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Research paper

Release characteristics of salmon calcitonin from dextran hydrogels for colon-specific delivery

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Abstract

Biodegradable dextran hydrogels were synthesized by crosslinking dextran (T-70) with epichlorohydrin (ECH) for the in vitro colon-specific delivery of salmon calcitonin (sCT). Crosslinking reaction was performed in 2.8 M NaOH solution both in the presence and absence of ethanol at 10 and 23 °C. Biodegradation kinetics of dextran hydrogels were studied and, in the presence of 0.7 IU ml^{-1} dextranase, dextran discs lost 71.0% and 56.5% of their dry weight within 80 h at pH 5.5 and 7.0, respectively. sCT was derivatized with the fluorescamine (FSM) at borate buffer (pH 9.0) and the quantitative determinations were performed using spectrofluorimetric method (λ_{ex}: 390 nm, λ_{em}: 475 nm). In vitro release studies for the hydrogels prepared in the presence of ethanol were carried out in simulated gastrointestinal fluids. Results indicated that 84.9% of the loaded-sCT was released for 17 h and dextran hydrogel prepared in the presence of ethanol may be a good delivery device for the colon-specific delivery of other peptide-type drugs as well as sCT.

Keywords: Dextran hydrogel; Epichlorohydrin; Salmon calcitonin; Fluorescamine; Derivatization colon-specific delivery

1. Introduction

The colon is largely being investigated as a site for administration of protein and peptides, which are degraded by digestive enzymes in the upper gastrointestinal tract. In addition, drug administration to the site of action for local diseases of the colon does not only reduce the dose to be administered but also decreases the side effects [1].

In this study, we proposed a colon-specific delivery device for the oral administration of salmon calcitonin (sCT). sCT is a polypeptide hormone comprised of 32 amino acids. The major physiological role of the sCT is to control the calcium concentration as well as its metabolism in the body. sCT, due to its ability to reduce osteoclast activity, is commonly used in the treatment of bone diseases such as Paget's disease, hypercalcemia and osteoporosis [2].

sCT is administered via parenteral and nasal routes since it is hydrolyzed by enzymes and acidic medium in the gastrointestinal tract. Because of its short half-life, current studies have been focused on delivery systems which serve to protect and stabilize the sCT and release it in a controlled way by using biodegradable and injectable systems [3,4]. Natural biodegradable polymers have also been widely used as delivery systems for peptides and proteins [5]. There are also some studies related with the oral delivery of sCT and hCT (human calcitonin) using non-biodegradable polymers including different grades of Carbopol® polymers and poly(*N*-isopropylacrylamide-co-butyl methacrylate-co-acrylic acid), a temperature and pH-sensitive hydrogel [6,7]. Crosslinked poly(methacrylic acid) grafted with poly(ethylene glycol) (PMAA-g-EG) gels were also used as possible oral delivery system for sCT [8]. However, because of the low intestinal absorption and susceptibility of sCT to the hydrolysis and digestion by acids

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and enzymes present in the stomach and small intestine, delivery of sCT is still a challenging problem.

Polysaccharide hydrogels have been extensively used as a colon-specific delivery system because of the fact that they degrade at the targeted sites [9]. They are also highly stable, safe, non-toxic and hydrophilic. Because of these special properties, dextran, as a polysaccharide, was determined as the delivery device for the sCT in this study. It is widely used in pharmaceutical field since it is water soluble and inert in biological systems. Besides, it is hydrolyzed by dextranases present in the colon. Because of the presence of these enzymes only in the colon, the use of biodegradable polymers for the colon-specific drug delivery seems to be a more site-specific approach compared to other approaches [10]. For the purpose of drug delivery, dextran hydrogels in different types and compositions were synthesized by several researchers [11–13].

For the quantitative determination of sCT, several methods have been used including HPLC with fluorescence detection [14], fluorescence spectroscopy, after derivatizing with 5-(and 6)carboxytetramethyl rhodamine,-succinimidylester [15] and post-column fluorescence HPLC methods [16,17].

In the proposed method, loaded and released-amounts of sCT into and from the dextran–ECH hydrogels were determined using a newly developed spectrofluorimetric method in which sCT was derivatized with fluorescamine (FSM), a derivatizing agent. It has several advantages over other amine derivatizing reagents, including its commercial availability in pure form, ease of handling, high fluorescence quantum yield and higher rate of reactivity with primary amines, peptides and proteins [16]. In addition, FSM and its hydrolysis products are non-fluorescent and in less than a minute excess reagent is destroyed [18].

The proposed dextran–ECH hydrogel has been successfully applied for the in vitro colon-specific delivery of sCT and a spectrofluorimetric method has been used for the quantitative determinations of sCT.

2. Materials and methods

2.1. Apparatus

A double-beam UV/vis Spectrophotometer, Shimadzu UV-160A, with data processing capacity was used to get spectrum of derivatized sCT (Tokyo, Japan). Shimadzu RF 5301 PC Spectrofluorimeter equipped with a 150 W Xenon-high pressure lamp was also used throughout the study (Tokyo, Japan). Excitation and emission slits were set to 5 nm. All release studies were carried out in a shaker agitating at 50 rpm, Nüve ST 402 (Ankara, Turkey). pH measurements were performed by using an Orion Model 720 A with a combined electrode (Beverly, MA 01915 USA). For the polymerization process, an incubator, Nüve ES 110, operating in the temperature range of −10 to 80 °C was used (Ankara, Turkey).

2.2. Chemicals

sCT (MW: 3432 Da.) was kindly donated by Novartis Company (Ringaskiddy, Ireland). The medical grade dextran (T-70), fluorescamine, FSM and dextranase (EC 3.2.1.11, 165 U mg^{-1}) from *penicillium species* were purchased from Sigma (Steinheim, Germany). Epichlorohydrin, ECH, ethanol and boric acid were obtained from Fluka (Steinheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany).

2.3. Preparation of dextran hydrogels

For the preparation of dextran hydrogel, 0.2000 g dextran polymer (T-70) was dissolved in 1.00 ml of 2.80 M NaOH solution and after adding 0.170 ml ECH, it was mixed thoroughly in a vortex mixer. This mixture was poured into Pyrex glass moulds and it lasted 2–3 days to obtain crosslinked dextran hydrogels at 10 °C and room temperature (23 ± 0.5 °C). Diameter and height of the dried-discs were 0.5 and 0.2 cm, respectively. In the second part, ECH was first mixed with equal volume of ethanol (0.170 ml) and then this mixture was added into aqueous dextran solution and mixed thoroughly in vortex mixer. This experiment was conducted at 10 °C in an incubator and crosslinking reaction was completed in 3–4 days. After the polymerization procedure was completed, dextran–ECH discs were put into distilled water and washed for 2 days in order to remove unreacted epichlorohydrin and dextran polymer as well as excess NaOH.

2.4. Swelling studies

Swelling properties of dextran–ECH hydrogels, in the form of disc, were explored by placing the dried samples into 50.0 ml reservoirs containing simulated gastric fluid (pH 2.0), simulated intestinal fluid (pH 7.4) and acetate buffer (pH 3.4). Swelling studies were conducted in a shaker agitating at 50 rpm at 37 °C. At certain time intervals, dextran–ECH discs were taken out of the solutions, and the swollen weight of each disc at time t (W_s) was determined by weighing in an electronic balance after blotting.

The percent water content for the dextran–ECH discs was calculated using the following equation:

$$\text{Percent water content} = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where W_d is the dry weight of disc.

2.5. Degradation kinetics of dextran–ECH hydrogels

Dry dextran–ECH discs were put into 5.0 ml, 0.7 IU ml^{-1}, dextranase solutions after weighing in an analytical balance. These studies were carried out at pH 5.5 and 7.0 phosphate buffer solutions (66 mM) in a shaker agitating at 50 rpm at 37 °C. At certain time intervals, discs were taken out of the dextranase-containing solutions,

dried at 37 °C until they reached constant weight and then placed again into dextranase solution after weighing. The degradability of the hydrogels was evaluated by percent degradation calculated as remaining dry weight in percent which was found using the following equation:

$$\text{Remaining dry weight}(\%) = \frac{W_t}{W_i} \times 100 \quad (2)$$

where W_i is the initial weight and W_t is the weight of hydrogel after time t.

2.6. sCT assay

Aliquots (50–500 μl) from 0.1 mg ml^{-1} sCT stock solution prepared in acetate buffer (pH 3.4) were placed into 10.0 ml volumetric flasks and mixed with 4.0 ml phosphate buffer (pH 7.4) and 3.0 ml borate buffer (pH 9.00, 100 mM), respectively. FSM solution, derivatizing reagent, in acetone (0.125 mg ml^{-1}), 0.75 ml, was added into each volumetric flask. It was shaken vigorously for 30 s and then derivatization was allowed to proceed for three more minutes after adjusting the volume to 10.0 ml with distilled water. Finally, fluorescence spectra and intensities of the derivatized sCT solutions were measured by setting excitation monochromator, emission monochromator and slit widths to 390, 475 and 5 nm, respectively. Standard solutions of the derivatized sCT were in the range of 0.5–5.0 μg ml^{-1}.

2.7. Characterization of derivatized sCT by UV/vis and fluorescence spectroscopy

Absorption spectrum of derivatized sCT was recorded in the range of 300 to 550 nm with UV/vis spectrophotometer and excitation and emission spectra were also obtained using spectrofluorimeter to prove that derivatization process was successfully performed.

2.8. sCT loading into the dextran–ECH hydrogels by soaking procedure

Previously dried dextran–ECH discs were placed into 6.0 ml of 0.1 mg ml^{-1} sCT solutions prepared using acetate buffer (pH 3.4). sCT loading process proceeded for 28 h in an incubator at 10 °C. 300 μl samples were taken from the loading medium before and after loading process to determine the initial and remaining amounts of sCT. Derivatization was performed with the FSM reagent and fluorescence values were obtained. Finally, by subtracting the remaining amount of sCT in the loading medium from the initial amount, quantity of loaded-sCT into each disc was determined by referring to the calibration curve.

2.9. In vitro sCT release studies

After drying at 23 °C, sCT-loaded discs were placed into 20.0 ml volumetric flasks and then release studies were conducted in 10.0 ml phosphate buffer solutions at pH 7.4. The release studies were also realized in the simulated gastrointestinal system. For that purpose, sCT- loaded discs were first immersed into simulated gastric fluid (pH 2.0) for 2 h, followed by simulated intestinal fluid (pH 7.4) for 3 h, and then followed by simulated colonic environment (pH 7.0) containing dextranase (0.7 IU ml^{-1}) for 14 h. All release studies were performed in a shaker agitating at 50 rpm at 37 °C. At certain time intervals, 4.0 ml of the releasing medium was taken by a micropipette and replaced with fresh buffer solution. The released amount of sCT was then determined using spectrofluorimeter after derivatizing sCT with FSM.

3. Results and discussion

3.1. Crosslinking reactions of the dextran polymer

Dextran polymer was crosslinked with the ECH since it is soluble in water and cannot be used as a delivery device without changing its chemical composition. Crosslinking reaction was carried out in a medium containing 2.8 M NaOH at 10 and 23 °C, respectively. It is known that ECH and ether polyols in basic medium are used in the synthesis of epoxy resins [19] and this type of reaction is conducted in the presence of NaOH, which is an acceptor for the HCl product of dehydrochlorination of chlorohydrine. In the synthesis of dextran–ECH hydrogel, dextran was used as a polyether polyol and an alcolate (dextran–ONa) formation occurs in the presence of NaOH. The chlorohydrin fragments formed are transformed to epoxy group by dehydrochlorination and finally, crosslinked-dextran hydrogel was obtained [11].

3.1.1. Effect of ethanol in the preparation of crosslinked dextran hydrogels

During the crosslinking reaction of dextran with ECH, it was determined that there was a mixing problem between aqueous dextran solution (polar) and ECH (non-polar), crosslinker, and this caused reaction of dextran with ECH to be failed in 2/3 of the moulds, inspite of the fact that reaction medium was mixed thoroughly in the vortex mixer. In order to get rid of this problem, we took the advantage of miscibility of ECH with ethanol and also that of ethanol with dextran solution. For this reason, ECH was first mixed with ethanol and then this mixture was added into the aqueous dextran solution. Ethanol helped ECH to mix better with the aqueous dextran solution and the reaction between ECH and dextran was improved, resulting in successfully conducted reactions in approximately all of the moulds.

3.1.2. Effect of temperature on crosslinking reaction

During the crosslinking studies of dextran with ECH, two different temperature values were tested. Crosslinking reactions in the absence of ethanol were conducted at 23 °C, room temperature. In the presence of ethanol, same

temperature value was tried but crosslinking reactions failed. So, it was decided to test a lower temperature, 10 °C. Depending on the temperature change, crosslinking reaction was achieved successfully in the presence of ethanol at 10 °C in all of the moulds. In order to understand whether main effect in the improvement of the reaction was due to the ethanol or temperature, same reaction was also performed in the absence of ethanol at 10 °C and it was observed that reaction between dextran and ECH failed again approximately in 2/3 of the moulds. From these results, it was concluded that reaction between dextran and ECH could be improved by the addition of ethanol into the reaction medium at 10 °C.

3.2. Swelling properties of the dextran–ECH hydrogels

For the characterization of synthesized dextran hydrogels, swelling properties of dextran–ECH hydrogels (at pHs 2.0, 3.4 and 7.4) prepared in the absence and presence of ethanol were compared. pH 2.0 and 7.4 were chosen to simulate gastric and intestinal fluids, respectively. In addition, swelling studies were also conducted in acetate buffer (pH 3.4) since it was the pH value used to load sCT into the dextran–ECH hydrogels. As far as drug loading is concerned, swelling properties are of importance because sCT is loaded into the dextran–ECH discs by embedding aqueous sCT solution. Swelling kinetics of the dextran–ECH hydrogels prepared in the absence and presence of ethanol at three different pHs are shown in Figs. 1a–c, respectively. Equilibrium water contents of hydrogels at pH 7.4 prepared in the presence and absence of ethanol were compared by Student's t test. Since $t_{\text{theoretical}} < t_{\text{calculated}}$ ($t_{\text{theoretical}} = 2.13$ and $t_{\text{calculated}} = 4.30$; $P = 0.05$), it was concluded that there was a significant decrease in the extent of equilibrium water contents of dextran–ECH hydrogels prepared in the presence of ethanol. For example, equilibrium water contents at pH 7.4 were 592 ± 32 and $829 \pm 90\%$ for the dextran hydrogels prepared with and without ethanol, Fig. 1c, respectively. That much high difference in equilibrium water contents, at pH 7.4, can be attributed to the effect of ethanol addition improving the reaction between crosslinker, ECH, and dextran polymer.

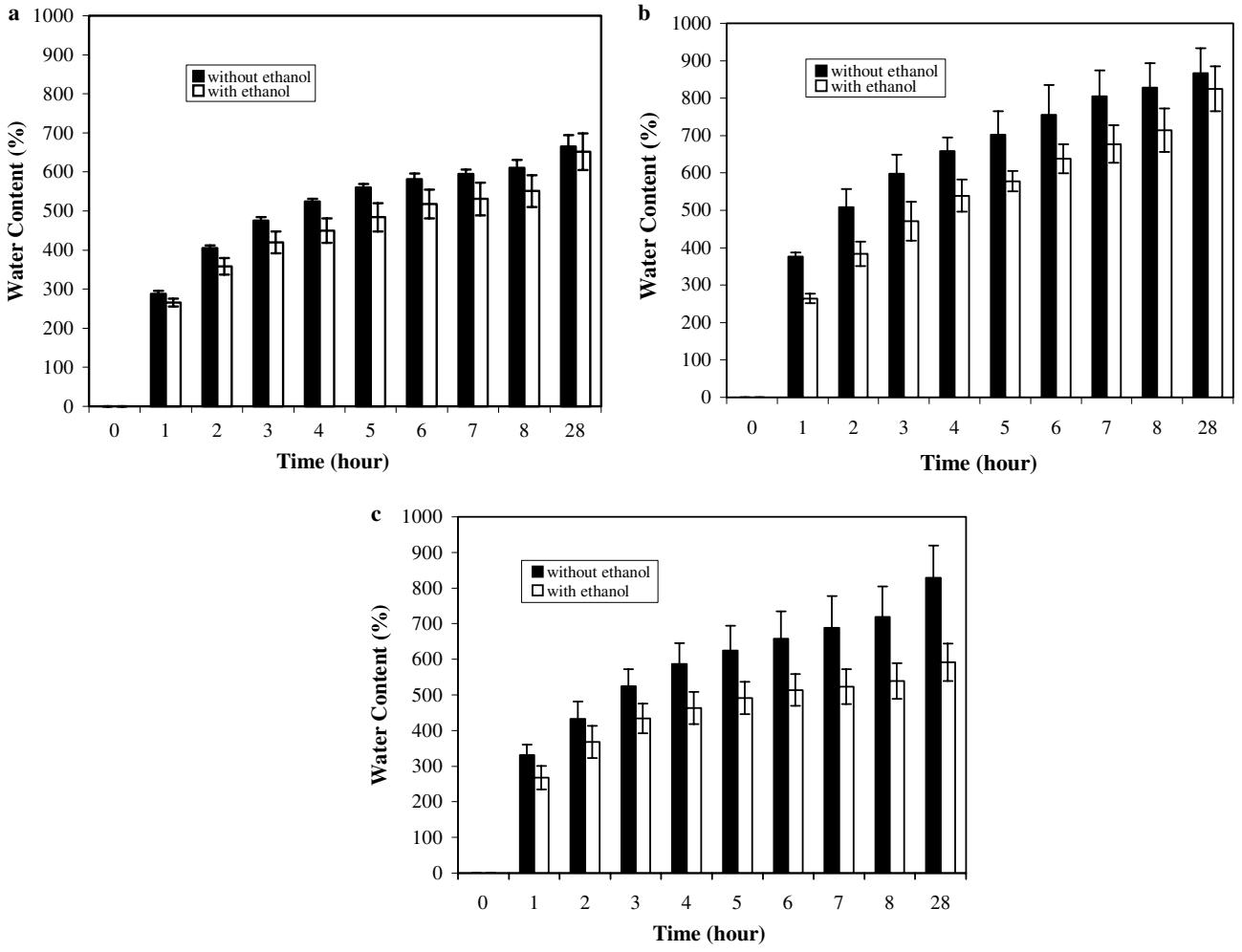

Fig. 1. Swelling kinetics of dextran–ECH discs prepared in the presence and absence of ethanol in (a) pH 2.0 phosphate buffer solution, (b) pH 3.4 acetate buffer solution and (c) pH 7.4 phosphate buffer solution.

When equilibrium swelling values in Fig. 1 were compared, highest swelling values were reached at pH 3.4, acetate buffer. This result showed that highest amount of sCT could be loaded into the dextran–ECH discs at this pH value because sCT loading is a swelling controlled process. From these results, it can be concluded that addition of ethanol into the reaction medium improved the reaction between ECH and dextran and this resulted in the lower swelling values for the dextran–ECH hydrogels.

3.3. In vitro biodegradation kinetics of dextran–ECH hydrogels

Because dextran–ECH hydrogel was prepared to release sCT into the colon by degradation of the hydrogel with dextranase present in the colon, the degradation profile of the dextran hydrogels in dextranase-containing medium is of special importance. Degradation behavior of gels was evaluated as the remaining dry weight of the hydrogels in percent of initial dry weight. Degradation was studied in two different pH values in order to test the degradation of dextran hydrogel, at the pH value dextranase has the highest activity (pH 5.5) and at the simulated colonic environment (pH 7.0). Dextranase degrades dextran chains, by degrading glucosidic linkages, with enzymatic hydrolysis into the oligosaccharides and then weight loss occurs via diffusion of these oligosaccharides from the hydrogel. As depicted in Fig. 2, dextran–ECH hydrogel, in the presence of 0.7 IU ml^{-1} dextranase, lost 71.0% of its dry weight at pH 5.5, while 56.5% weight loss was observed at pH 7.0 within 80 h. This result clearly indicated that degradation of dextran hydrogel in dextranase solution was significantly affected by the medium pH. There are also some studies in the literature investigating degradation kinetics of dextran hydrogels [20]. In these studies, dextran was crosslinked with 1,6-hexamethylene diisocynate and it was found that dextran hydrogel lost 76% of its dry weight after 48 h incubation and after 72 h of incubation it was completely degraded at pH 7.8. In another study, initial particle volume of hydroxyethyl methacrylated dextran hydrogel was reduced by 46% after 3 days and by 95% after 7 days in the presence of dextranase at pH 8.0 [21]. So, it is apparent that our results are consistent with the literature values.

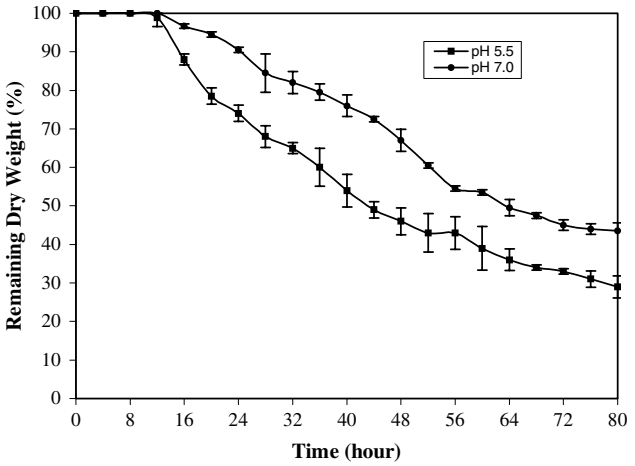

Fig. 2. Biodegradation kinetics of dextran–ECH discs prepared in the presence of ethanol in dextranase (0.7 IU ml^{-1}) containing buffer solutions having different pH values.

3.4. Derivatization of sCT with FSM

There are many instances where the molecule of interest is nonfluorescent or where the intrinsic fluorescence is not adequate for the desired experiment. sCT contains only one aromatic amino acid, tyrosine, but it has not enough intrinsic fluorescence. For this reason, quantitative determination of sCT has been performed using HPLC with absorption detection [22]. It has UV absorption at 210–220 nm due to peptide bond; but there is a selectivity problem at this wavelength range. In addition, it may also be determined at the 250–280 nm wavelength range; however, in this case, sensitivity problem arises. In order to get rid of sensitivity and selectivity problems, it was decided to derivatize sCT with FSM, a derivatizing reagent, to determine it using spectrofluorimetric method. FSM has high fluorescence quantum yield and FSM and its hydrolysis products are non-fluorescent [18]. Derivatization reaction of sCT with FSM is represented in Fig. 3. In this reaction, FSM reacted with sCT amino group at the N-terminus in a borate buffer (pH 9.0) reaction medium. Although previous FSM assays for peptides and proteins have employed phosphate buffer at pH 7–8 [18], we have used borate buffer for the derivatization of sCT since it has greater buffering capacity and it provides high reaction rate for the FSM and sCT [23].

Several experiments were carried out for the optimization of derivatization and calibration conditions for the sCT. One of the significant results of these optimization studies was that mixing the reaction medium in a vortex mixer resulted in deviations in the calibration of derivatized sCT, resulting in low values of correlation coefficients. So, when reaction medium in a volumetric flask was mixed, for a short period of time, by shaking with hand, instead of vortex mixer, relatively high correlation coefficient ($R^2 = 0.9955$) value was obtained. We concluded that mixing the reaction medium in a vortex mixer had a negative influence on the chemical stability of sCT since peptides differ from conventional chemical entities in their sensitivity to numerous experimental conditions, such as temperature,

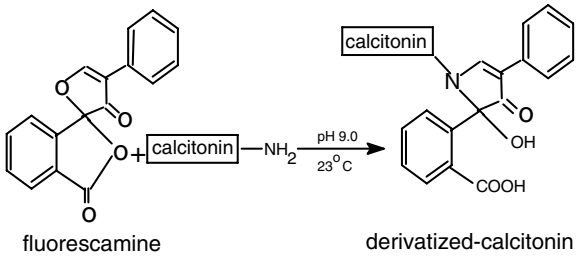

Fig. 3. Derivatization reaction of sCT with fluorescamine at borate buffer, pH 9.0.

pH and shaking. Another important result was the influence of borate buffer. Borate buffer in the range of 3.0–8.5 ml (100 mM) was used during the optimization study. High fluorescence signal and correlation coefficient values were obtained using 3.0 ml of borate buffer. This result was also consistent with the study conducted by Zhu et al. in that fluorescence intensity decreased three times when the concentration of borax was increased threefold in the derivatization of peptides with FSM [24]. These conclusions show that the rate of FSM hydrolysis is increased at high buffer concentrations. Another parameter in the optimization study was the concentration of FSM in the reaction solution. FSM solutions in the range of 0.75–2.5 ml of 0.125 mg ml^{-1} were tried and lowest blank signal and high fluorescence signal for the derivatized sCT were obtained using 0.75 ml of 0.125 mg ml^{-1} FSM solution.

3.5. Characterization of derivatized sCT

In order to see whether derivatization reaction was successful or not, absorption spectrum of the derivatized sCT was recorded and as can be seen from Fig. 4, there is an absorption maximum at 390 nm. In addition, excitation and emission spectra were also obtained and

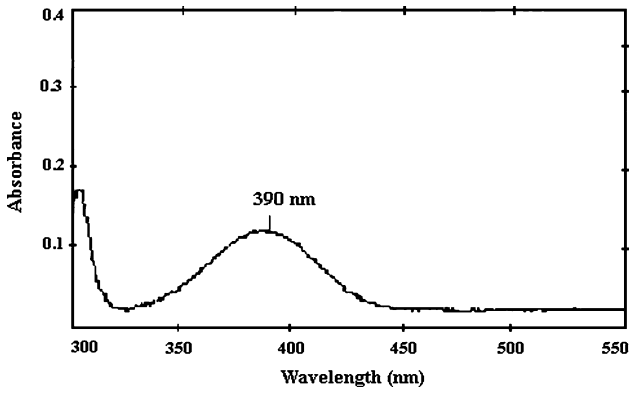

Fig. 4. Absorption spectrum of derivatized sCT (50 μg ml^{-1}).

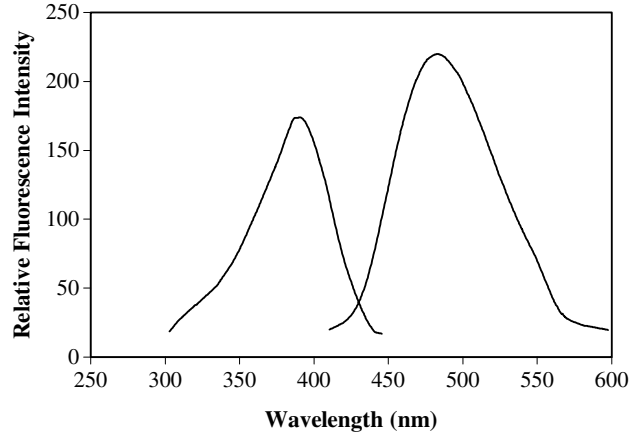

Fig. 5. Excitation ($\lambda_{max} = 390$ nm) and emission ($\lambda_{max} = 475$ nm) spectra of derivatized sCT (3 μg/ml).

are represented in Fig. 5. These three spectra showed that derivatization of sCT was successfully achieved and excitation and emission wavelengths for the derivatized-sCT were determined to be 390 and 475 nm, respectively. Although 50 μg ml^{-1} sCT was used, approximately 17 times more concentrated than the one used in getting excitation and emission spectra, to obtain absorption spectrum, resulting signal from the spectrophotometer was relatively low. This result explains why we decided to use spectrofluorimetric method instead of spectrophotometric method for the determination of sCT.

3.6. sCT loading into dextran–ECH discs

sCT was loaded into the dextran–ECH discs prepared in the presence and absence of ethanol by soaking procedure. Average weight, diameter and height of the discs were 420 ± 15 mg, 0.5 and 0.2 cm, respectively. Before doing the loading process, optimum loading conditions were determined. Since the temperature was the effective parameter in the stability of sCT, loading process was carried out at 10 °C. Loading process was performed in 10 mM acetate buffer in which sCT has the highest stability [25]. Furthermore, the pH of the acetate buffer was adjusted to 3.4 because the most stable pH for the sCT is in the range of 3–4 [26]. As far as swelling properties of the dextran–ECH hydrogels in three different media (pH 2.0, 3.4 and 7.4) were concerned, highest percent equilibrium swelling value (at 28th hour) was obtained in acetate buffer (pH 3.4), Fig. 1. For this reason, it was apparent that highest amount of sCT would be loaded in acetate buffer medium. Since the equilibrium swelling values for the dextran–ECH hydrogels (825%) prepared in the presence and absence of ethanol (867%) at pH 3.4 were very close to each other, loaded amounts of sCT into the discs were approximately the same. Mean loaded amounts of sCT into the discs prepared in the absence and presence of ethanol were 125 ± 5 and 127 ± 10 μg $disc^{-1}$, respectively. The results were reported as the average values of the three loading studies.

3.7. Release behavior of sCT from the dextran–ECH discs

3.7.1. Effect of ethanol on sCT release

sCT release from the dextran–ECH hydrogels prepared in the presence and absence of ethanol was compared. As shown in Fig. 6, sCT release from the dextran–ECH disc, prepared in the absence of ethanol, lasted 12 h and 98% of the loaded-sCT was released at pH 7.4. On the other hand, sCT release from the dextran–ECH disc prepared in the presence of ethanol was 67.6% in 12 h and reached 89.8% in 21 h. Results clearly indicated that longer release profile was obtained with the dextran hydrogels prepared in the presence of ethanol, owing to the low swelling values obtained as a result of improved reaction between dextran and ECH. In addition, long release profile is of importance as far as effectiveness of patient care and short half-life of sCT are concerned.

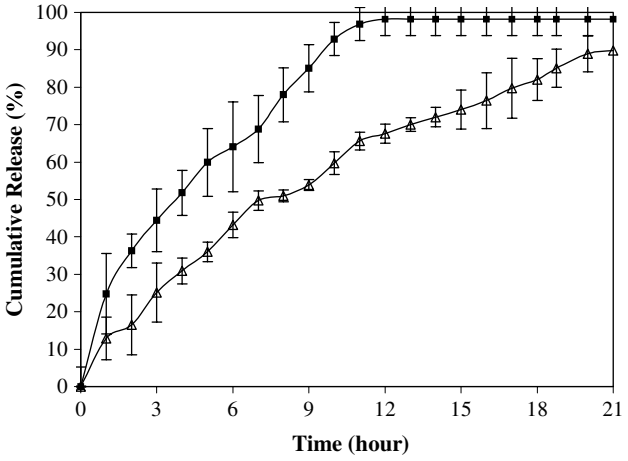

Fig. 6. Cummulative release of sCT from dextran–ECH discs prepared in the presence of ethanol (△) and in the absence of ethanol (■) at phosphate buffer, pH 7.4.

3.7.2. In vitro sCT release in the simulated human gastrointestinal system

In this part, effect of dextranase in the release profile of sCT from dextran–ECH discs prepared in the presence of ethanol was investigated using simulated gastric, intestinal and colonic environments. Two independent release studies were conducted in the presence and absence of dextranase, respectively. As shown in Fig. 7, at the end of the fifth hour, one of the sCT-loaded discs was put into the simulated colonic fluid-containing dextranase, 0.7 IU ml^{-1} while the other one stayed in phosphate buffer solution (pH 7.4) containing no dextranase. As it can be seen, when no dextranase was present, up to 5 h, release rate was slow. However, after transferring the disc to the colonic medium containing 0.7 IU ml^{-1} dextranase, release rate increased rapidly and reached 49% due to degradation of dextran hydrogel into oligosaccharides in 7 h. On the other hand, release rate was only 33.2% in the medium containing no dextranase in 7 h. The rapid increase in the release rate was ascribed to the degradation of dextran hydrogel by dextranase in the simulated colonic medium. Release lasted 17 h and cumulative release was found to be 84.9% in medium with dextranase. But, sCT release in the medium containing no dextranase was 63.2% in 17 h. Since release measurements were not performed in the simulated gastric medium for 2 h, it degrades at this pH value, some amount of the loaded sCT may have released here or may be entrapped in the dextran hydrogels. Similar studies were conducted by other researchers using hydrogels composed of poly(methacrylic acid) (PMAA) grafted with poly(ethylene glycol) which can be used as drug delivery carriers for sCT [8] and they found that sCT release from the p(MAA-g-EG) hydrogel continued for 100 h and approximately 100% of sCT was released in phosphate buffer solution (pH 7.0). Another group prepared poly(ether-ester)s composed of hydrophilic poly(ethylene glycol)-terephtalate (PEGT) blocks and hydrophobic poly(butylene terephthalate) (PBT) blocks as matrix for the controlled release of sCT [27]. sCT release from this polymer matrix lasted 2.5 days and reached approximately 10–65% depending on the copolymer composition at pH 7.4. As far as those released amounts of sCT from the polymer matrices were compared to our values, it was seen that cumulative sCT release, in percent, in our study was consistent with the ones in the literature.

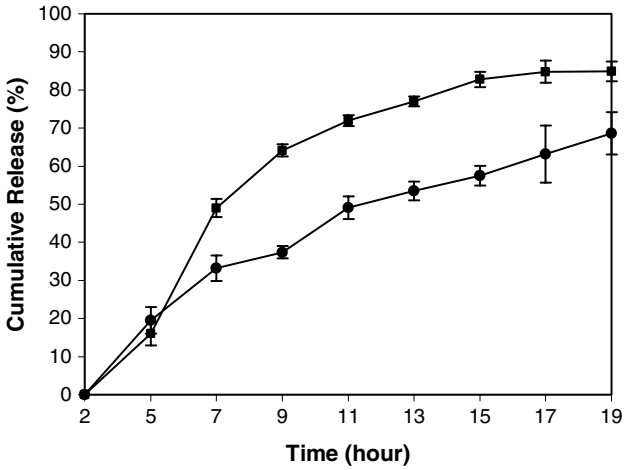

Fig. 7. Release profile of sCT from dextran hydrogels prepared in the presence of ethanol in media with (■) and without (●) dextranase.

In this paper, we demonstrated that degradable dextran hydrogels were suitable systems for the colon-specific delivery of sCT. The extent and the swelling rate of the hydrogels were reduced with the help of ethanol and as a result of this, longer release profile was obtained. Furthermore, release rate increased sharply by the degradation of glucosidic linkages of dextran hydrogels in colonic medium containing dextranase. Release characteristics of sCT from dextran hydrogels are comparable to the ones described in the literature and dextran hydrogels synthesized may be suitable delivery devices for other peptide-type drugs as well as sCT. Furthermore, derivatization of sCT with FSM was successfully achieved and a new easy, fast, sensitive and selective spectrofluorimetric method was developed for the determination of sCT. This fluorimetric method can also be applied for the determination of other pharmaceuticals containing primary amine groups.

Acknowledgements

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