pHs 1.2 (a) and 7.4 (b).

![Figure 6](image6.png)

**Figure 6.** Schematic illustration of alginate beads formation in a calcium chloride solution [14].
linking of beads after 10 min of reaction was not completed.

**Effect of Chitosan Concentration**

Figure 7 shows the swelling behaviour of alginate-chitosan beads in acidic (pH 1.2) and basic (pH 7.4) media at different concentrations of chitosan. Swelling degree at pH 1.2 slightly increased by increasing chitosan concentration due to presence of -NH$_3^+$ groups in the network which induces a higher osmotic pressure for swelling of the network. But the effect of chitosan concentration on the degree of swelling at pH 7.4 was not considerable (slight decrease), because the osmotic pressure for swelling of the network is usually driven by counterions which neutralize carboxyl groups, -COO$^-$ [34]. But, Liu et al. [24] reported that swelling ratio of alginate-chitosan microcapsules decreased by increasing chitosan concentration.

**Effect of NOCC Addition into Alginate Solution**

Figure 8 shows the changes of swelling behaviour of the alginate-chitosan beads with addition of NOCC into alginate solution. The swelling degree of beads at both pHs decreased by the presence of NOCC in the network due to the hydrogen bond formation and ionic interactions between carboxyl groups on alginate and amino groups on NOCC and chitosan. These results differ from those obtained by Lin et al. [14] and Chen et al. [27]. They have reported that swelling ratio of physically or chemically cross-linked alginate-NOCC hydrogels increased at both acidic and basic media with increasing the amount of NOCC.
Figure 9. Variation of swelling degree of air and freeze-dried alginate-NOCC-chitosan beads with time at pHs 1.2 (a) and 7.4 (b).

Figure 10. Variation of swelling degree of chitosan coated and uncoated alginate-NOCC beads with time at pHs 1.2 (a) and 7.4 (b).

Effect of Drying Method
Figure 9 shows the variation of swelling degree versus time for air and freeze-dried alginate-chitosan beads. The swelling degree of freeze-dried beads with a porous structure at both pHs was higher than that of air-dried ones. At pH 7.4, the swelling degree of air and freeze-dried alginate-NOCC-chitosan beads were 14.9 and 24, respectively (Figure 9b). Similar behaviour was reported for alginate-chitosan beads by Silva et al. [36].

Effect of Chitosan Coating
Figure 10 shows the dynamic swelling behaviour of the coated and uncoated alginate-NOCC beads, at pHs 1.2 and 7.4. The swelling degree of uncoated alginate-NOCC beads was higher than that of chitosan coated beads in both media. The reduced swelling degree of the chitosan coated bead at pH 1.2 can be attributed to the presence of strong hydrogen bonding between -OH and -COOH groups of the network [27]. The swelling degree of the coated beads is also limited due to deprotonation of -NH$_3$ groups [24], particularly in outer shell which results in decreased chemical potential of the network for solvent uptake as a whole [34].

Mechanism of Solvent Uptake
A very simple model which is common to determine the mechanism of solvent diffusion into a polymeric system is given by:

$$\frac{M_t}{M_\infty} = k.t^n$$

where $M_t$ is the amount of solvent uptake at time $t$, $M_\infty$ is the equilibrium amount of solvent uptake, and $k$ and $n$ are constants.
$M_\infty$ is the total amount of solvent uptake, $k$ is a constant incorporating the structural and geometric characteristic of the device, and $n$ is the uptake exponent indicative of the mechanism of solvent uptake.

The experimental data for swelling of air-dried alginate-chitosan and alginate-NOCC-chitosan beads, as suitable candidates for drug release, were plotted in terms of $\ln(M_t/M_\infty)$ vs. $\ln(t)$ to determine the value of $n$. The values of $n=0.73$ for the former and $n=0.86$ for the latter were obtained indicating anomalous or non-Fickian behaviour of solvent uptake. Anomalous transport occurs due to a coupling of Fickian diffusion and polymer relaxation as the gels swell [37].

**CONCLUSION**

Alginate-chitosan and alginate-NOCC-chitosan beads were prepared and their swelling behaviour investigated in this study. Swelling degree at pH 7.4 was considerably higher than that at pH 1.2, which indicates the pH sensitivity of these networks. Swelling degree at both pHs 1.2 and 7.4 decreased by increasing concentration of calcium chloride solution and cross-linking time length due to the increased degree of network cross-linking. Swelling degree of beads also decreased by chitosan coating and presence of NOCC due to the hydrogen bond formation and ionic interaction of functional groups of the polymer chains.

Drying method had a profound effect on swelling behaviour and solvent uptake of freeze-dried beads with a porous structure, was higher than that of air-dried beads. The swelling behaviour of alginate-NOCC-chitosan beads with a considerable difference in acid and basic media renders them as a suitable polymeric carrier for colon-specific drug delivery system.

**REFERENCES**


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