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Novel thiolated carboxymethyl chitosan-*g*-β-cyclodextrin as mucoadhesive hydrophobic drug delivery carriers

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Abstract

A novel thiolated carboxymethyl chitosan-*g*-β-cyclodextrin (CMC-*g*-β-CD) drug delivery carrier was synthesized and characterized. Thiolated CMC-*g*-β-CD was synthesized using two steps. First, carboxymethyl β-cyclodextrin (CM β-CD) was grafted onto carboxymethyl chitosan (CMC) using water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as the condensing agents. Next, the resultant product was further grafted with cysteine methyl ester hydrochloride (CMEH). Its structure was confirmed by FT-IR and ^{1}H NMR spectral analysis. X-ray diffraction (XRD) analysis on the resulting product showed that the peaks at $2\theta = 10°$ and 20° decreased greatly in thiolated polymers, indicating that these polymers are more amorphous in nature. The swelling study showed that the water uptake of thiolated CMC-*g*-β-CD was higher than that of the unmodified chitosan control. The adhesive properties of thiolated CMC-*g*-β-CD were evaluated *in vitro* on a freshly excised mouse mucosa, and a fivefold increase in the adhesion time was found in thiolated CMC-*g*-β-CD when compared with the unmodified chitosan control. The drug release profile showed that thiolated CMC-*g*-β-CD tablets provided a slower release of the entrapped hydrophobic model drug, ketoprofen, than the chitosan control, and the release behavior was influenced by the amounts of thiol groups present on the polymer chains. These results suggest that thiolated CMC-*g*-β-CD with improved mucoadhesive properties may potentially become an effective hydrophobic drug delivery system with controlled drug release capability.

Keywords: Carboxymethyl chitosan; β-Cyclodextrin; Mucoadhesive; Drug delivery

1. Introduction

Over the past few years, mucoadhesive polymers have received considerable attention as excipients for various drug delivery systems due to their ability to prolong the residence time of dosage forms as well as to enhance drug bioavailability (Duchene, Touchard, & Peppas, 1988; Lehr, 1996; Smart, Kellaway, & Worthington, 1984). Because of a prolonged residence time of the mucoadhesive delivery systems at the site of drug absorption, the frequency of dosing can be reduced and as a result the patient compliance improved. Due to these advantages, many attempts have been made to improve the mucoadhesive properties of polymeric carriers.

In earlier studies, various natural and synthetic polymers were explored as mucoadhesive excipients. The mucoadhesive properties of these polymers were based on the formation of non-covalent bonds such as hydrogen bonds and ionic interactions with the mucus layer (Peppas & Mikos, 1990). Recently, it has been shown that polymers with thiol groups provide much higher adhesive properties than those polymers generally considered to be mucoadhesive (Bernkop-Schnurch, Schwarz, & Steininger, 1999). The enhancement of mucoadhesion can be explained by the formation of covalent bonds between the polymer and the mucus layer, which are stronger than non-covalent bonds. These thiolated polymers, or the so-called thiomers, are supposed to interact with cysteine rich sub-domains of

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mucus glycoproteins via disulfide exchange reactions (Snyder, Reddy, Cennerazzo, & Field, 1983). Thus, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus layer by the formation of disulfide bonds, the bridging structure most commonly encountered in biological systems.

A number of cationic thiomers (e.g., chitosan–cysteine, chitosan–thiobutylamidine, and chitosan–thioglycolic acid) and anionic thiomers (e.g., poly(acylic acid)–cysteine, poly(acrylic acid)–cysteamine, carboxymethyl cellulose–cysteine, and alginate–cysteine) have been studied thus far (Bernkop-Schnurch, Konig, Leitner, Krauland, & Brodnik, 2004; Jayakumar, Nwe, Tokura, & Tamura, 2007; Jayakumar, Reis, & Mano, 2007). Due to the immobilization of thiol groups on these polymers, their mucoadhesive properties have been improved (Bernkop-Schnurch, 2005). However, the poor interaction of these thiolated polymers with hydrophobic drug molecules often resulted in a faster drug release, which will affect their potential applications in pharmaceutical fields. Therefore, in this work, an attempt has been made to synthesize a thiolated CMC-*g*-β-CD as mucoadhesive drug delivery carrier with controlled drug release capability by grafting CM β-CD and CMEH onto CMC.

CMC is a water-soluble and biodegradable polymer, which is derived from chitosan. It is often used as a pharmaceutical excipient because of its very safe toxicity profile (Lu et al., 2007; Prabaharan & Mano, 2007). It offers the advantage of easy chemical modifications due to the primary amino group at the C_2-position and the carboxyl group at the C_6-position of each polymer subunit (Jayakumar, Prabaharan, Reis, & Mano, 2005; Prabaharan, Reis, & Mano, 2007). These reactive sites enable the grafting of a large variety of properly functionalized molecules. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide with the outstanding ability to form inclusion complexes with a large variety of organic and inorganic guests (Prabaharan & Mano, 2006; Uekama, 2002).

One of the most remarkable applications of β-CD is its use as drug carriers in controlled release systems. As drug carriers, β-CD allows the solubilization, stabilization, and transport of hydrophobic drugs together with several pharmacological benefits such as the reduction of unwanted side effects (Hedges, 1998; Uekama, Hirayama, & Irie, 1998). During the complex formation with drug molecules, no covalent bonds exist between the β-CD and its guest, thus complexation can be considered as a dynamic process. The drug molecules included within the β-CD cavity therefore may be dissociated upon dilution, displaced by a more suitable guest, or transferred to a matrix for which it has a higher affinity, such as a biological membrane (Loftsson & Brewster, 1996).

Due to these favorable properties of CMC and β-CD, thiolation of CMC-*g*-β-CD may lead to a molecular carrier that possesses the cumulative effects of inclusion, size specificity, controlled release, and transport properties of CMC-*g*-β-CD, as well as the mucoadhesive ability of CMEH. Therefore, the aim of this study is to synthesize and characterize novel thiolated CMC-*g*-β-CD as mucoadhesive drug carriers. For the drug delivery study, tablets were prepared from thiolated CMC-*g*-β-CDs since they can provide an accurate dosage and are easy to manufacture and handle for the peroral administration. The feasibility of these tablets as mucoadhesive delivery carriers for the transfection of the hydrophobic model drug, ketoprofen, was investigated. The swelling and mucoadhesive properties of the tablets also were evaluated.

2. Experimental

2.1. Materials

Chitosan (viscosity average molecular weight 20 kDa, degree of *N*-deacetylation 75–85%) was purchased from Sigma Chemical Company and used as received. β-CD, Isopropyl alcohol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Sigma–Aldrich and used without further purification. Chloroacetic acid, L-cysteine methyl ester hydrochloride (CMEH), and sodium hydroxide were supplied by Acros Organics, USA, and used as supplied. All other chemicals used were of analytical reagent grade.

2.2. Synthesis of CMC

CMC was prepared according to a method reported in the literature (Chen, Tian, & Du, 2004). Chitosan (8.0 g, 49.7 mmol glucoseamine) was added into 40 g of 50 wt% NaOH solution and placed in a refrigerator at −18 °C overnight for alkalization. After the excessive alkali solution was extruded, chitosan was placed in a 250 ml reactor containing 40 ml of isopropyl alcohol. Then, 10 g (105.7 mmol) of chloroacetic acid dissolved in 40 ml of isopropyl alcohol was added into the reactor dropwise. Afterwards, this reaction mixture was refluxed at 65 °C under extensive stirring. After 4 h, the reaction was stopped and the solvent was discarded. The mixture was neutralized using HCl and dialyzed in tubings (molecular weight cutoff 2 kDa) against distilled water for 3 days to remove the impurities and unreacted materials. Finally, the frozen product was lyophilized at −50 °C and 0.05 mbar (Labconco, USA) and stored at room temperature. From the relative peak intensities between the protons of the carboxymethyl groups (4.4 ppm) and the protons at C_2 of monosaccharide residue (3.0 ppm), the degree of substitution (DS) of carboxymethyl groups on the primary hydroxyl sites of the modified chitosan (CMC) was determined as ~35% using ^{1}H NMR.

2.3. Synthesis of CM β-CD

CM β-CD was prepared according to a method reported in the literature (Prabaharan & Mano, 2005). Briefly, a

mixture of β-CD (5.675 g, 5 mmol) and sodium hydroxide (5 g, 0.125 mol) in 20 ml of distilled water was treated with 0.4725 g (5 mmol) of chloroacetic acid at 50 °C for 5 h. After neutralization, the obtained product was precipitated with excess amount of acetone and dried at 40 °C under vacuum. The DS of carboxymethyl groups on the primary hydroxyl sites of the CM β-CD was estimated as ~14% using ^{1}H NMR.

2.4. Grafting CM β-CD onto CMC

CM β-CD was grafted onto CMC in the presence of EDC and NHS. A solution of CM β-CD (1.193 g, 1 mmol) in 40 ml of distilled water was first activated with 0.192 g (1 mmol) of EDC and 0.115 g (1 mmol) of NHS for 30 min. With this mixture, 0.181 g (1 mmol) of CMC dissolved in 10 ml of distilled water was added dropwise. The reaction was maintained at pH 7 and room temperature under constant stirring. After 8 h, the product formed was dialyzed in tubings (molecular weight cutoff 2 kDa) against distilled water for 3 days and freeze-dried.

2.5. Conjugation of CMEH onto CMC-g-β-CD

A solution of CMC-*g*-β-CD (1.4 g in 50 ml of distilled water) was treated with 0.192 g (1 mmol) of EDC and 0.115 g (1 mmol) of NHS for 30 min. in order to activate the carboxyl groups of CMC-*g*-β-CD. Different amounts CMEH were then added as listed in Table 1. The pH of the reaction mixture was adjusted using NaOH and HCl. After a reaction period of 8 h at room temperature under constant stirring, the resulting product was dialyzed against distilled water for 3 days and freeze-dried. The product was stored at 4 °C until further use.

2.6. Characterization

The FT-IR spectra of the products were recorded with a double-beam Mattson Galaxy Series FTIR-3000 spectrometer in the range of 4000–400 cm^{-1} using KBr pellets. ^{1}H NMR spectrum of the samples was recorded on a Bruker DPX 300 spectrometer using tetramethylsilane as an internal standard and D_2O as a solvent at 25 °C. XRD experiments were performed in a XDS-2000 (Scintag Inc, USA) diffractometer using Cu K_α radiation source. Absorbance measurements were carried out in an Ocean Optics UV–visible spectrophotometer model SD-2000. The calibration curve of absorbance against different concentrations of Ketoprofen was made at 267 nm.

2.7. Determination of thiol groups

The amount of thiol groups present on thiolated CMC-*g*-β-CDs was determined spectrophotometrically using Ellman's reagent as reported in the literature (Kafedjiiski et al., 2007). The quantity of free thiol groups was calculated from a standard curve obtained by solutions with increasing concentrations of L-cysteine hydrochloride hydrate.

2.8. Drug loading and tablet preparation

Three hundred mg of thiolated CMC-*g*-β-CD was dissolved in 30 ml of distilled water and homogenized with 40 mg of ketoprofen dissolved in 5 ml of ethanol under constant stirring for 1 h then the mixture was freeze-dried. Thereafter, the dried samples were compressed into 30 mg, 5.0 mm diameter flat-faced tablets using a compression molding machine. The compaction force (11 kN) was kept constant during the preparation of all tablets. A similar method is followed for the preparation of control unmodified chitosan tablets loaded with drug. In order to determine the initial drug content of the tablets, a known weight of drug-loaded tablets was extracted with ethanol for 24 h under stirring. After centrifugation, the ketoprofen in the supernatant was assayed by UV–visible spectrophotometer.

2.9. Swelling studies

The swelling behaviors of the chitosan and thiolated CMC-*g*-β-CD tablets were studied in phosphate buffered saline (PBS) solutions at pH 6.0 and 37 °C. The tablets were accurately weighed (W_0) and immersed in solutions. At predetermined time intervals the swollen tablets were weighed after they were wiped with soft paper tissue (W_t). The degree of swelling for each sample at time t was calculated by using the expression: $(W_t - W_0)/W_0 \times 100$, where W_t and W_0 are the weights of the tablets at time t and in the dry state, respectively.

2.10. Drug release studies

Tablets (30 mg) were suspended in 100 ml of PBS solution at pH 6.0 contained in a glass bottle. This dissolution

Table 1
Types of thiolated polymers and their reaction conditions and thiol groups contents

Polymer type	CMC-*g*-β-CD (g/50 ml)	CMEH (g)	EDC/NHS (mmol)	pH	Thiol groups (μmol/g polymer ± SD)
Thiolated CMC-*g*-β-CD1	1.4	0.172	1/1	7	356.33 ± 38.80
Thiolated CMC-*g*-β-CD2	1.4	0.086	1/1	7	235.82 ± 15.46
Thiolated CMC-*g*-β-CD3	1.4	0.086	1/1	5	71.21 ± 12.91
Thiolated CMC-*g*-β-CD4	1.4	0.086	1/1	9	189.17 ± 7.34

medium was stirred at 100 rpm in a horizontal laboratory shaker and maintained at 37 °C in a water bath. Samples (2 ml) were periodically removed and the volume of each sample was replaced by the same volume of fresh medium. The amount of released ketoprofen was analyzed with a spectrophotometer at 267 nm. The drug release studies were performed in triplicate for each of the samples.

2.11. In vitro mucoadhesive studies

The time period of binding of thiolated CMC-*g*-β-CDs and control chitosan tablets to the mucosa was determined according to the literature with slight modification (Bernkop-Schnurch & Steininger, 2000). These tablets were attached to a freshly excised intestinal mouse (4 months old) mucosa. Thereafter, the tablets attached with mucosa were entirely immersed with 200 ml of, pH 6.0, PBS at 37 °C and agitated at 100 rpm. The detachment of the test tablets was determined visually during an observation time of 4 h. The experiments were conducted in triplicate for each of the samples.

3. Results and discussion

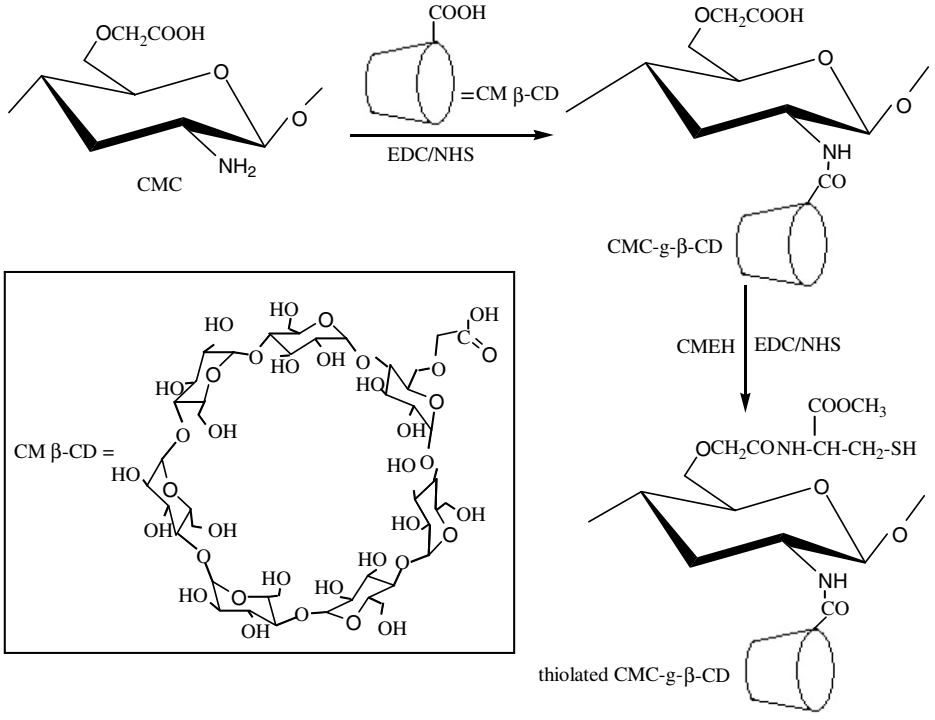
3.1. Synthesis of thiolated CMC-g-β-CD

Thiolated CMC-*g*-β-CDs were synthesized using a two-step procedure. The first step involves the formation of amide bond between the primary amino groups of CMC and the carboxyl groups of CM β-CD using EDC and NHS. In the second step, carboxyl groups of the resultant CMC-*g*-β-CD were conjugated with the amino groups of CMEH (Scheme 1). Due to the presence of β-CD, the resultant polymers will have an ability to release the encapsulated drug molecules in a controlled manner to the surrounding mucosal tissues. It was reported that the pH-value during the thiolation reactions has a great impact on the amount of thiol groups bounded onto the polymer back bone (Kast & Bernkop-Schnurch, 2001). Therefore, in this work, the conjugation of CMEH onto CMC-*g*-β-CD was performed at pH 5, 7, and 9 in order to determine the optimum pH-value for the maximum covalent attachment of thiol groups.

The results showed that at pH 5 the amount of covalently attached thiol groups was low due to the poor efficiency of EDC coupling reaction at this pH-value. The grafting reactions, which were performed at pH 7, led to the highest yield in the thiolated polymer. At this pH, the efficient EDC-mediated reaction between CMEH and CMC-*g*-β-CD may be responsible for this observation. In contrast, at pH 9 the yield of polymer-bound thiol groups decreased again. The reason for this observation may be the oxidation of the thiol groups during the reaction (Bernkop-Schnurch, Leitner, & Moser, 2004). Table 1 shows the types of thiolated polymers prepared under different reaction conditions and their thiol groups contents. Thiolated CMC-*g*-β-CD1 and 2 were used for further characterization studies.

3.2. FT-IR and ^1H NMR analysis

The FT-IR spectrum of chitosan (Fig. 1a) shows a broad —OH stretch absorption band between 3500 and



Scheme 1. Synthesis of thiolated CMC-*g*-β-CD.

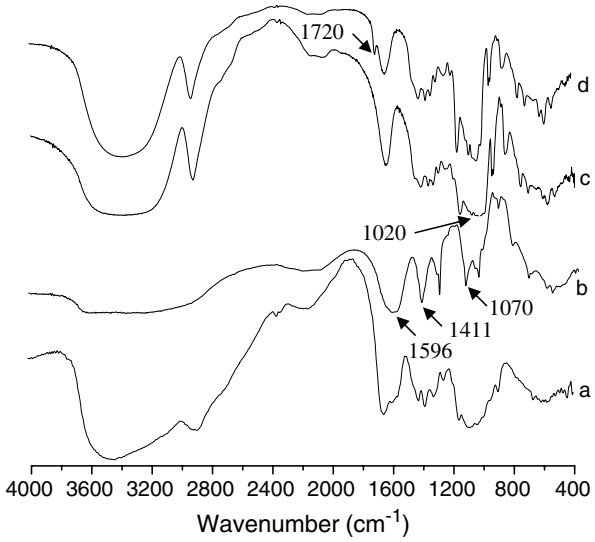


Fig. 1. FT-IR spectrum of (a) chitosan, (b) CMC, (c) β-CD, and (d) CM β-CD.

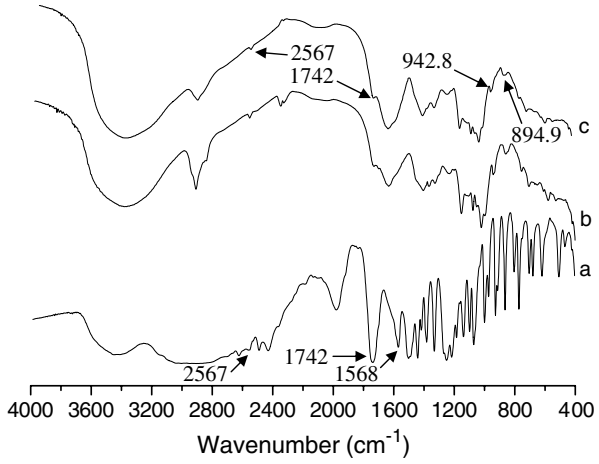


Fig. 2. FT-IR spectrum of (a) CMEH, (b) thiolated CMC-*g*-β-CD1, and (c) thiolated CMC-*g*-β-CD2.

3100 cm^{-1} and the aliphatic C—H stretch between 2990 and 2850 cm^{-1}. As the —OH stretch band and the aliphatic C—H stretch band are aligned, they appear as a broad band from 3500 and 2850 cm^{-1} in the spectrum. The other major absorption band between 1220 and 1020 cm^{-1} represents the free primary amino group (—NH_2) at C_2 position. The peak at 1647 cm^{-1} represents acetylated amino group of chitin, which indicates that the sample is not fully deacetylated. The peak at 1384 cm^{-1} represents the —C—O stretch of primary alcoholic group (—CH_2—OH). In the IR spectrum of CMC (Fig. 1b), the strong peaks at 1596 and 1411 cm^{-1} could be assigned to the respective asymmetry and symmetry stretching vibration of COO^-. Also, the C—O adsorption peak of the secondary hydroxyl group becomes stronger and moves to 1070 cm^{-1}. The results indicate that the carboxymethyl substitution occurs at the C_6 position of chitosan. The IR spectrum of β-CD (Fig. 1c) shows its characteristic strong —OH absorption band at 3500 cm^{-1} and a strong —C—O— band around 1020 cm^{-1}. In the IR spectrum of CM β-CD (Fig. 1d), the strong peak at 1720 cm^{-1} could be assigned to the stretching vibration of carbonyl group. This peak is not observed in β-CD. The results indicate that the carboxymethylation occurs at C_6 position.

In the IR spectrum of CMEH (Fig. 2a), the absorption peak at 1568 cm^{-1} can be assigned to the bending vibration of primary amine group and at 1742 cm^{-1} to the C=O stretching vibration of ester group. In addition, a weak peak at 2567 cm^{-1} is ascribed to the SH group. The spectra of thiolated CMC-*g*-β-CDs (Fig. 2b and c) show not only the characteristic peaks of CMC, but also the characteristic absorption bands of CM β-CD and CMEH. The relative intensity of the 3500 cm^{-1} band of thiolated CMC-*g*-β-CDs is much higher than the intensity of 3500 cm^{-1} band of CMC. The increased intensity could be attributed to the presence of more —OH groups in thiolated CMC-*g*-β-CDs resulting from the coupling of CM β-CD. Further, the characteristic peak of the β-pyranyl vibration of CMC at 894.9 cm^{-1} and the characteristic peak of the α-pyranyl vibration of CM β-CD at 942.8 cm^{-1} both appeared in thiolated CMC-*g*-β-CDs. In addition, characteristic peaks of CMEH also appeared at 1742 and 2567 cm^{-1}. The absorption peak at 1644 cm^{-1}, due to carbonyl stretching of primary amide band, also confirms the grafting of CM β-CD and CMEH onto CMC.

Fig. 3 shows the ^1H NMR spectra of CMC and thiolated CMC-*g*-β-CDs. The spectrum of CMC (Fig. 3a) shows the protons of 2-amino-2-deoxy-b-D-glucopyranosyl residues at 3.0, 3.3–4.0, and 4.8 ppm. The chemical shift at 4.4 ppm was the protons of carboxymethyl groups at the C_6 position of the CMC. This indicated that carboxymethyl substitute occurred on some of the primary hydroxyl sites of the modified chitosan structure. Fig. 3b and c shows the spectrum of thiolated CMC-*g*-β-CD1 and 2, respectively. The signals at 3.0–4.0 ppm are multiplet and those were originated from CMC, CM β-CD, and CMEH. The signals due to methylene protons of CMEH appeared at 2.65–2.8 ppm in the spectrum of thiolated CMC-*g*-β-CDs. Also, new peaks at 5.0–5.2 ppm were observed due to the presence of D-glucopyranosyl residues of the β-CD moiety. These results clearly confirmed that CMC has been successfully grafted with both CM β-CD and CMEH. From the relative peak intensities of anomeric protons of CMC (4.8 ppm), CM β-CD (5.0 ppm), and methylene protons of CMEH (2.65 ppm), the DS of CM β-CD in thiolated CMC-*g*-β-CDs was estimated as ~77%. The DS of CMEH in thiolated CMC-*g*-β-CD1 and 2 was determined as ~21% and ~18%, respectively.

3.3. X-ray diffraction analysis

XRD analysis was used to study the crystalline structures of thiolated CMC-*g*-β-CDs since the crystalline structures affect various properties such as water uptake and

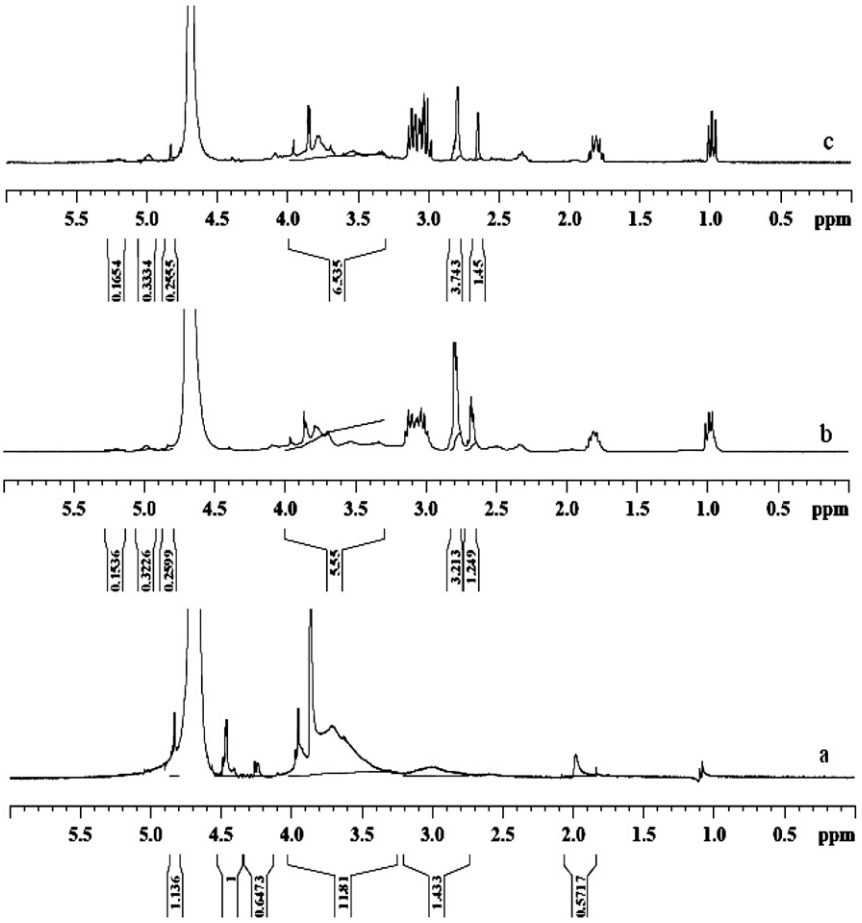


Fig. 3. ^{1}H NMR spectrum of (a) CMC, (b) thiolated CMC-*g*-β-CD1, and (c) thiolated CMC-*g*-β-CD2.

biodegradability of the polymers. The XRD patterns of chitosan and thiolated CMC-*g*-β-CDs are presented in Fig. 4. Chitosan has shown characteristic intense peaks at $2\theta = 10°$ and 20° due to its crystalline nature. The peak at $2\theta = 10°$ was assigned to crystal forms I. The strongest peak appears at $2\theta = 20°$, which correspond to crystal forms II (Pham, Rinaudo, & Desbriers, 1994). Whereas in thiolated CMC-*g*-β-CDs, the intensities of these peaks were decreased and a new broad peak appeared at around $2\theta = 27°$. These results demonstrated that after the chemical modification of chitosan followed by grafting with CMEH and β-CD, the original crystallinity of chitosan was destroyed. This indicates that thiolated CMC-*g*-β-CDs are more amorphous in nature, which can be used to improve the biodegradability and mucoadhesive properties of the polymers.

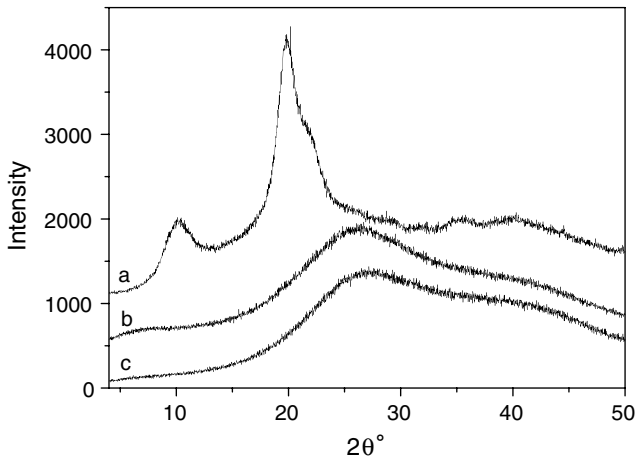


Fig. 4. XRD pattern of (a) chitosan, (b) thiolated CMC-*g*-β-CD1, and (c) thiolated CMC-*g*-β-CD2.

3.4. Swelling behavior

The swelling behavior of mucoadhesive polymers greatly influences their adhesive, cohesiveness, and drug release properties (Mortazavi & Smart, 1993). With the absorbing, swelling, and capillary effects, mucoadhesive polymers should take water from the underlying mucosal tissue, which leads to considerably strong adhesion (Duchene & Ponchel, 1992). To evaluate this effect, swelling studies were carried with chitosan and thiolated CMC-*g*-β-CD tablets. The water uptake of chitosan and thiolated CMC-*g*-β-CD tablets in acidic (pH 6.0) buffer solution at 37 °C is shown in Fig. 5. It is evident that the character of swelling curves for both chitosan and thiolated CMC-

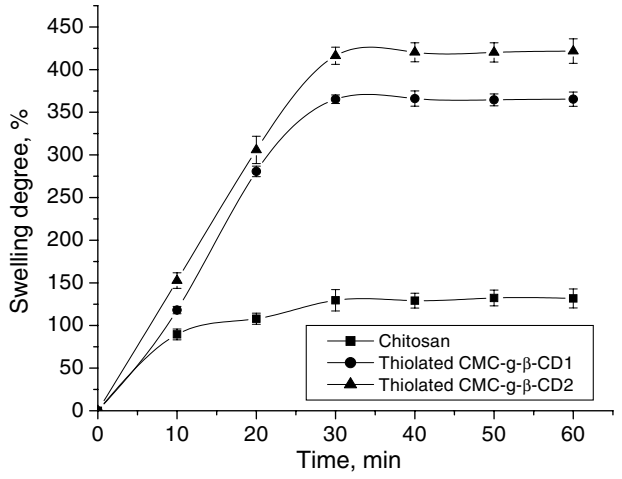


Fig. 5. Swelling behavior of chitosan and thiolated CMC-*g*-β-CD tablets at pH 6.0 and 37 °C.

g-β-CD tablets changes significantly over time and swollen tablets retained their integrity and water uptake even after 30 min. The swelling degree and the rate of swelling of thiolated CMC-*g*-β-CD tablets were found to be higher than that of chitosan tablets. Moreover, the swelling of thiolated CMC-*g*-β-CD tablets reached a stable equilibrium more slowly than chitosan tablets. A rapid swelling behavior of thiolated CMC-*g*-β-CD tablets would favor the interdiffusion process between the polymer and the mucus layer, which could result in a stronger adhesion. The increased swelling behavior of thiolated CMC-*g*-β-CDs may be due to the chemical modification of chitosan followed by grafting with CMEH and β-CD on its backbone. It is well known that the chemical modification can increase the swelling property of chitosan by decreasing its crystallinity (Jayakumar et al., 2005). Among the thiolated CMC-*g*-β-CDs, the swelling degree of thiolated CMC-*g*-β-CD2 was found to be slightly higher than that of thiolated CMC-*g*-β-CD1. This result indicates that the amount of CMEH groups present on the polymer chain also influence the swelling behavior. A marginal decrease in the swelling degree of thiolated CMC-*g*-β-CD1 may be due to the presence of more CMEH groups that led to a higher cross-linking density between the polymer chains through the disulfide bondings.

3.5. Mucoadhesive studies

The mucoadhesion studies with chitosan and thiolated CMC-*g*-β-CD tablets were carried out using freshly excised intestinal mouse mucosa in, pH 6.0, PBS solution. This method should correlate better with the *in vivo* situation, as it concurrently imitates the adhesion and cohesiveness of the polymer in a physiological medium (Kafedjiiski, Foger, Werle, & Bernkop-Schnurch, 2005). Table 2 shows that the adhesion time of thiolated CMC-*g*-β-CD tablets was higher than that of control chitosan tablets. The adhesion time of the thiolated CMC-*g*-β-CD tablets on mucosal tissue increased with increasing amounts of CMEH attached on the polymer from 126 up to 203 min. Tablets based on control chitosan remained on the mucosa for only 41 min. These results clearly showed the strong influence of covalently attached CMEH on the mucoadhesive properties of the polymer. Because the immobilized thiol groups are capable of forming disulfide bonds with mucus glycoproteins by thiol/disulfide exchange reactions and/or a simple oxidation process (Bernkop-Schnurch, Kast, & Richter, 2001), the mucoadhesiveness of the thiolated CMC-*g*-β-CD tablets increased fivefold compared with that of the unmodified chitosan tablet.

Table 2
Mucoadhesive properties of chitosan and thiolated CMC-*g*-β-CDs

Polymer	Time, min (±SD)	Improvement ratio
Chitosan	41 (±6)	1
Thiolated CMC-*g*-β-CD1	203 (±11.7)	5.0
Thiolated CMC-*g*-β-CD2	126 (±6)	3.1

Another possible mechanism for the improved mucoadhesive properties of these thiolated polymers is based on their *in situ* cross-linking properties. During and after the interpenetration process, disulfide bonds are formed within the polymer matrix, leading to additional anchors via chaining up with the mucus gel layer (Bernkop-Schnurch, 2005). In this study, the improvement ratio was calculated by the adhesion time of the thiolated CMC-*g*-β-CD tablets versus the adhesion time of the chitosan tablets.

3.6. Drug release studies

The release of ketoprofen from chitosan and thiolated CMC-*g*-β-CD tablets was carried out in an acidic (pH 6.0) buffer solution at 37 °C. Fig. 6 shows the release profiles of ketoprofen from chitosan, thiolated CMC-*g*-β-CD1, and two tablets loaded with 12.75, 12.92, and 13.12

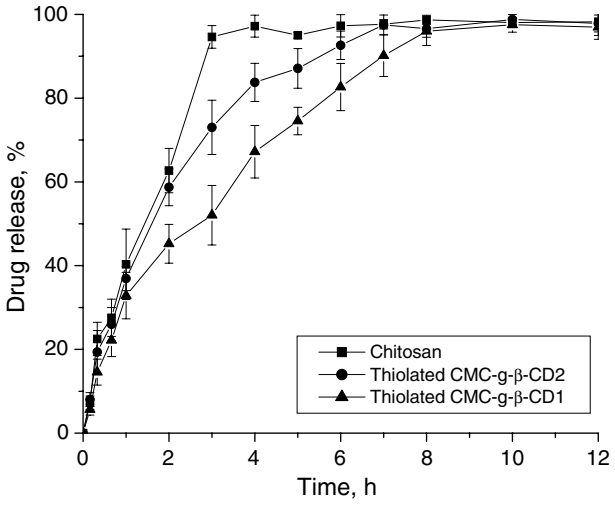


Fig. 6. Release of ketoprofen from chitosan and thiolated CMC-*g*-β-CD tablets.

wt% of drug as a function of time, respectively. The results showed that the drug release rate from all types of the tablets was quite fast during the initial period of time. About 35–40% of the drug was released in about 30 min. Such an abrupt release was due to the free drug remaining at the surface that was not entrapped efficiently within the polymer matrix. After this initial burst, thiolated CMC-*g*-β-CD tablets encapsulated with the drug presented a slow and steady release into the buffer and that equilibrium was reached after 7 h.

In contrast to these results, chitosan tablets, which are unable to form inclusion complexes, released the ketoprofen much faster: most of the drug was released within 3 h. The slow release of hydrophobic drug from thiolated CMC-*g*-β-CD tablets could be attributed to host–guest complex formation between ketoprofen and β-CD present on the polymer chain. There are different factors involved in the complex formation of thiolated CMC-*g*-β-CD with ketoprofen, including (1) the van der Waals interaction between the hydrophobic moiety of the guest molecules and the β-CD cavity; (2) the hydrogen bonding between the polar functional groups of drug molecules and the hydroxyl groups of β-CD; and (3) the release of high-energy water molecules from the cavity during the complex formation (Bibby, Davies, & Tucker, 2000; Ross & Rekharsky, 1996).

Fig. 6 also shows that the rate of drug release from thiolated CMC-*g*-β-CD tablets depends on the contents of their CMEH groups. The rate of release from thiolated CMC-*g*-β-CD2 tablets was found to be slightly higher than that of thiolated CMC-*g*-β-CD1. This can be explained by considering the rate of diffusion from the swollen tablets. As previously discussed, due to the higher disulfide cross-linking density, there is a limited swelling of thiolated CMC-*g*-β-CD1 tablets when compared with thiolated CMC-*g*-β-CD2 tablets, which inhibits the diffusion of drugs at a faster rate as it occurs in thiolated CMC-*g*-β-CD2.

The mechanisms of drug release from polymeric systems have been discussed in detail previously (Baker, 1987; Seiller, Martini, & Benita, 1996; Washington, 1996). The three most common mechanisms by which the release of drug from these systems occurs are erosion, diffusion, and swelling followed by diffusion. Erosion may take place via hydration or hydrolysis of the bulk with the polymer being slowly degraded starting at the periphery of the particle. Diffusion can occur through the non-hydrated polymer matrix, but it will generally be facilitated as the polymer gradually swells when it comes into contact with the body fluids.

In the case of thiolated CMC-*g*-β-CD tablets, it was observed that drug release is faster during the initial period of time. Thereafter, a constant and much slower release rate is observed. It seems that the release obeys a swelling controlled release mechanism, especially at this initial period of release. After this initial period, in which the swelling equilibrium is achieved, the release is most probably followed by a diffusion-controlled mechanism. Also, in this diffusion step the release of the drug from the thiolated CMC-*g*-β-CD tablets was slower than in chitosan due to the higher interaction between the drug and the β-CD groups, as previously discussed.

4. Conclusions

Novel thiolated CMC-*g*-β-CDs were successfully prepared by grafting CM β-CD and CMEH onto CMC using an EDC and NHS-mediated coupling reaction. The product was confirmed by FT-IR and ^{1}H NMR spectroscopy and characterized using XRD analysis. The covalent attachment of CMEH onto CMC-*g*-β-CD leads to strongly improved mucoadhesive properties of the polymer by forming inter and/or intramolecular disulfide bonds, which is verified by mucoadhesive studies. The improved mucoadhesive properties could provide stability and prolonged residence time of the polymer-drug molecules on the mucosal tissues. The swelling property of thiolated CMC-*g*-β-CDs was found to have increased because of the chemical modification. This improved swelling character can be contributed to the mucoadhesiveness of the thiolated polymers. The results of ketoprofen release experiments indicate that this system seems to be a very promising vehicle for the administration of controlled release of hydrophobic drugs.

The ability of β-CD present in the thiolated polymers to form host–guest inclusion complexes with the drug molecules and disulfide cross-linking between the polymer chains could be the main reasons for the slow and steady release of the drug from thiolated CMC-*g*-β-CD tablets. It is believed that the release of drug may be controlled by swelling followed by a diffusion-controlled mechanism. Overall, it is evident that thiolated CMC-*g*-β-CD could be used as controlled drug delivery carriers with the improved mucoadhesive properties.

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