In Vitro Release Kinetics of Bovine Serum Albumin from Highly Swellable Dextran Hydrogels

Dilek İmren,[1] Menemşe Gümüşderelioğlu,[2] Ali Güner[3]

[1]*Chemical Engineering Department, Cumhuriyet University, Sivas, Turkey 58140*
[2]*Chemical Engineering Department, Hacettepe University, Beytepe, Ankara, Turkey 06800*
[3]*Chemistry Department, Hacettepe University, Beytepe, Ankara, Turkey 06800*

Received 11 January 2009; accepted 22 June 2009
DOI 10.1002/app.31042
Published online 10 September 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Hydrogels synthesized from a polysaccharide-based polymer, dextran, in the presence of two crosslinking agents, *N,N′*-methylenebisacrylamide and epichlorohydrin, were evaluated for the oral colon-specific delivery of polypeptide drugs. These novel dextran hydrogels had significantly greater swelling ratios than recently developed dextran hydrogels. A model protein, bovine serum albumin, was loaded into 50% (by weight) crosslinker-containing dextran hydrogels in two ways: during the crosslinking reaction and by a soaking method. The loading capacity was varied between 22 and 25 mg/g of dry gel, depending on the loading procedure. *In vitro* release experiments were performed with a simulated gastrointestinal system in the presence and absence of dextranase. The diffusion exponents were calculated by means of a semiempirical power-law equation for the release of protein from swellable hydrogel discs. Bovine serum albumin was mainly released by Fickian diffusion, and this indicated that its hydrodynamic diameter (7.7 nm) was smaller than the hydrogel mesh size (~19 nm). The release of bovine serum albumin from both hydrogel types was substantially higher than expected, especially in the presence of dextranase, and this was attributed to the high swellability of the hydrogels. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 740–747, 2010

Key words: biopolymers; diffusion; drug delivery systems; hydrogels; proteins

INTRODUCTION

Pharmaceutically active proteins are increasingly being used as drugs for the treatment of various diseases. The effective application of these drugs requires special delivery methods. Because the acidic environment and the proteolytic enzymes of the gastrointestinal tract (GIT) cause the rapid degradation of protein drugs, simple oral administration is not possible. In addition, proteins have a short half-life in the blood circulation, and this necessitates repeated injections of a drug to have a therapeutic effect.[1] An option for overcoming the aforementioned problems is the use of delivery systems consisting of polymeric networks in which the proteins are loaded and from which they are gradually released. Colon-specific drug delivery is of special interest for protein drugs that are unstable in the upper GIT because the amount of digestive enzymes in the colon is significantly lower than that in the upper GIT.[2,3]

In the past 2 decades, many systems have been proposed to deliver drugs specifically to the colon.[4–9] Among them, dextran hydrogels are mostly preferred because they are degradable by special kinds of microbial enzymes called dextranases, which are present in the colon.[10–13] It is known that dextran is easily crosslinked with various functional organic and inorganic compounds with the formation of swollen aqueous gels.[14–17] Degradable dextran hydrogels have been prepared by the co-entrapment of dextranase in the gels[18] and by the induction of hydrolyzable groups into the crosslinks.[12] In addition, pH-responsive dextran hydrogels have been developed to protect proteins from enzymatic hydrolysis in the upper GIT and enhance drug release in the colon via extensive gel swelling and degradation.[19–21] Biodegradable and pH-sensitive hydrogels have been widely applied for protein delivery,[22–24] and there is increasing interest in these hydrogels.[25,26] The release behavior of a number of proteins from both degradable and pH-sensitive dextran hydrogels has been studied by several researchers.[18,27–29]

Recently, Güner et al.[30] studied crosslinking reactions of dextran with epichlorohydrin (ECH), phosphorus oxychloride, *N,N′*-methylenebisacrylamide (MBAm), and ^{60}Co γ-irradiation. In our previous

Correspondence to: M. Gümüşderelioğlu (menemse@hacettepe.edu.tr).

Contract grant sponsor: Turkish Scientific and Research Council; contract grant number: MİSAG-247.

study, we prepared dextran hydrogels by crosslinking dextran with ECH, glutaraldehyde, and MBAm.[31] Doğan et al.[32] investigated the controlled release of epidermal growth factor and basic fibroblast growth factor from dextran hydrogels crosslinked with ECH. The objective of this article is to compare the suitability of dextran hydrogels prepared with two different crosslinkers, MBAm and ECH, as possible drug carriers for colon-specific drug delivery by an investigation of the release of a model protein, bovine serum albumin (BSA). The factors affecting the protein release profile were studied and evaluated.

EXPERIMENTAL

Materials

The dextran used in this study (T-70) was purchased from Sigma (Schnelldorf, Germany). The weight average and number-average molecular weights were determined by the manufacturer to be 70,000 and 46,800 g/mol, respectively. The crosslinking agents MBAm and ECH were obtained from Sigma. Sodium hydroxide was used in the preparation of an alkaline medium for the crosslinking reactions, and it was supplied by Sigma. Hydrochloride acid (37%) and 2-amino-2hydroxymethyl-1,3-propanediol (Tris) buffer used in the protein release medium were obtained from Merck (Darmstadt, Germany). Dextranase D-1508 from *Penicillium* spp (EC 3.2.1.11; specific activity = 500 U/mg of protein) and sodium azide were obtained from Sigma. All chemicals were analytical-grade and were used as received.

Synthesis and characterization of the dextran hydrogels

The synthesis of the dextran hydrogels from dextran–crosslinker mixtures was carried out through intermolecular side-chain reactions of dextran hydroxyl groups with monomeric crosslinking agents such as MBAm and ECH in alkaline solutions with an appropriate temperature program. In a typical experiment, dextran was dissolved in distilled water containing 2.8*M* NaOH at a concentration of 20 (w/v) %. MBAm or ECH (50 wt %) was added to the dextran solution, which was stirred magnetically for 10 min. The polymer mixture was then poured into glass tubes 6 mm in diameter. In the case of MBAm, the crosslinking reaction was completed in 24 h at 25 ± 0.5°C. For ECH-containing gels, crosslinking was completed in 2 h at 25°C and then in 60 h at 37.5°C. The hydrogels were removed from the tubes and cut into discs (diameter = 6 mm, thickness = 3 mm). Then, they were washed thoroughly in distilled water with stirring for 7 days to remove the uncrosslinked dextran and excess of crosslinkers. Washed hydrogel discs were dried *in vacuo* (EV 018, Nüve, Ankara, Turkey) at 25°C until no weight loss could be detected. The dry discs were kept hermetically until further use.

The time-dependent swelling ratios of the hydrogels were determined at pHs 2.0 and 7.0. The preweighed dry hydrogel discs were immersed in 25-mL Tris buffer solutions at 37°C. Changes in the weights of the hydrogel discs were recorded at time intervals until equilibrium swelling was reached. The swelling of the hydrogels was expressed as the swelling ratio with the following equation:

$$\text{Swelling ratio} = [(M_t - M_0)/M_0] \quad (1)$$

where M_0 is the dry weight of the hydrogel (initial weight) and M_t is the weight of the swollen gel at time t. In addition, the initial swelling rate of the hydrogels was determined by means of second-order kinetics proposed by Schott.[33]

A scanning electron microscope (JSM-840 A, JEOL, Tokyo, Japan) was used to analyze the surfaces and cross sections of the dextran hydrogels. The hydrogel disc samples were prepared for microscopy via drying *in vacuo* from the swollen state. The dry disc samples were mounted onto stubs and sputtered with gold.

The molecular weight between crosslinks (M_c) was determined by density measurements according to the well-known Flory–Rehner equation[34] as modified by Peppas and Merrill[35] for gels in which crosslinks are introduced in solution. The details of the synthesis method and the characterization of the dextran hydrogels have been reported by us previously.[31]

BSA as a model protein

BSA (weight-average molecular weight = 65,000 Da; Fraction V, Sigma) was used as a model protein. The hydrodynamic diameter of BSA was calculated with the Einstein–Stokes equation:

$$D_0 = kT/3\pi\eta d \quad (2)$$

where η is the viscosity of the solvent; k is the Boltzmann constant; T is the absolute temperature; and d and D_0 are the hydrodynamic diameter and diffusion coefficient of the protein; respectively. The reported D_0 value was 5.9×10^{-7} cm^2/s for BSA.[36,37] With the Einstein–Stokes equation, the hydrodynamic diameter of BSA was calculated to be 7.7 nm.

A BSA assay was performed with a fluorescence spectrophotometer (RF 1501, Shimadzu, Tokyo, Japan). All measurements were made with freshly prepared solutions of BSA in PBS with 1-cm^2 quartz cells. The intrinsic tryptophan fluorescence was

observed from protein solutions at 280-nm excitation and 332-nm emission wavelengths. The calibration results showed that concentrations up to 3 μg/mL fit linear curves, and the minimum detectable concentration was 0.1 μg/mL.

Preparation of BSA-loaded dextran hydrogel discs

Crosslinked dextran discs containing BSA were prepared according to two frequently used methods. The first method was the incorporation of proteins after the formation of the hydrogel network by soaking. Hydrogel discs containing 50% (w/w) crosslinker were allowed to swell until equilibrium was reached at 37°C in a Tris buffer (pH 7.0) containing three different BSA concentrations (0.5, 1.0, and 5.0 mg of BSA/mL of buffer). Experiments in which the loading time was varied showed that 48 h was sufficient for complete loading. The concentrations of the loading solutions were determined fluorimetrically. The amount of protein loaded onto each hydrogel disc was calculated from the difference between the original and final reservoir concentrations, and it was presented as milligrams of protein per gram of dry hydrogel. This solution loading technique is commonly used to incorporate solutes at concentrations below the maximum solubility limit, as described by Carelli et al.[38] At the end of 48 h, the hydrogel discs were dip-rinsed in a buffer to wash off any protein solution on the gel surface, and free surface water was blotted with filter paper. The gels were then dried at room temperature *in vacuo* until the weight was constant and then stored in an evacuated desiccator for 24 h.

The second method was the incorporation of proteins during the formation of the hydrogel network (during the crosslinking reaction). Dextran was dissolved in a Tris buffer (pH 7.0) containing 10 mg of BSA/mL. The crosslinking agent, MBAm or ECH, was added afterwards. The mixture was poured into a glass tube and allowed to react in an incubator with the same temperature program given before for the hydrogel preparation; this yielded BSA-loaded dextran hydrogel discs. The discs were removed from glass tubes, blotted with filter paper, and dried at room temperature *in vacuo* until the weight was constant. The dry protein-loaded discs were kept hermetically until further use.

In vitro release study of BSA from dextran hydrogel discs

The release of BSA from the hydrogels was investigated in a model gastrointestinal system containing 0.02% sodium azide under perfect sink conditions. Protein-loaded dry hydrogel discs were immersed in a Tris buffer at pH 2.0 for 2 h (simulating gastric medium). Then, the samples were transferred to a Tris buffer at pH 7.0 (simulating intestinal medium). Incubation was conducted by rotation at 50 rpm on a shaker (S400, Nüve) thermostated at 37°C. Samples were withdrawn at 15-min intervals initially and at 1-h intervals later and replaced with fresh release medium. The released amount of BSA from each hydrogel disc was determined with a fluorescence spectrophotometer in the same way as the loading process.

The release studies were also realized in the presence of dextranase under the same conditions described previously. In the fourth hour of the release experiment, a dextranase solution in the range of 0.01–0.5 U/mL was added to the release medium. The cumulative amount of released BSA from each hydrogel disc was determined in the same way described previously.

All experiments (loading and release) were realized in BSA-precoated vessels to prevent the adsorption of BSA onto vessel walls, and they were repeated at least three times. The results are reported as average values.

RESULTS AND DISCUSSION

Synthesis and characterization of the dextran hydrogels

Dextrans are colloidal, hydrophilic, and water-soluble substances, are inert in biological systems, and do not affect cell viability. Because of these properties, they have been used for many years as carrier systems for a variety of therapeutic agents, including antibiotics, anticancer drugs, peptides, proteins, and enzymes. Several approaches to preparing dextran hydrogels have been adopted.[14–21] The dextran hydrogels that were used in this study were prepared with MBAm and ECH acting as the crosslinkers. The crosslinking reaction of dextran–MBAm can be realized with the simultaneous opening of acrylic double bonds in the presence of NaOH. Because MBAm is a polyfunctional monomeric crosslinker, dextran is crosslinked with MBAm through a side-chain reaction of dextran hydroxyl groups with amine groups of MBAm.[30,31] In the first stage of the reaction of dextran with ECH, the opening of epoxy groups with the formation of free chlorohydrin fragments in the side chain of linear macromolecules proceeds. The chlorohydrin fragments that are formed can be easily transformed into an epoxy functionality by dehydrochlorination in the presence of NaOH. A dehydrochlorination reaction between two macromolecules containing OH and Cl substitutes, respectively, is realized, providing the formation of a crosslinking structure.[30,31]

TABLE I
Structural Characteristics and Swelling Properties of the Synthesized Dextran Hydrogels

Hydrogel	M_c (g/mol)	Hydrogel mesh size (nm)	Initial swelling rate		Equilibrium swelling ratio (g of water/g of gel)	
			pH 2.0	pH 7.0	pH 2.0	pH 7.0
Dextran–MBAm	7385	19.23	0.120	0.161	12.00	14.17
Dextran–ECH	7128	19.02	0.088	0.133	9.20	12.55

In our previous study,[31] we prepared a number of dextran hydrogels by varying the amounts of the crosslinker in the polymer mixture. After taking into account the swelling and mechanical properties of all those hydrogels, in this study, we decided to use only the gels prepared with a 50 wt % crosslinker concentration.

The synthesized dextran hydrogels demonstrated typical characteristic properties (Table I). The value of M_c for the dextran hydrogels was estimated according to the Flory–Rehner model. The M_c values for both kinds of hydrogels are higher than 5000 g/mol; thus, the hydrogels synthesized here can be considered loosely crosslinked networks. The hydrogel mesh size is an important parameter for predicting the release behavior of a macromolecule (e.g., protein release from hydrogels). The calculated mesh size of the hydrogels is about 19 nm; this means that it will not likely result in a screening effect for most protein drugs because the hydrodynamic diameters of most proteins are smaller than 19 nm (e.g., that for BSA was calculated to be 7.7 nm). The equilibrium swelling ratios of both hydrogel types, dextran–MBAm and dextran–ECH, are higher in a basic medium than an acidic medium; however, dextran–MBAm hydrogels have higher swelling ratios and higher initial swelling rates than dextran–ECH hydrogels (Table I). The state of equilibrium is reached within 4 h. Although dextran–MBAm hydrogels show average equilibrium swelling ratios between 12.0 (at pH 2) and 14.0 (at pH 7), dextran–ECH hydrogels show ratios between 9.0 (at pH 2) and 12.5 (at pH 7). Both hydrogels (crosslinked with MBAm or ECH) show higher swelling ability at pH 7 than pH 2. This behavior may be explained by the basic character of hydrogels.[30] At an acidic pH, a hydrogel has an affinity to protons. Then, protonation leads to the formation of intermolecular hydrogen bonds by hydrogen-acceptor groups (etheric oxygen, hydroxyl, and amide groups) of the hydrogel. As a result, the hydrogel does not show high swelling behavior at pH 2. However, in a neutral medium (pH 7), protonation does not occur; thus, water diffusion occurs, and swelling reaches higher values than those at pH 2. The equilibrium swelling values of both hydrogel types used in our study are significantly greater than those observed by Chiu et al.[20] for the acrylic acid containing dextran hydrogels and about 10 times higher than those observed by Hennink et al.[11] for glycidyl methacrylate containing dextran hydrogels.

Loading of dextran hydrogels with BSA as a model protein drug

Protein drugs are loaded into hydrogels by one of the two common methods. In the first methods, hydrogels are prepared in the presence of drugs (the crosslinking procedure). However, this method has several disadvantages, such as the toxic effects of initiators, monomers, and crosslinkers on the system and the activity loss of protein drugs due to the temperature increase during polymerization. On the other hand, this method is often satisfactory for loading a high protein dose. The second method includes the loading of drugs by the swelling of hydrogels in a drug-containing solution (the soaking procedure). Unfortunately, this method leads to poor loading because of the diffusion limitations, which depend on the mesh size of the hydrogel network. In this study, we decided to use both methods for BSA loading. The hydrogel preparation method used here includes only the crosslinking reaction, which is realized in the presence of a crosslinker and NaOH at room temperature. Thus, most of the disadvantages of the first method discussed previously should be eliminated. The high swelling rates and swelling ratios of the dextran hydrogels synthesized here also eliminate the drawbacks of the second procedure.

Dextran–MBAm and dextran–ECH hydrogels were loaded with 25 mg of BSA/g of dry hydrogel during crosslinking. This is the highest amount of loadable protein that does not cause the disintegration of the hydrogel structure.

The soaking procedure was realized with three concentrations of the loading solution (i.e., 0.5, 1.0, and 5.0 mg/mL BSA solutions). However, dextran–MBAm hydrogels lost their stability in the loading solution containing BSA. Güner et al.[30] showed that the stability of crosslinks formed by ECH is higher than that of crosslinks formed by MBAm. This is the

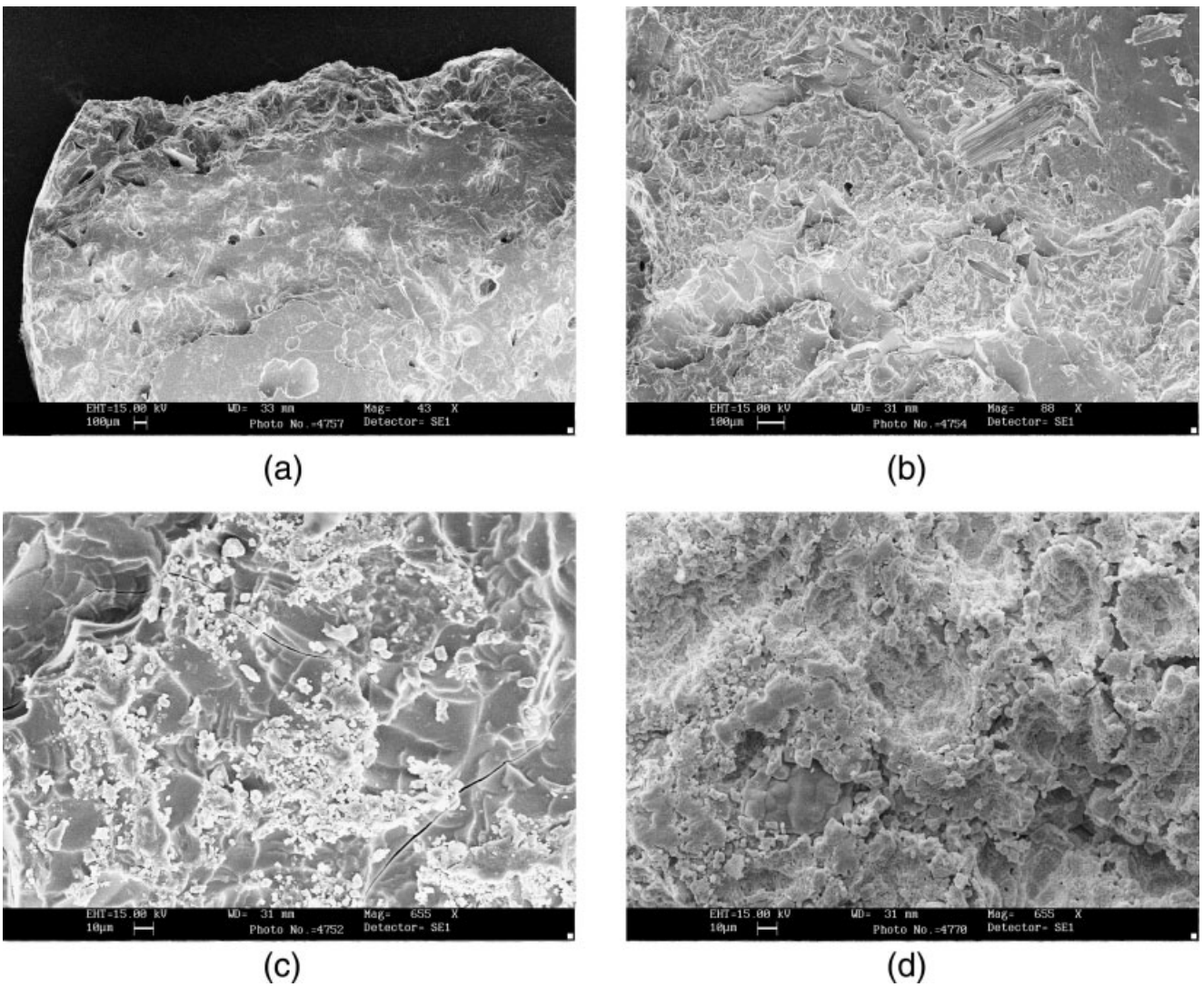

Figure 1 SEM images of BSA-loaded dextran hydrogel discs: (a) a general view of a BSA-loaded dextran–ECH hydrogel during a crosslinking reaction (magnification = 43×), (b) a cross section of the same hydrogel (magnification = 88×), (c) a cross section of the same hydrogel (magnification = 655×), and (d) a dextran–MBAm hydrogel (magnification = 655×).

reason that, in the presence of BSA in the loading medium (pH 7), BSA interacts strongly with the amide groups of MBAm. As a result, the hydrogels disintegrate. Thus, only dextran–ECH hydrogels were loaded with BSA with the soaking procedure. Increased solute loading concentrations above 1.0 mg/mL did not change the amount of the drug loaded in each hydrogel. The results showed that approximately 22 ± 0.6 mg of BSA was loaded into each gram of dry hydrogel. Taking into account the pH-dependent water sorption properties of the hydrogels, we loaded BSA at pH 7, which allowed high swelling and thus high loading capacity without causing denaturation of BSA molecules. The loading of hydrogels by swelling in a protein-containing buffer solution depends on the size of the hydrogel mesh versus the size of the protein and on the swelling properties of the hydrogel. BSA diffusion into the dextran–ECH hydrogel is significantly realized because the mesh size of the hydrogel (19.02 nm) is larger than the hydrodynamic diameter of BSA (7.7 nm).

Characterization of BSA-loaded dextran hydrogel discs

The swelling properties of BSA-loaded hydrogels were determined at 37°C in a Tris buffer (pHs 7 and 2). The results showed that there was no detectable difference between the swelling ratios of the protein-loaded and unloaded hydrogels. This may be explained by the absence of osmotic force, which is generated by the water sorption of dissolved protein molecules.

Scanning electron microscopy (SEM) pictures of protein-loaded hydrogel discs are presented in Figure 1(a–d). Figure 1(a) illustrates the appearance of a disc material loaded with BSA during the dextran–ECH

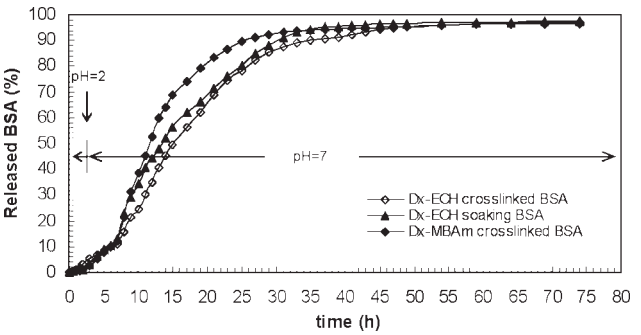

Figure 2 Cumulative release of BSA from dextran (Dx) hydrogels in the model GIT in the absence of dextranase.

hydrogel formation. The material is porous in its native state. The SEM photograph of the internal structure of the same disc shows a porous, filamentous matrix; this allows the transport of BSA molecules through the matrix [Fig. 1(b)]. Figure 1(c) presents a cross-sectional view of the dextran–ECH hydrogel at a higher magnification (655×). It is possible to see the BSA particles within the hydrogel. The solubility of BSA molecules in water is 0.56 g/mL.[39] Although we studied below the maximum solubility limit of BSA during the loading process, the presence of BSA particles within the hydrogel may be attributed to the effect of nonionic polymers on the solubility of globular proteins. Guo and Narsimhan[40] investigated the effect of dextran, a nonionic polymer, on the solubility of BSA, a globular protein. They showed that the solubility of BSA sharply decreased with increasing concentrations of dextran. In the case of dextran–MBAm hydrogels, the structure becomes less ordered and more porous [Fig. 1(d)].

In vitro release of BSA from dextran hydrogels in the GIT model

In the absence of dextranase

Figure 2 presents the cumulative percentage release of BSA from dextran hydrogels as a function of time. It can be clearly seen that the loading procedure did not change the release profile. However, the BSA release rates for the dextran–MBAm hydrogels are higher than those for the dextran–ECH hydrogels because of the highly swellable characteristic of the dextran–MBAm hydrogels. The most important point in Figure 2 is that the cumulative release of BSA was minimal (2%) in a pH 2.0 medium (stomach) for all the hydrogel samples: at the end of the second hour, both hydrogels reached less than 50% of their equilibrium swelling ratios (≈6.0). In addition, 80–90% of BSA was released in 30 h (the minimum retention time in the intestinal tract).

The values of the diffusion exponent (n) were calculated with the semiempirical power-law equation [eq. (3)], as suggested by Ritger and Peppas.[41] This equation is appropriate for the release of drugs from swellable matrices, and it has been frequently used in the literature to describe the drug transport mechanism:

$$M_t/M_\infty - kt^n \tag{3}$$

where M_t is the cumulative amount of released protein at time t, M_∞ is the amount of loaded protein present in the hydrogel network, M_t/M_∞ represents the fractional release of the protein at time t, k is a constant characteristic for the protein-loaded matrix system, and n is a characteristic of the release mechanism.

The n and k values determined from the initial portion of log–log plots of F versus time ($F \leq 0.6$) are presented in Table II together with the regression coefficients. For swellable cylindrical devices, Fickian diffusion corresponds to $n = 0.45$, whereas polymer relaxation occurs at higher n values. n varies between 0.52 and 0.56 for BSA release at pH 7 from all the hydrogel systems (Table II). This demonstrates that the most important mechanism for BSA release is Fickian diffusion at pH 7.

The diffusion coefficients of BSA were calculated with eq. (4) for monolithic devices with cylindrical geometry for $M_t/M_\infty \leq 0.60$:[42]

$$M_t/M_\infty = 4(D_m t/\pi r^2)^{1/2} \tag{4}$$

where D_m is the diffusion coefficient of the protein in the hydrogel and r is the radius of the hydrogel cylinder in the equilibrium state.

TABLE II
Diffusion Parameters Related to BSA Release from Dextran Hydrogels at pH 7.0

Hydrogel	Amount of loaded BSA (mg/g of dry gel)	n	k	$D_m \times 10^7$ (cm^2/s)	R^2
Dextran–MBAm/ crosslinking BSA	25.00 ± 0.09	0.52 ± 0.002	0.046	1.708	0.990
Dextran–ECH/ crosslinking BSA	25.00 ± 0.09	0.56 ± 0.002	0.056	1.531	0.990
Dextran–ECH/ soaking BSA	22.00 ± 0.06	0.53 ± 0.003	0.058	1.542	0.992

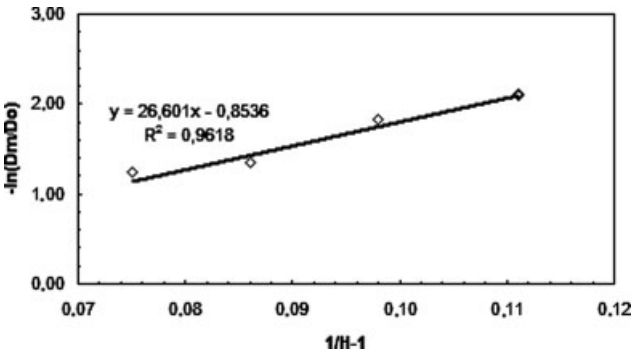

Figure 3 Logarithm of the normalized diffusion coefficients of BSA in dextran–MBAm and dextran–ECH hydrogels as a function of the inverse of the hydrogel hydration.

The D_m values that were calculated from the slopes of F versus time curves are also presented in Table II. The D_m values were quantitatively analyzed with the free volume theory.[43] This theory gives a relationship between the diffusion coefficients of a solute in a hydrogel and the degree of hydration of the gel:

$$\mathrm{Ln}(D_m/D_o) = \ln(\varnothing) - (kr^2/V_{f,\mathrm{water}})(1/H - 1) \qquad (5)$$

where D_o is the diffusion coefficient of the solute in water, Ø indicates the screening effect of the network, r is the hydrodynamic radius of the solute, $V_{f,\mathrm{water}}$ is the free volume in water, k is a constant, and H is the hydration of the hydrogel (the volume fraction of water in the gel).

The free volume theory predicts a linear relationship between $\ln(D_m/D_o)$ and $1/H$ in the absence of any screening effect. For this reason, Figure 3 shows that the diffusion of BSA in the dextran–MBAm and dextran–ECH hydrogels can be described by the free volume theory. This suggests that there is no screening effect, indicating that the mesh size of the hydrogels is larger than the hydrodynamic diameter of BSA at all hydration levels. We also estimated the mesh sizes of the hydrogels from the swelling behavior by the application of the Flory–Rehner theory. The results of both approaches are compatible.

At the beginning of the release experiment, hydrogels are in a nonswollen state, and the mesh sizes are therefore smaller. At the time of maximum swelling, an approximate mesh size of 19 nm was calculated for the dextran–MBAm and dextran–ECH hydrogels. Because it is greater than the hydrodynamic diameter of BSA (7.7 nm), BSA is released by a diffusion-controlled mechanism.

In the presence of dextranase

The release of BSA from dextran hydrogels in the GIT model containing various concentrations of dextranase (0.01–0.5 U/mL) was studied. The results are shown in Figure 4(a–c) together with release data in the absence of dextranase.

When no dextranase is present, the cumulative BSA release is about 90% from all the hydrogels at the end of the 35-h incubation period and is controlled by simple diffusion. In the presence of high concentrations (0.5 U/mL) of dextranase, a fast release of BSA can be observed from all the hydrogels, and 90% of BSA is released in 13, 15, and 21 h from dextran–MBAm, dextran–ECH (crosslinking), and dextran–ECH (soaking) hydrogels, respectively. In other words, the release of BSA from dextran hydrogel discs is enhanced in the presence of dextranase. This can be explained by the enzymatic degradation of the dextran backbone, which results in increased permeability of

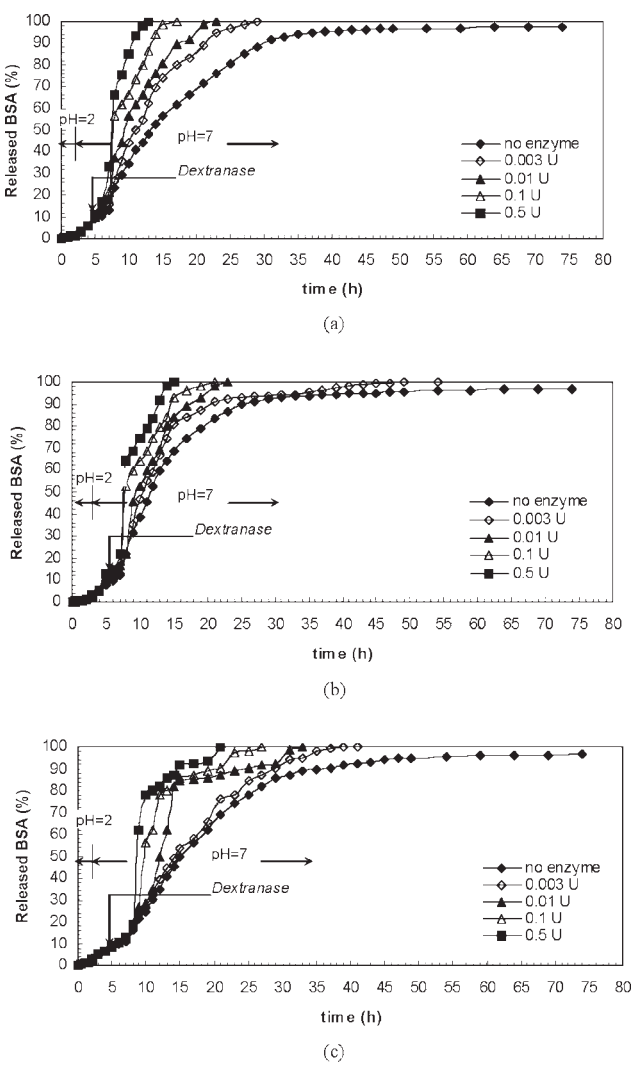

Figure 4 Comparison of the profiles of BSA release from dextran hydrogels in the presence and absence of dextranase in the model GIT: (a) dextran–ECH hydrogels loaded during crosslinking, (b) dextran–ECH hydrogels loaded by soaking, and (c) dextran–MBAm hydrogels loaded during crosslinking.

the hydrogels. Furthermore, it has been observed that the release rate is independent of the loading method. In addition, the release rate changes with respect to the enzyme concentration. Hydrogels prepared from 50% (w/w) solutions of dextran and a crosslinker were dissolved completely in 0.5 U/mL dextranase between 75 and 80 h in the release experiment.

In this study, a BSA assay was performed: the intrinsic tryptophan fluorescence was observed at 280-nm excitation and 332-nm emission wavelengths. Because of the sensitivity of tryptophan emissions to medium conditions, conformational change and denaturation can be easily detected from the fluorescence spectra. During BSA loading and release experiments, these wavelengths were periodically checked, and no deviation was observed. This result suggested that the secondary structure of BSA was preserved during the loading and release processes.

CONCLUSIONS

The results presented in this study demonstrate that highly swellable dextran hydrogels synthesized in the presence of MBAm and ECH can be used for the colon-specific release of proteins. Nearly equal amounts of BSA were loaded into the hydrogels by crosslinking and soaking procedures. This indicated that the loading procedure does not affect the release profile of BSA. The release rate depends on and can be manipulated by the type of crosslinker, release medium pH, and presence of dextranase in the release medium. The diffusion of BSA from these highly swellable gels could be effectively described by the free volume theory. Both hydrogels provide a sufficient lag time for protein release to prevent premature release in the small intestine. Furthermore, a protein can be released completely within the period of colonic residence.

References

1. Manning, C.; Patel, K.; Borchardt, R. T. Pharm Res 1989, 6, 903.
2. Yang, L.; Chu, C.; Fix, J. A. Int J Pharm 2002, 235, 1.
3. Sinha, V. R.; Trehan, A. J Controlled Release 2003, 90, 261.
4. Kopečková, P.; Kopeček, J. Makromol Chem 1990, 191, 2037.
5. Kinget, R.; Kalala, W.; Vervoort, L.; Van den Mooter, G. J Drug Target 1998, 6, 129.
6. Sinha, V. R.; Mittal, B. R.; Bhutani, K. K.; Kumria, R. Int J Pharm 2004, 269, 101.
7. Chen, L. G.; Liu, Z. L.; Zhuo, R. X. Polymer 2005, 46, 6274.
8. Mladenovska, K.; Raicki, R. S.; Janevik, E. I.; Ristoski, T.; Pavlova, M. J.; Kavrakovski, Z.; Dodov, M. G.; Goracinova, K. Int J Pharm 2007, 342, 124.
9. Kaur, K.; Kim, K. Int J Pharm 2009, 366, 140.
10. Simonsen, L.; Howga, L.; Mortensen, P. B.; Brondsted, H. Eur J Pharm Sci 1995, 3, 329.
11. Hennink, W. E.; Franssen, O.; van Dijk-Wolthuis, W. N. E.; Talsma, H. J Controlled Release 1997, 48, 107.
12. de Jong, S. J.; van Eerdenbrugh, B.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. J Controlled Release 2001, 71, 261.
13. Pitarresi, G.; Casadei, M. A.; Mandracchia, D.; Paolicelli, P.; Palumbo, F. S.; Giammona, G. J Controlled Release 2007, 119, 328.
14. Howgaard, L.; Brøndsted, H. J Controlled Release 1995, 36, 159.
15. Kim, S. H.; Won, C. Y.; Chu, C. C. Carbohydr Polym 1999, 40, 183.
16. Zhang, X.; Wu, D.; Chu, C. C. Biomaterials 2004, 25, 4719.
17. Maia, J.; Ferreira, L.; Carvalho, R.; Ramos, M. A.; Gil, M. H. Polymer 2005, 46, 9604.
18. Franssen, O.; Vandervennet, L.; Roders, P.; Hennink, W. E. J Controlled Release 1999, 60, 211.
19. Kim, S. H.; Won, C. Y.; Chu, C. C. J Biomed Mater Res 1999, 46, 160.
20. Chiu, H. C.; Wu, A. T.; Lin, Y. F. Polymer 2001, 42, 1471.
21. Chiu, H. C.; Hsiue, G. H.; Lee, Y. P.; Huang, L. W. J Biomater Sci Polym Ed 1999, 10, 591.
22. Qui, Y.; Park, K. Adv Drug Delivery Rev 2001, 53, 321.
23. Chen, X.; Qian, Z. Y.; Gou, M. L.; Chao, G. T.; Zhang, Y. D.; Gu, Y. C.; Huang, M. J.; Wang, J. W.; Pan, Y. F.; Wei, Y. Q.; Chen, J. P.; Tu, M. J. J Biomed Mater Res A 2008, 84, 589.
24. Ichikawa, H.; Torres-Lugo, M.; Peppas, N. A. AAPS Pharm Sci 2000, S3287.
25. Chao, G. T.; Deng, H. X.; Huang, Q.; Jia, W. J.; Huang, W. X.; Gu, Y. C.; Tan H. P.; Fan, L. Y.; Liu, C. B.; Huang, A. L.; Lei, K.; Gong, C. Y.; Tu, M. J.; Qian, Z. Y. J Polym Res 2006, 13, 349.
26. Chao, G. T.; Qian, Z. Y.; Huang, M. J.; Kan, B.; Gu, Y. C.; Gong, C. Y.; Yang, J. L.; Wang, K.; Dai, M.; Li, X. Y.; Gou, M. L.; Tu, M. J.; Wei, Y. Q. J Biomed Mater Res A 2008, 85, 36.
27. Kamath, K. R.; Park, K. Polym Gels Networks 1995, 3, 243.
28. Franssen, O.; Vos, O. P.; Hennink, W. E. J Controlled Release 1997, 44, 237.
29. Hiemstra, C.; Zhong, Z.; Steenbergen, M. J.; Hennink, W. E.; Feijen, J. J Controlled Release 2007, 122, 71.
30. Güner, A.; Akman, Ö.; Rzaev, Z. M. O. React Funct Polym 2001, 47, 55.
31. İmren, D.; Gümüşderelioğlu, M.; Güner, A. J Appl Polym Sci 2006, 102, 4213.
32. Doğan, A. K.; Gümüşderelioğlu, M.; Aksöz, E. J Biomed Mater Res B 2005, 74, 504.
33. Schoot, H. J Macromol Sci Phys 1992, 31, 1.
34. Flory, P. J.; Rehner, R. J Chem Phys 1943, 11, 521.
35. Peppas, N. A.; Merril, E. W. J Polym Sci Polym Chem Ed 1976, 14, 459.
36. Merril, E. W.; Dennison, K. A.; Sung, C. Biomaterials 1993, 14, 1117.
37. Carvalho, J. M.; Coimbra, M. A.; Gama, F. M. Carbohydr Polym 2009, 75, 322.
38. Carelli, V.; Di Colo, G.; Nannipieri, E.; Serafini, M. F. Int J Pharm 1989, 55, 199.
39. Zhang, X.; Wyss, U. P.; Pichora, D.; Amsden, B.; Goosen, M. F. A. J Controlled Release 1993, 25, 61.
40. Guo, R.; Narsimhan, G. Presented at the Conference of Food Engineering, Chicago, IL, 1995.
41. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 37.
42. Baker, R. W.; Lonsdale, H. K. In Advances in Experimental Medicine and Biology; Tanquary, A. C.; Lacey, R. E., Eds.; Plenum: New York, 1974; Vol. 47, p 15.
43. Sato, S.; Kim, S. W. Int J Pharm 1984, 22, 229.