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Synthesis, characterization of biodegradable dextran–allyl isocyanate–ethylamine/polyethylene glycol–diacrylate hydrogels and their in vitro release of albumin

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Abstract

Based on dextran–allyl isocyanate–ethylamine (Dex-AE) and polyethylene glycol–diacrylate (PEGDA), a pH sensitive biodegradable hydrogel with improved protein release was prepared through UV photo-crosslinking. The Dex-AE precursor was prepared through a two-step chemical modification and characterized by FT-IR and ^{1}H NMR. The effects of reaction conditions on the synthesis of Dex-AE were studied. The interior morphology data by scanning electron microscopy (SEM) revealed that an increase in Dex-AE content led to an initial decrease in pore size of the microstructure of the Dex-AE/PEGDA hydrogels, but a further increase in Dex-AE content resulted in a relatively looser network structure. The swelling data indicated that the swelling ratio depended on the precursor feed ratio and was correlated to the morphology of the microporous network structure. The pH sensitive property of the Dex-AE/PEGDA hydrogels was studied in different pH buffer solutions and was found that these hydrogels were pH sensitive due to the presence of ample pendant amino groups. The ionic strength data demonstrated that the Dex-AE/PEGDA hydrogels exhibit the highest swelling ratio in pure water, but the swelling ratio became lower as the ionic strength of the media increased. The in vitro albumin release from these hydrogels was examined in different pH (3.0, 7.4) buffer solutions. Both the protein loading and release data indicated that the Dex-AE/PEGDA hydrogels had more protein loading and sustained release capability than pure PEGDA hydrogel, and this ability increased as the Dex-AE composition increased; the Dex-AE/PEGDA hydrogel also showed a faster BSA release in a lower pH than a higher pH medium.

Keywords: Dextran; Polyethylene glycol; Hydrogel; Biodegradable; Albumin

1. Introduction

Polyethylene glycol (PEG) is a unique amphiphilic polymer, which has been explored for many biomedical applications because of its well-known hydrophilicity, biocompatibility, and nonbiodegradability. PEG has been employed to improve biocompatibility (Chung, Kim, Kim, & Rhee, 2003; Zhang, Li, Dong, Zhao, & Zhang, 2002), promote peptide immobilization (Hern & Hubbell, 1998; Wang, Tan, Kang, & Neoh, 2002), prolong protein drug circulating time (Greenwald, Choe, McGuire, & Conover, 2003; Koumenis et al., 2000), increase bioactivity (Muslim et al., 2001), and reduce immunogenicity (Hu, Zhai, He, Mei, & Liu, 2002). Interestingly, PEG is not biodegradable but it can be readily excreted from the body via kidney and liver, and forms nontoxic metabolites, which makes it more suitable for drug delivery. Some PEG-based hydrogels are already used as drug carriers and wound care products. Due to the above properties, PEG-based products have been approved by FDA for human intravenous (i.v.), oral and dermal applications (Greenwald et al., 2003).

The potential value of proteins as therapeutics has long been recognized. A wide range of protein-based drugs have become commercially available as therapeutic agents.

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However, many therapeutic proteins suffer from short circulating half time and low stability in vivo, and therefore sustained release of proteins has been difficult. In order to reduce protein loss through kidney filtration, Abuchowski, McCoy, Palczuk, Vanes, and Davis (1977) increased the molecular weight of proteins by conjugating PEG onto proteins, which was later termed as PEGylation. PEGylation can increase the molecular weight of proteins up to 70 kDa, and has significantly increased the circulating half time of proteins in vivo, reduced immunogenicity and antigenicity, and retention of a large portion of proteins' bioactivity. It has been postulated that these effects are due to a shell of PEG macromolecules around the proteins (Greenwald et al., 2003). Such a sheltering could sterically hinder the reaction of immune cells and protects sheltered proteins from proteolytic inactivation.

For years, hydrogels have been proved to be another effective way for protein controlled release (Wu, Zhang, & Chu, 2003; Zhang & Chu, 2002a, 2002b, 2002c, 2002d; Zhang & Chu, 2001). Hydrogels are three-dimensional crosslinked network that can swell dramatically in the presence of water/biological fluids and retain the absorbed liquid while maintaining their structure. Due to their structural similarity to natural living tissues (Ratner & Hoffman, 1976), hydrogels have found various applications in the fields of cell culture, tissue engineering, and controlled drug release. Most hydrogels are not limited to work only as the carriers for controlled release; they can also protect proteins from their hostile environment. In addition, hydrogels also have a good compatibility with proteins (Chen, Jo, & Park, 1995; Molina, Li, Martinez, & Vert, 2001), which makes hydrogels more suitable for protein delivery. Recently, physically crosslinked hydrogels have shown some promising applications in the biomedical fields (Ikada, Jamshidi, Tsuji, & Hyon, 1987; Inoue, Chen, Hoffman, & Nakamae, 1998; Jeong, Kim, & Bae, 2002; Nakamae, Miyata, & Hoffman, 1994). However, the more general approach based on chemical crosslinking for designing biodegradable hydrogels continues to be highly desirable because of their easy manipulation by controlling the crosslinking agents, initiator concentrations, ratio, and concentration of precursors.

Intelligent hydrogels have received more attention than other hydrogels. These types of hydrogels are capable of responding to external stimuli, such as temperature (Chen & Hoffman, 1995; Hoffman, 1987; Peppas & Klier, 1991; Zhang & Chu, 2003, 2004a, 2004b; Zhang, Lewis, & Chu, 2005; Zhang, Sun, Chu, 2004; Zhang, Sun, Wu, & Chu, 2004), pH (Chen et al., 1995; Peppas & Klier, 1991; Zhang, Wu, & Chu, 2004), electric field (Kim, Yoon, Lee, & Kim, 2003; Liu & Calvert, 2000), and photo field (Mamada, Tanaka, Kungwatchakun, & Irie, 1990). Generally, an ideal controlled release mechanism for a drug carrier is the zero-order drug release; namely, the release rate is independent of time. However, it is more desirable if drugs could be administered under temporal modulations, such as temperature, pH, light, and so on to meet some physiological need (Li & D'Emanuele, 2001). It is known that all pH sensitive hydrogels have either acidic or basic groups, which can respond to pH environment by gaining or losing protons. The stomach has a low pH (<3), which is quite different from the neutral intestinal environment, and this has drawn more attention to pH sensitive hydrogels.

Dextran is a biodegradable polysaccharide and composed of linear α-l,6-linked D-glucopyranose residues with a low percentage of α-1,2, α-1,3, and α-l,4 linked side chains (Stenekes, Talsma, & Hennink, 2000). Dextran is a colloidal, hydrophilic, biocompatible, and nontoxic polymer, and can be biodegraded by dextranase (Franssen, Vandervennet, Roders, & Hennink, 1999). From the structure point of view, dextran has chemically active functional groups (i.e., –OH group) that can be chemically modified to form hydrogels via crosslinking. Because of these properties, dextran and their hybrids have been intensively investigated as drug carriers. For examples, dextran-based biomaterials have been employed in cell immobilization (Massia, Stark, & Letbetter, 2000) and gene transfection (Azzam, Eliyahu, Makovitzki, & Domb, 2003) and as carriers for a variety of pharmaceutically active drugs (Chu, 2003; Kim, Jeong, Kim, Lee, & Kim, 2001; Kim & Chu, 2000a, 2000b; Won & Chu, 1998, 2000; Zhang & Chu, 2002a, 2002b, 2002c, 2002d).

In this study, the PEG derivative (PEG–diacrylate, PEGDA) was chemically incorporated into dextran derivative (dextran–allylisocyanate–ethylamine, Dex-AE) via photo-crosslinking to form hybrid biodegradable hydrogels having pH sensitivity. The chemical structure and morphology of the resulting hybrid hydrogels and the effects of reaction time and temperature, the molar ratio of reactants and catalyst on the degree of substitution were examined. The swelling property and protein release profiles of these hybrid hydrogels were studied as a function of the feed ratio of PEGDA to Dex-AE precursors and pH. Bovine serum albumin (BSA) was chosen as the model protein for the controlled release study.

2. Experimental section

2.1. Materials

Dextran (Dex) of MW 43,000, allyl isocyanate (AI), magnesium chloride, and sodium chloride were purchased from Sigma Chemical Company (St. Louis, MO). Dextran was dried in a vacuum oven for 24 h at 50 °C before use. Dimethyl sulfoxide (DMSO), dibutyltin dilaurate (DBTDL), 2-bromoethylamine hydrobromide (BEAHB), triethylamine, acryloyl chloride, and polyethylene glycol (PEG) of MW 8000 and other chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI), and dried in a vacuum oven for 24 h at room temperature before use. 2-Hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-l-propanone (HHPMP) was donated by Ciba Specialty Chemicals Corporation. Bovine serum albumin (BSA) of MW 69,000 was purchased from Sigma Chemical

Company (St. Louis, MO). The buffer solution (PBS) of pH 3 was purchased from Fisher Scientific (Fair Lawn, NJ), and PBSs with pH 7 and 10 were purchased from GFS chemicals (Powell, OH).

2.2. Synthesis of dextran–allyl isocyanate–ethylamine (Dex-AE)

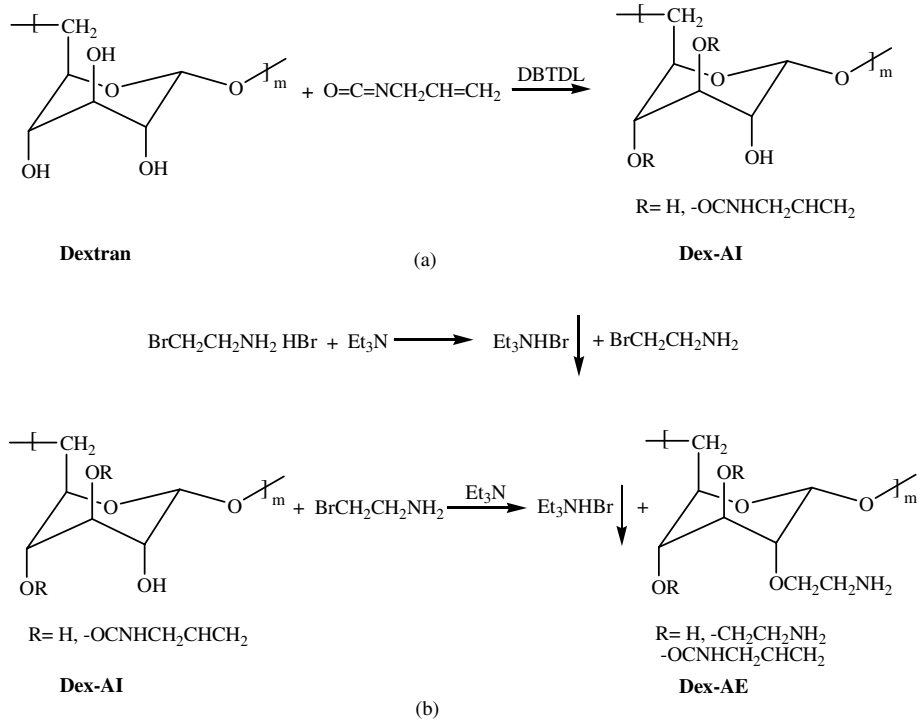
The synthesis of Dex-AE is shown in Scheme 1. The procedures involved two basic steps: the incorporation of allyl isocyanate followed by the incorporation of ethylamine. Dextran-based precursor (Dex-AI) was synthesized and characterized according to our previously published paper (Zhang, Won, & Chu, 2000). Briefly, dextran reacted with allyl isocyanate (AI) in the presence of DBTDL catalyst first. At room temperature (22 °C), dry dextran (e.g., 2 g) was dissolved in anhydrous DMSO (24 mL) under dry nitrogen gas. DBTDL catalyst (0.73 mL) was injected into the solution at room temperature dropwise, and then AI (1.09 mL) was added dropwise. The reaction mixture was stirred at a predetermined temperature (22 °C) and time (6 h). The resulting polymer was precipitated in cold excess isopropanol. The product was further purified by dissolution and precipitation in DMSO and isopropanol, respectively. This resulting Dex-AI (degree of substitution DS 0.25, Table 1) was dried at room temperature under vacuum for a week and stored in a cold dark place before further use.

Dex-AE was synthesized from Dex-AI so that free amine groups could be introduced into dextran-AI. In the preparation of Dex-AE, Dex-AI reacted with 2-bromoethylamine hydrobromide (BEAHB) in the presence of triethylamine. A series of Dex-AE of different DS (the number of ethylamine group per anhydroglucose unit) were synthesized and characterized.

An example of Dex-AE synthesis is given here. At a predetermined temperature (50 °C), dry Dex-AI (2.0 g) was dissolved in anhydrous DMSO (20 mL) under dry nitrogen gas. Triethylamine (11.2 mL) was injected into the above solution. BEAHB (7.5 g) was dissolved in DMSO (10 mL) and then added to the above solution dropwise. This reaction solution was stirred at 50 °C for pre-determined periods (i.e., 2, 4, 6, and 8 h). The reaction mixture was then filtered to remove the precipitated Et_3NH_4Br. The resulting Dex-AE polymer was obtained by precipitating the filtered solution into excess cold isopropyl alcohol. The product was further purified at least three times by dissolution and precipitation with DMSO and cold isopropyl alcohol, respectively. The final product was dried at room temperature under vacuum overnight before further use. The reaction conditions like catalyst, temperature, and time for the synthesis of Dex-AE are listed in Table 1.

2.3. Synthesis of polyethylene glycol–diacrylate

Polyethylene glycol–diacrylate (PEGDA) was synthesized according to published procedures (Wu et al., 2003). In brief, PEG reacted with acryloyl chloride in the presence of triethylamine as a catalyst. At 40 °C, dry PEG (12 g) was dissolved in anhydrous benzene (150 mL) under nitrogen atmosphere. Triethylamine (1.78 mL) and acryloyl chloride (1.22 mL) were subsequently added. The



Scheme 1. Synthesis of Dex-AE.

Table 1
Effect of reaction conditions on the degree of substitution of Dex-AE

Sample	Molar ratio of the reactants[a]		Reaction temperature (°C)	Reaction time (h)	DS[c]
	BEAHB/Dex-AI[b]	Triethylamine/BEAHB[d]			
Dex-AE-1	1.5	2.0	50	4	0.65
				6	1.05
				8	1.25
Dex-AE-2	3.0	2.0	50	4	1.92
				6	2.05
				8	2.15
Dex-AE-3	2.0	2.0	50	6	1.95
Dex-AE-4	1.5	2.0	40	6	1.06
Dex-AE-5	1.5	2.0	60	6	0.85
Dex-AE-6	1.5	2.5	50	6	1.45
Dex-AE-7	1.5	1.5	50	6	1.05

[a] MW of the repeat unit is used when calculating the ratio.
[b] The DS of Dex-AI is 0.25.
[c] Degree of substitution per anhydroglucose unit.
[d] 2-Bromoethylamine hydrobromide.

reaction mixture was stirred for 3 h at 80 °C. The resulting polymer was precipitated in hexane. It was further purified 3 times by dissolution and precipitation with benzene and hexane, respectively.

2.4. Preparation of Dex-AE/PEGDA hydrogel

The preparation of Dex-AE/PEGDA hydrogels is shown in Scheme 2. The objective of this step is to prepare the new biodegradable dextran-based hydrogels through the photo-crosslinking of Dex-AE and PEGDA precursors using a 365 nm long wave UV lamp. The initiator, Irgacure 2959 (2-hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone) was first dissolved in hot water in a vial. The Dex-AE and PEGDA precursors at a predetermined feed ratio were added to the above initiator solution and stirred well at room temperature. The solution obtained was irradiated by a 365 nm long wave 16 W UV lamp for 20 h until gelation. The resulting hydrogels were washed in distilled water for 48 h to remove unreacted precursors. The final gel was dried in a vacuum oven at 60 °C for 2 days before further use. The feed composition and the sample ID are listed in Table 2.

2.5. Characterization

The dextran, dextran-AI, and dextran-AI-EA were all characterized for their chemical structure by FT-IR and ^{1}H NMR. For FT-IR characterization, all samples were dried in a vacuum oven for at least 24 h and then compressed into pellets with KBr powder (1/10, w/w) for FT-IR characterization (Nicolet Magna 560, Madison, SI). For ^{1}H NMR characterization, samples were dissolved in deuterated DMSO (DMSO-d_6) at a concentration of 25% (w/v) and their spectra were recorded on a Varian INOVA 400 MHz spectrometer (Palo Alto, CA). The DMSO peak at 2.50 ppm was used as the reference line. The degree of substitution (DS) was estimated from the ratio of the normalized, integrated intensities of the sum of the hydroxyl group peaks to the normalized, integrated intensities of the anomeric proton peak according to the published procedures (Kim & Chu, 2000a, 2000b; Zhang, Wu et al., 2004).

2.6. Swelling study of Dex-AE/PEGDA hydrogels

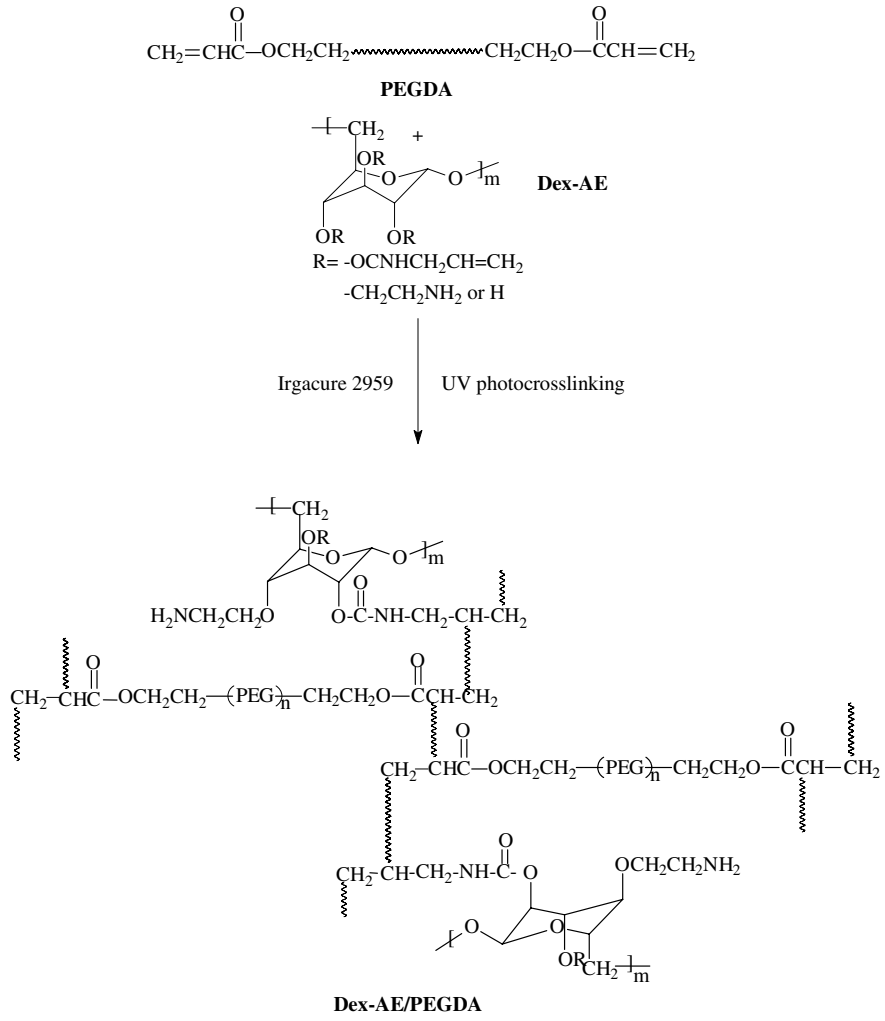
The swelling property of the Dex-AE/PEGDA hydrogels was gravimetrically determined. Dry hydrogel specimens of known weight (W_d) were immersed in distilled water at room temperature (25 °C). The swollen hydrogels were removed from water at predetermined intervals and weighed (W_t) after wiping off excess water on the surface with a wet filter paper. The swelling ratio was then calculated according to the formula below:

$$\text{swelling ratio } (\%) = [(W_r - W_d)/W_d] \times 100.$$

The hydrogel reached an equilibrium swelling state when there was no difference in swelling ratio between two adjacent intervals.

2.7. Morphological study of Dex-AE/PEGDA hydrogels

The interior morphology of the swollen Dex-AE/PEGDA hydrogels at room temperature was studied using a scanning electron microscope (SEM, Leica Cambridge Stereoscan 440, Cambridge, UK). The hydrogel specimens were immersed in distilled water for 24 h and the swollen hydrogels were removed from the solution, quickly frozen in liquid nitrogen, and then freeze-dried in a Virtis Freeze Drier (Gardiner, NY) under vacuum at −50 °C for 3 days until the samples became completely dry prior to morphological observation. The freeze-dried hydrogels were fractured carefully to reveal the interior and mounted onto aluminum stubs with double side carbon tape and sputter-coated (JFC-1200 Fine Coater, Japan) with Au for 40 s. The SEM examination on the interior morphology was conducted at 25 kV and 12 nA.



Scheme 2. Synthesis of Dex-AE/PEGDA hydrogels from Dex-AE and PEGDA.

Table 2
Feed ratio and formation of Dex-AE/PEGDA hydrogels

	Sample ID[a]						
	DEG1	DEG2	DEG3	DEG4	DEG5	DEG6	DEG7
Dex-AE[b] (g)	0	10	30	50	70	90	100
PEGDA	100	90	70	50	30	10	0
Irgacure 2959[c] (g)	0.015	0.015	0.015	0.015	0.015	0.015	0.015
H_2O (mL)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Conversion (%)	83.17	81.13	74.57	69.37	51.60	±[d]	/[e]

[a] All reactions were carried out for 20 h at room temperature.
[b] Dex-AE-2 at 6 h was used in this study.
[c] Initiator: 2-hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-l-propanone.
[d] Gels formed but not good.
[e] No gels formed under this condition.

2.8. pH and electrolyte sensitivity of the Dex-AE/PEGDA hydrogels

2.8.1. Effect of pH on the swelling ratio of Dex-AE/PEGDA hydrogels

The pH sensitivity was investigated by studying its swelling behavior in a wide range of pH buffer solutions (pH 3.0, 7.0, and 10.0). The Dex-AE/PEGDA hydrogels were immersed in buffer solutions for 24 h at room temperature in order to attain an equilibrium state. The swollen hydrogels were removed, wiped, and weighed, and their equilibrium swelling ratios were calculated the same way as defined above.

2.8.2. Effect of electrolytes on the swelling of Dex-AE/PEGDA hydrogels

The electrolyte effect on the swelling property of these Dex-AE/PEGDA hydrogels was further investigated in a series of salt solutions of different concentrations (1, 2.5, and 5.0 M of NaCl and 5.0 M $MgCl_2$). Distilled water was used as the control medium. DEG3 was chosen as the typical model hydrogel sample because its composition was between the minimum and maximum precursor ratios of Dex-AE to PEGDA. The swollen hydrogels were removed at predetermined intervals and weighed after wiping off the excess water on the surface with a wet filter paper and the swelling ratio was calculated the same way as given above.

2.9. In vitro albumin release study

2.9.1. Loading capacity

BSA was post-loaded into the Dex-AE based hydrogels. Ten percent BSA solution was first prepared by dissolving BSA in PBS solution (pH 7.4). Each hydrogel was weighed and then immersed into 15 mL of the BSA-containing PBS solution for 48 h at room temperature. After the removal of the immersed hydrogel at the end of 48 h, the solution on the hydrogel surface was blotted by a wet filter paper. These hydrogels were dried at 60 °C under vacuum for at least 48 h until there was no weight change. The difference in weight between the BSA-loaded hydrogel and the original hydrogel was the initial amount of loaded BSA. The loading capacity is defined as the ratio of the amount of loaded BSA to weight of dry hydrogels, and is summarized in Table 3.

2.9.2. Cumulative release

Each BSA-containing Dex-AE/PEGDA hydrogel was immersed in 15 mL PBS (pH 3.0 and 7.4) at 37 °C. At predetermined intervals, the hydrogel was removed and placed in fresh 15 mL PBS at 37 °C. The albumin concentration in PBS medium (after the removal of the immersed hydrogel) at each interval was monitored by the absorption of the medium at $\lambda = 490$ nm on a Perkin-Elmer Lambda 2 UV/VIS spectrometer (Norwalk, CT). The concentration of BSA released at a particular interval was calculated from a BSA standard calibration curve. All release studies were carried out in triplicate. The results were presented in the terms of cumulative release as a function of time:

$$\text{Cumulative amount released}(\%) = \left(\sum_{t=0}^{t=t} M_t/M_0\right) \times 100,$$

where $\sum_{t=0}^{t=t} M_t$ is the cumulative amount of released BSA from the hydrogel at time t, and M_0 is the initial amount of loaded BSA in the hydrogel and is given in Table 3.

3. Results and discussion

3.1. Synthesis of Dex-AE

The synthesis of Dex-AE involved two steps and is shown in Scheme 1. The first step is the synthesis of Dex-AI (Scheme 1a), which was reported in our previous study (Zhang et al., 2000). In this study, the DS of the Dex-AI was 0.25 per anhydroglucose unit. According to our previous study, there were enough double bonds in the precursor at this level of DS for crosslinking. At the same time, there were also enough unreacted hydroxyl groups in the Dex-AI that could further react with BEAHB to form Dex-AE (Scheme 1b).

Fig. 1 shows the FT-IR spectra of dextran, Dex-AI, Dex-AE, PEGDA, and Dex-AE/PEGDA hydrogel. Compared with the spectrum of dextran (A), the FT-IR spectra of Dex-AI (B), and Dex-AE (C) show the typical double-bond absorption bands at 1645 cm^{-1}, and blown-up version of peaks 1 and 2 clearly shows the C=C stretch vibration in Dex-AI and Dex-AE. This confirmed that the C=C double bonds were successfully incorporated into the dextran. For the vinyl group of Dex-AI, there are three typical vibrations: C=C stretch vibration at around l645 cm^{-1}, C–H stretch vibration at around 3010 cm^{-1} and C–H bending vibration at around 950 cm^{-1}. Both the C–H stretch and bending vibrations are weak and overlapped with Dex-AE and PEGDA, thus they could not be used to identify the consumption of C=C bonds during gelation process (Section 3.3). Although both amide (from Dex-AI) and amine groups (from Dex-AE) have –NH– bands and they overlaps some in FT-IR spectra, the difference between Dex-AI (spectrum B) and Dex-AE (spectrum C) is still evident. For example, the FT-IR spectra of Dex-AE showed that amide II shifted from 1540 cm^{-1} (in Dex-AI) to 1532 cm^{-1}, and 3380 cm^{-1} band (in Dex-AI) was also shifted to 3343 cm^{-1} due to the N–H stretching vibration of the amine group. The 1650 cm^{-1} band in the dextran spectrum (A) was due to the presence of trace amounts of adsorbed water that was difficult to remove in dextran (Zhang et al., 2000). After the formation of Dex-AE/PEGDA hydrogel (spectrum E), the characteristic peaks of C=C bonds (peaks 1 and 2 in spectra B and C) that were evident before

Table 3
BSA loading capacity of Dex-AE[b]/PEGDA hydrogels having different precursor feed ratios

	Sample ID				
	DEG1	DEG2	DEG3	DEG4	DEG5
Composition	0/100	10/90	30/70	50/50	70/30
Loading capacity[a]	0.1746	0.1021	0.1074	0.1094	0.1057

[a] g BSA/g hydrogel.
[b] The DS of Dex-AE is 2.05.

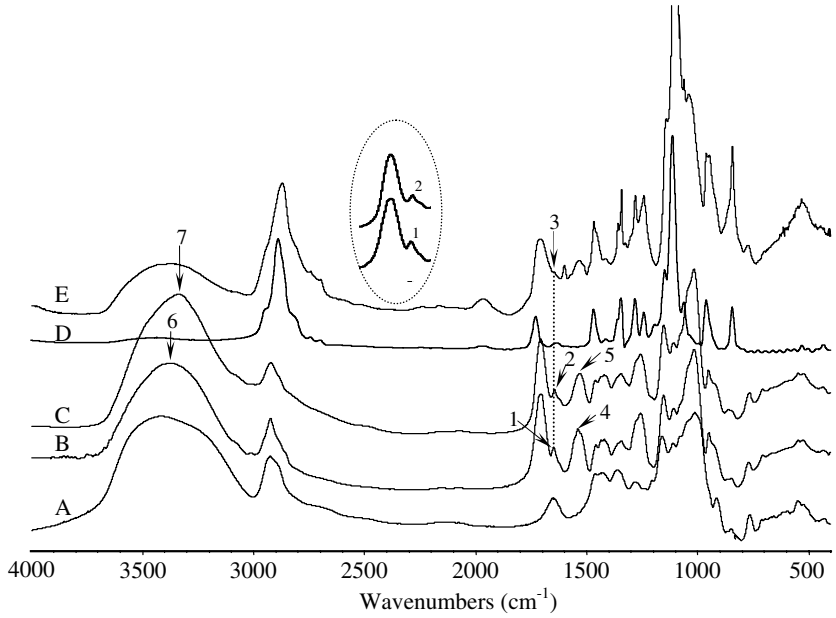


Fig. 1. FTIR spectra of (A) Dextran; (B) Dex-AI; (C) Dex-AE (DS 2.05); (D) PEGDA; (E) Dex-AE/PEGDA (30/70) hydrogel. (1,2&3: ~1645 cm^{-1}; 4: ~1540 cm^{-1}; 5: ~1532 cm^{-1}; 6: ~3380 cm^{-1}; 7: ~3343 cm^{-1}).

the photo-crosslinking reactions disappeared, an indication of the required consumption of these C=C bonds for hydrogel formation.

Fig. 2 shows the ^1H NMR spectra of dextran (A), Dex-AI (B), and Dex-AE (C). As shown in spectrum (A) for dextran, the characteristic resonance peak at 4.68 ppm (peak 1) was the anomeric proton, which had no reactivity and did not change during both reactions (dextran to Dex-AI and Dex-AI to Dex-AE); the other three adjacent peaks from 4.49 to 4.91 ppm were assigned to the three hydroxyl protons. The newly appeared peaks in Dex-AI (spectrum B) were from the protons of the AI segment. The peaks at 7.33 and 7.19 ppm could be assigned to the carbamate (–NH–) proton in Dex-AI. The vinyl end group peaks in Dex-AI (–CH=CH_2) appeared at 5.76 and 4.99–5.15 ppm, respectively. The peak at 3.59 ppm was assigned to the –CH_2– of the AI pendent group. Because of the different reactivity of the three hydroxyl groups in the dextran anhydroglucose unit, i.e., $C_2 > C_4 > C_3$ (Won & Chu, 1998; Zhang et al., 2000), the peak intensities of these signals were different.

Spectrum (C) shows the Dex-AE characteristic ^1H peaks after ethylamine was incorporated into the remaining hydroxyl groups in Dex-AI. Compared to Dex-AI, Dex-AE had more hydroxyl group protons substituted. Spectrum (C) shows that the peaks corresponding to the hydroxyl group protons that link to C_2 and C_4 disappeared; this means these protons were completely substituted by ethylamine. At the same time, the ^1H peak corresponding to C_3 bearing hydroxyl group also shows a decreased intensity when comparing to Dex-AI, and this lower peak intensity is due to further substitution by ethylamine. The broad peaks around 3.40 ppm in spectrum C can be assigned to the –CH_2–CH_2– protons in the ethylamine segment, which overlapped with some protons from the anhydroglucose rings. Due to the rapid exchange with its adjacent protons, the amine protons were hard to identify.

Several reaction parameters, such as time, temperature, and molar ratio of reactants to catalyst, were examined for the synthesis of Dex-AE, and the effect of these reaction conditions on the degree of substitution of Dex-AE are summarized in Table 1 and described below. In this study, the DS of Dex-AI was 8.33. The DS of Dex-AE was calculated from the difference between Dex-AE and Dex-AI.

3.1.1. Effect of the 2-bromoethylamine hydrobromide (BEAHB) concentration on the synthesis of Dex-AE

The effect of BEAHB on the synthesis of Dex-AE is shown in Table 1. A comparison between Dex-AE-1, Dex-AE-2, and Dex-AE-3 (at 50 °C 6 h reaction period and at a triethylamine/BEAHB molar ratio 2.0) clearly shows that an increase in the BEAHB to Dex-AI molar ratio from 1.5 (Dex-AE-1) to 3.0 (Dex-AE-2) led to an increase in the incorporation of the ethyl amine group into Dex-AI from DS 1.05 (Dex-AE-1) to 2.05 (Dex-AE-2) per anhydroglucose unit; and the most significant increase in DS occurred between 1.5 and 2.0 ratio of BEAHB to Dex-AI. For example, a 86% increase in DS (from 1.05 to 1.95) occurred as the BEAHB to Dex-AI ratio increased from 1.5 (Dex-AE-1) to 2.0 (Dex-AE-3); however, a further increase in the BEAHB/Dex-AI ratio from 2.0 to 3.0 led to only 5% increase in DS (from 1.95 to 2.05). This indicates that an increase in BEAHB/Dex-AI molar ratio beyond 2.0 may not be beneficial for a significant increase in DS.

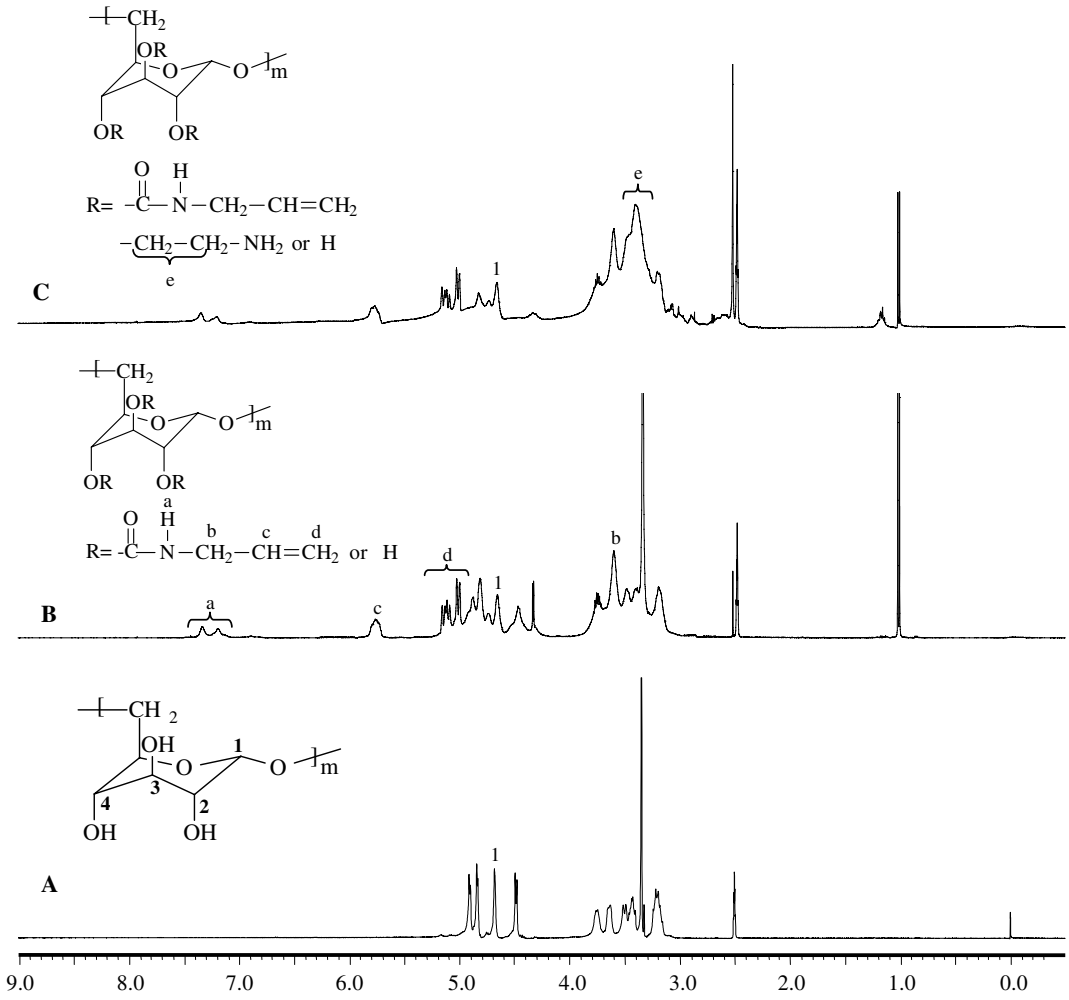


Fig. 2. ^{1}H NMR spectra of: (A) dextran; (B) Dex-AI; (C) Dex-AE (DS 2.05). Degree of substitution (DS) of Dex-AE is 2.05.

3.1.2. Effect of catalyst on the synthesis of Dex-AE

The triethylamine acted as a catalyst by reacting with the resultant hydrobromide, forming triethylamine hydrobromide, then precipitating during the reaction, and thus facilitating the reaction. Table 1 shows that an increase in the triethylamine/BEAHB ratio led to an increase in the DS. For example, at a constant BEAHB/Dex-AI ratio (1.5) and a 6 h reaction period, the DS increased from 1.05 (Dex-AE-1 and Dex-AE-7) to 1.45 (Dex-AE-6) when the triethylamine/BEAHB ratio increased from 1.5 to 2.5. However, there was no DS increase until the triethylamine/BEAHB ratio increased to 2.0. This could be understandable from the BEAHB structure. As shown in Scheme 1(b), each BEAHB molecule ($NH_2CH_2CH_2Br{\cdot}HBr$) consists of one hydrobromide molecule, and each BEAHB needs one triethylamine to consume the hydrobromide to form $BrCH_2CH_2NH_2$ for its subsequent reaction with Dex-AI. The formed salt by-product, i.e., triethylamine hydrobromide did not dissolve in DMSO, and it would eventually precipitate out. Due to this salt precipitation, most hydrobromide in the solution was removed. Because the reaction from Dex-AI to Dex-AE was reversible, the precipitation of Et_3NHBr reduced the hydrobromide concentration and hence moved the reaction of Dex-AI to Dex-AE toward greater completion.

3.1.3. Effect of temperature and reaction time on the synthesis of Dex-AE

The amounts of AE grafted onto the dextran backbone increased with an increase in reaction time. For example, Dex-AE-1 showed an increase in DS from 0.65 at 4 h to 1.25 at 8 h of reaction time. At a higher BEAHB/Dex-AI ratio (e.g., 3.0) like Dex-AE-2, however, a prolonged reaction time did not increase DS as much as the lower ratio case (e.g., Dex-AE-1). For example, at a lower BEAHB/Dex-AI ratio (i.e., 1.5), DS increased 92.3% (from 0.65 to 1.25) from 4 to 8 h; while at a higher ratio 3.0 (Dex-AE-2), DS increased only 10.9% (from 1.92 to 2.15) during the same time frame.

To investigate temperature effect on the synthesis of Dex-AE, three temperatures (40, 50, and 60 °C) were used: unlike other reaction parameters discussed above, the DS of Dex-AE decreased slightly with an increase in temperature. At a 6 h reaction period and constant molar ratio of

reactants (e.g., Dex-AE-4, Dex-AE-1, and Dex-AE-5), the DS were 1.06, 1.05, and 0.85, at 40, 50, and 60 °C, respectively.

In general, higher reaction temperatures can activate more molecules and hence promote reactions; however, in the reaction of Dex-AI to Dex-AE, more triethylamine could escape from the solution mixture at a higher reaction temperature. This may be attributed to the fact that triethylamine is a fairly volatile liquid (freezing point −115 °C and boiling point 88.8 °C at 1 atmosphere), which becomes more volatile at higher temperatures. As a result, the volatilization will reduce the amount of triethylamine from the reaction solution, which causes the reduction of hydrobromide through salt (triethylamine hydrobromide) precipitation difficult, leading to the reversible reaction hard to move along from Dex-AI to Dex-AE. Therefore, increasing temperature had an adverse effect on the DS of Dex-AE in this study.

3.2. Synthesis of Dex-AE/PEGDA hydrogel

The preparation of Dex-AE/PEGDA hydrogel is shown in Scheme 2, and the effect of feed ratios of Dex-AE (DS 2.05) to PEGDA precursors is given in Table 2. During the formation of the hydrogel, the Dex-AE reacted as a precursor as well as the crosslinking agent since a Dex-AE has many pendant double bonds (via AI unit) in its repeated units. Table 2 illustrates that the conversion (wt.%) of the synthesized hydrogels decreased with an increase in the content of the Dex-AE from DEGl (83.17%) to DEG5 (51.60%). If the content of Dex-AE moiety was too high (e.g., DEG6 and DEG7), the formation of the hydrogels became difficult. DEG6 and DEG7 hybrid network could not be formed under the specified condition, and hence they will not be discussed for the remaining of this paper. The reduction in conversion efficiency with an increase in Dex-AE content as well as the inability of forming hydrogel for a pure Dex-AE precursor (DEG7) may suggest that the reactivity of the C=C bonds in AI was lower than the C=C bond in PEGDA. Guo and Chu (2005) reported that the conjugation interaction between carbonyl group and the C=C double bond would facilitate the stability of the free radicals during polymerization; hence facilitate the attack on the carbon double bond. Thus, the C=C bonds in PEGDA were more reactive than the C=C bonds in Dex-AE, i.e., capable of forming hydrogels from pure PEGDA. The formed PEGDA gel could limit the chain mobility of Dex-AE and hence the reactivity of the C=C bonds in Dex-AE, which could reduce the gel forming capability of Dex-AE further as evident in the reduction of gel conversion efficiency with an increase in Dex-AE feed ratio (Table 2). The Dex-AE/PEGDA hydrogels formed showed pH sensitivity due to the presence of free amine groups in Dex-AE precursor, and the details will be described in the latter part of this paper.

3.3. FT-IR spectra of Dex-AE/PEGDA hydrogel

The FT-IR spectra of the Dex-AE/PEGDA hybrid network samples are showed in Fig. 3. The FT-IR data showed the effect of feed ratio of Dex-AE (DS 2.05) to PEGDA precursor on the spectra (from DEG2 to DEG5). From spectra B (DEG3) to D (DEG5), the double bond band at 1645 cm^{-1} completely or mostly disappeared due to the consumption of C=C bonds during photo-cross-

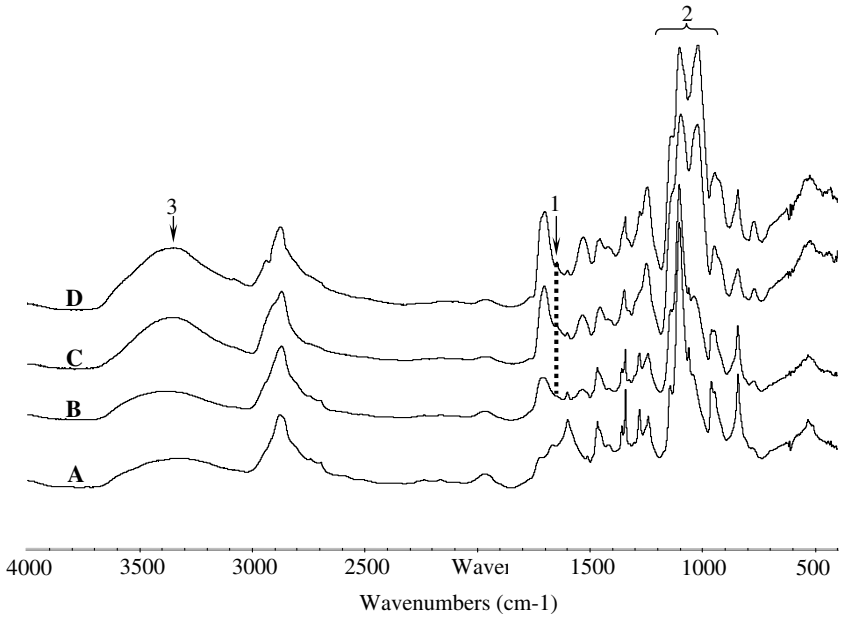


Fig. 3. FT-IR spectra of Dex-AE/PEGDA hydrogel at different precursor feed ratios. (A) 10/90; (B) 30/70; (C) 50/50; (D) 70/30. (1: C=C; ~1645 cm^{-1}; 2: ~1101 to ~ 1020 cm^{-1}; 3: ~3340 cm^{-1}). DS of Dex-AE is 2.05.

linking reaction. Spectrum A (DEG2) showed a visible double bond band and this is because DEG2 hydrogel had the lowest Dex-AE content (10%) in this study, and its spectrum was mostly dominated by the PEGDA component (90%) (see spectrum D in Fig. 1 for PEGDA). The two peaks at 1101 and 1020 cm^{-1} were assigned to the C–O asymmetric stretching vibration of ether group (–C–O–C–) and hydroxyl group (–C–O–H), respectively. Attributed to the dominant role of PEGDA in those hybrid hydrogels having low Dex-AE content (DEG2 and DEG3) given above, the peak at 1020 cm^{-1} caused by the C–O asymmetric stretching vibration from dextran can hardly been seen in spectra A (DEG2) and B (DEG3), and this peak intensity increased as the Dex-AE content increased from spectra A (10% Dex-AE) to D (70% Dex-AE). Each FT-IR spectrum in Fig. 3 had a broad band in the range of 3700–3100 cm^{-1}, which was assigned to N–H stretching vibration of Dex-AE. As the Dex-AE moiety increased, the intensity of this peak increased from DEG2 (spectrum A) to DEG5 (spectrum D). Both the C–O and N–H stretching vibrations confirmed that the Dex-AE content increased from DEG2 to DEG5.

3.4. Interior morphology of the Dex-AE/PEGDA hydrogels

The interior morphology of these freeze-dried, swollen hydrogel samples as a function of the precursor feed ratio is shown in Fig. 4. The swollen hydrogel network became denser and more compact as the Dex-AE (DS 2.05) composition increased (from DEG1 to DEG3). Evidenced by the increased pore size, however, a further increase in the Dex-AE content (i.e., DEG4 and DEG5) led to a more open and looser network structure. Fig. 5 shows the change of average hydrogel pore size as a function of the Dex-AE content. From DEG1 to DEG3, the pore size decreased from around 14 to 8 μm, and the network become denser and more compact. The initial reduction in pore size with an increase in Dex-AE content from 0% to 30% could be attributed to a higher level of crosslinking from the increasingly available crosslinkable unit (AI) for photo-crosslinking when Dex-AE moiety increased from DEG1 to DEG3.

As the Dex-AE moiety increased beyond 30% (by weight), the hydrogel network became more open, i.e., the pore size became bigger. This phenomenon could be attributed to the incomplete crosslinking in DEG4 and DEG5 as evident in their reduced conversion efficiency shown in Table 2. As discussed in Section 3.2, the lower reactivity of the C=C bonds in the AI of Dex-AE than the C=C bonds in PEGDA caused the lower conversion in DEG4 and DEG5, i.e., reduced level of crosslinking in DEG4 and DEG5. Due to the reduced level of crosslinking in DEG4 and DEG5, a larger porous microstructure was formed and shown in Fig. 4.

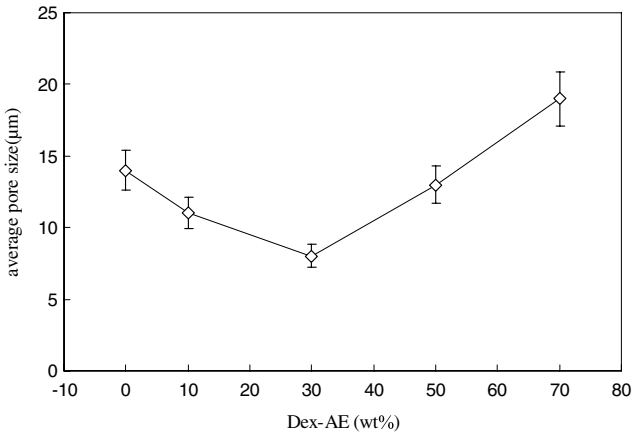


Fig. 5. Pore size of Dex-AE/PEGDA hydrogels as a function of Dex-AE content (DS 2.05).

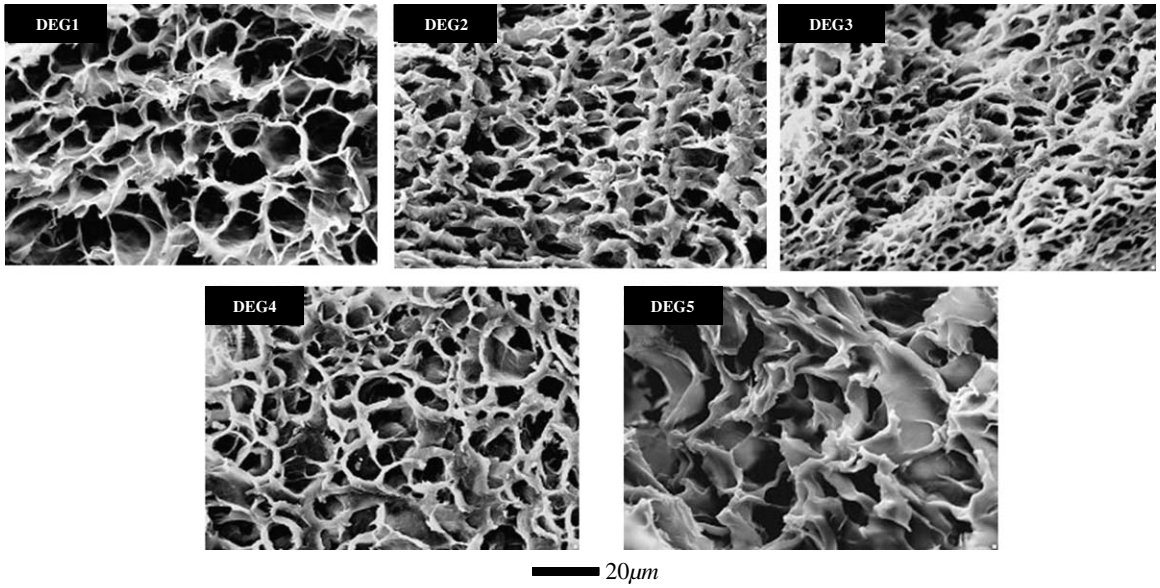


Fig. 4. SEM micrographs of Dex-AE/PEGDA hydrogels. DS of Dex-AE is 2.05.

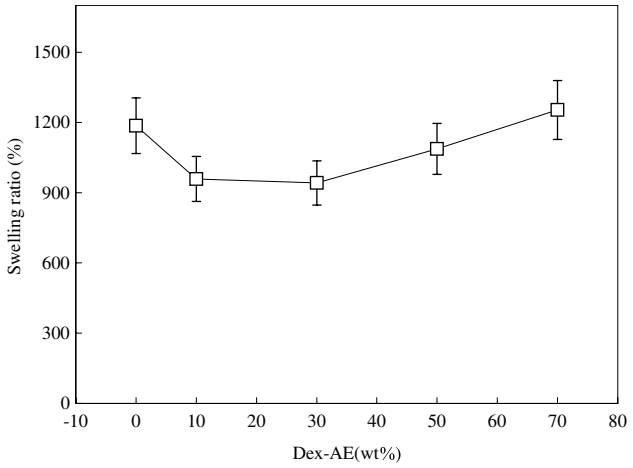


Fig. 6. Swelling ratio of Dex-AE/PEGDA hydrogels at room temperature. DS of Dex-AE is 2.05.

3.5. Swelling study of Dex-AE/PEGDA hydrogels

Fig. 6 shows the effect of Dex-AE (DS 2.05) to PEGDA feed ratio on the swelling ratios of Dex-AE/PEGDA hydrogels at room temperature. The data show that an initial increase in Dex-AE content (from DEGl to DEG3) led to a reduced swelling of Dex-AE/PEGDA hydrogels, reached a minimum swelling between 10% and 30% Dex-AE content, and a further increase in Dex-AE content (from DEG3 to DEG5), however, resulted in an increase in swelling. For instance, the swelling ratio decreased 25% from around 1200% (DEGl) to 900% (DEG3) when the Dex-AE content increased from 0% (DEGl) to 30% (DEG3), and then increased 39% (to 1250%) as the Dex-AE content reached 70% (DEG5).

The dependence of swelling ratio of Dex-AE/PEGDA hydrogels on Dex-AE content is in good agreement with the morphological images (Fig. 4) and average pore size data (Fig. 5). The morphological data showed that an increase in the content of the Dex-AE from DEGl to DEG3 led to a compact network structure due to a higher crosslinking level of the corresponding hydrogels, thus causing a reduction in its swollen or water uptake capacity due to the decreased pore volume. However, in the case of DEG3 to DEG5, the swelling ratio dramatically increased, which is believed to be attributed to presence of the distinctive more open and large pore network structure. This suggests that the swelling ratio of this Dex-AE/PEGDA hydrogels mainly depends on the characteristic of their network structure.

3.6. Environmental sensitivity of the Dex-AE/PEGDA hydrogels

3.6.1. Effect of pH on the swelling ratio of Dex-AE/PEGDA hydrogels

To investigate pH effect on the swelling kinetics of the Dex-AE/PEGDA hydrogels, a series of buffer solutions of different pHs (3.0, 7.0, and 10.0) were used. Fig. 7 shows the results of the swelling ratios of these hydrogels at three different pHs. The results indicate that their swelling ratios were influenced by pH, and the level of pH effect depended on the Dex-AE (DS 2.05) content. Over the feed ratios studied, these hybrid hydrogels swelled faster and retained more water in a lower pH medium than in a higher pH medium. For example, at pH 3.0, the swelling ratio of DEG3 was 1072%, but 808% at pH 10.0, a 25% reduction. This pH effect became more significant when the Dex-AE content reached 50% (by weight) and beyond. For instance, at pH 3.0, the swelling ratio of DEG5 (70% Dex-AE content) was 1710%, which was 28% more than at pH 7.0 (1338); while for DEG3 (30% Dex-AE content), the swelling ratio increased 1 only 8% over the same pH range.

All pH sensitive polymers have either acidic or basic groups. They are capable of responding to environmental pH changes by losing or gaining protons. The observed increase in swelling with a decrease in pH shown in Fig. 7 is mainly attributed to the presence of free pendant amine groups in Dex-AE. Primary amines (pK_a 10.5) are Lewis basic, which can accept a proton and thus become protonated in an acid medium. The protonation of amine groups could lead to disruption of the interaction of the amine groups with their surrounding water molecules, thus generated a higher proton concentration within the Dex-AE based hydrogels than in their surrounding medium. This concentration gradient could give rise to an osmotic pressure and resulted in a water inflow into the hydrogels, i.e., higher swelling ratio. The electrostatic repulsion of the protonated amine group could also lead to an expansion of hydrogel network structure and resulted in an increase in swelling as demonstrated by others (Herber, Olthuis, & Bergveld, 2003; Kim & Chu, 2000a, 2000b). Dex-AE/PEDGA hydrogels having higher Dex-AE content would have more free amine group for higher osmotic pressure and electrostatic repulsion and hence larger pH effect as observed.

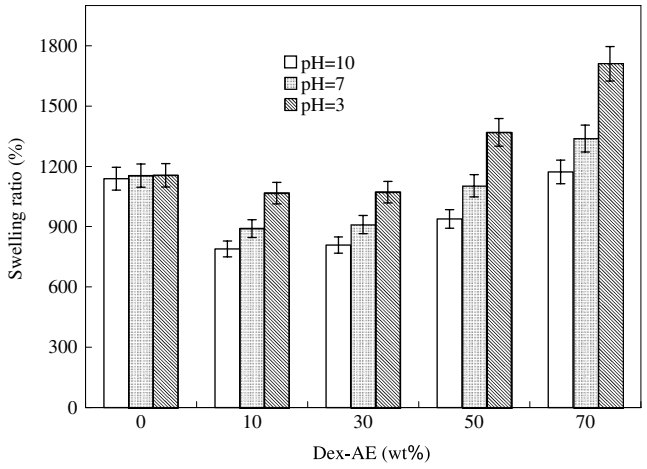


Fig. 7. Effect of pH on the swelling ratio of Dex-AE/PEGDA hydrogels at three different pHs. DS of Dex-AE is 2.05.

3.6.2. Effect of ionic strength on the swelling of Dex-AE/PEGDA hydrogels

The ionic strength on a concentration basis is defined as follows:

$$I = \frac{1}{2}\sum C_A \cdot Z_A^2,$$

where C_A is the molar concentration of ion A, Z_A is the charge number of ion A. The ionic strength of each salt solution was calculated as the sum of all ion strength in each solution.

A representative effect of ionic strength on the swelling of Dex-AE/PEGDA hydrogels (e.g., DEG3 and DS 2.05) is shown in Fig. 8. Generally, an increase in ionic strength led to a decrease in swelling of DEG hydrogels. The highest swelling was achieved in pure water, while the lowest swelling was observed in the 5.0 M of MgC_2 solution ($I = 15$). For instance, DEG3 swelled and retained up to 8.4 times water of its original weight in pure water, while it only reached 4.0 times of its original weight in the 5.0 M of NaCl solution ($I = 5$), and only 2.0 times of its original weight in 5.0 M of $MgCl_2$ solution ($I = 15$). Obviously, DEG3 showed a reduced swelling ratio in the 5.0 M of $MgCl_2$ solution than the NaCl solution of same concentration.

The effect of ionic strength on the swelling of hydrogels has also been investigated by Zhang et al. (2000) and Kim et al. (1999) in their studies of Dex-AI/PDLLA and Dextran–maleic acid hydrogels. They demonstrated that the presence of electrolytes like NaCl and MgC_2 in swelling medium had a significant effect on the swelling behavior of those hydrogels, and the degree of such an effect depended on ionic strength of the electrolytes in swelling medium.

This effect of electrolytes on swelling could be attributed to osmotic pressure. Osmotic pressure results from the difference in ion concentration between the interior of a hydrogel network and its surrounding medium. An increase in the ionic concentration (or ionic strength) of the surrounding medium will increase concentration gradient between the network and its surrounding medium (Bajpai & Giri, 2003), which, in turn, would increase osmotic pressure across the boundary. This increase in osmotic pressure could be further augmented by the presence of higher ionic strength which could reduce ion mobility. For example, in the case of 5.0 M $MgCl_2$ ($I = 15$), the Cl^- had a lower mobility due to its higher concentration, which led to a higher concentration of these ions in surrounding solution. In addition, each Cl^- is surrounded by a layer of its counterions Mg^{2+}, i.e., ion atmosphere, in which the mobility of Cl^- is limited due to the electrostatic interaction with its counterions Mg^{2+}. The more the ion charge, the more the ion atmosphere effect. Unlike the monovalent Na^+, Mg^{2+} is divalent and it has more electrostatic interaction with Cl^-, which reduced more mobility of Cl^-. Thus, this reduction in ion mobility in the surrounding medium could give rise to a higher osmotic pressure which, in turn, could facilitate the diffusion of pure water molecules from inside swollen Dex-AE/PEGDA hydrogel into its surrounding medium, i.e., less water retention and lower swelling ratio of the hydrogels in a high ionic strength medium as observed.

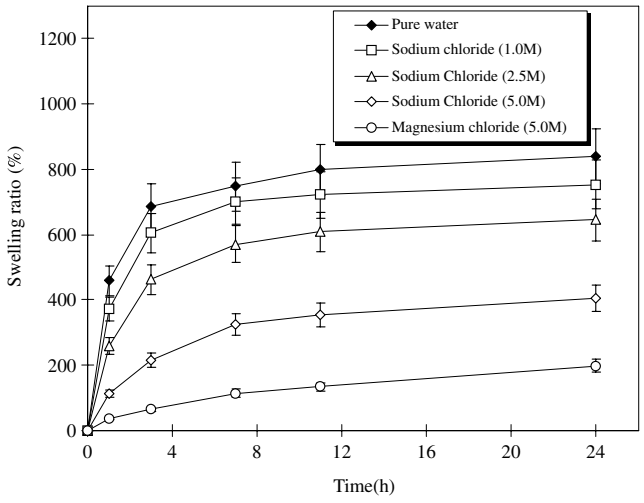


Fig. 8. Effect of ionic strength of media on the swelling Dex-AE/PEGDA hydrogel (DEG3) in NaCl and $MgCl_2$ solutions. DS of Dex-AE is 2.05.

3.7. In vitro albumin release study

3.7.1. Loading capacity

The BSA loading capacity in Dex-AE/PEGDA hydrogels is shown in Table 3 (Dex-AE DS 2.05). From Table 3 we can see that hydrogel DEG1 (pure PEGDA) had the highest BSA loading capacity (0.1746) among all the hybrid hydrogels; there was no significant difference in the BSA loading capacity of DEG2 to DEG5.

The loading capacity difference could be attributed to the chemical structure difference between DEG1 and other DEGs. Since PEG has the lowest level of protein absorption capability (Greenwald et al., 2003), BSA can diffuse into the interior of DEG1 hydrogel quickly. However, the hybrid hydrogels have many amine groups from Dex-AE, and these groups have stronger interactions with BSA than pure PEG. This is because that the free amine groups from Dex-AE groups could become protonated and positively charged and exert electrostatic attraction with the negatively charged BSA (Palacio, Ho, Prádanos, Hernández, & Zydney, 2003). These strong interaction would limit the migration of BSA into the interior of the hydrogel, i.e., less BSA was loaded in hybrid hydrogels than in pure PEGDA hydrogels.

3.7.2. Cumulative release at pH 7.4

The cumulative amounts of BSA released from the Dex-AE/PEGDA hydrogels in pH 7.4 PBS are shown in Fig. 9. The data show that all DEG hydrogels had lower BSA release rates and amounts than pure PEGDA (DEG1). The above-suggested active interaction between the Dex-AE (DS 2.05) component of the DEG hydrogels and BSA may be partially responsible for this slower release

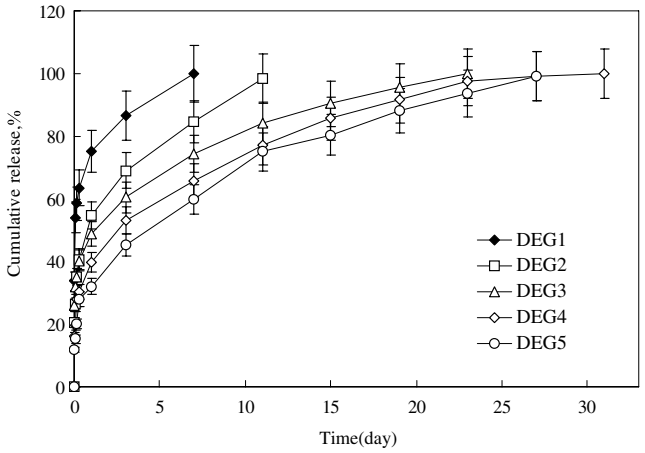


Fig. 9. Cumulative release of BSA from Dex-AE/PEGDA hydrogels at 37 °C and pH 7.4. DS of Dex-AE is 2.05.

phenomenon. All hydrogel samples have an initial burst release. During the first 8.0 h of the initial burst release, the cumulative releases were 63% for DEG1, 44% for DEG2, 41% for DEG3, 31% for DEG4, and 28% for DEG5. All the Dex-AE incorporated DEG hydrogels had lower release rates and amounts than DEG1. After this burst release period, all the release rates slowed down, and their release rates are almost in the order of DEG5 < DEG4 < DEG3 < DEG2 < DEG1. DEG1 had a faster release rate than other DEGs though it had a higher loading capacity, and the BSA was completely released at the end of seventh day. The release became more sustained as Dex-AE component increased in the hydrogels. DEG4 had the longest time and all BSA was released at the end of 31st day.

Burst release is a common phenomenon and its potential reasons were first discussed by Huang and Brazel (2001). According to them, there are five possible reasons that cause burst release, including processing conditions, surface characteristics of host materials, sample geometry, host/drug interactions (surface adsorption), and morphology and porous structure of dry material. In this study, the initial burst release could be attributed to those BSA located near the surface of the DEG hydrogels and these BSA would be the first one to come out from the DEG host carrier. In addition, BSA concentration gradient could be another factor responsible for BSA initial burst release. During the early stage of immersion, the BSA concentration gradient between the hydrogel surface and its surrounding medium could build a very strong driving force for BSA diffusion. Under such a strong driving force, those BSA located near surface could easily diffuse into the buffer solution. Zhang and Chu (2001) suggested that the burst release was also controlled by swelling in their study of indomethacin from dextran/polylactide hydrogels.

The structure of the hydrogel may also be one of the parameters that can control the sustained release of BSA. The SEM images (Fig. 4) indicate that the DEG network structure became more compact and rigid from DEG1 to DEG3 and then became open again with the increase of Dex-AE component (DEG4 and DEG5), which was further confirmed by the average pore size data (Fig. 5). Due to the compact network structure, a lower level of swelling was found and hence a slower rate of BSA release, i.e., more sustained release were observed for DEG2–DEG3 than DEG1. The qualitative correlation between swelling ratio and drug release from hydrogels have been first suggested by Kim et al. in the study of Doxorubicin release from dextran/methacrylate hydrogels (Kim & Chu, 2000a, 2000b). It appears that this relationship is partially held for BSA release from our current Dex-AE/PEGDA hydrogel system having relatively lower amounts of Dex-AE (DEG2–DEG3).

Apparently, the network structure alone cannot explain the BSA release profile, especially for hydrogel DEG5 (Fig. 9). DEG5 showed the slowest and most sustained BSA release though it has largest pore size. The discrepancy between our experimental finding of BSA release from DEG5 and the qualitative correlation between swelling ratio and drug release rate might be attributed to the chemical factor, i.e., chemical interaction between BSA and the hydrogel macromolecules. Since both DEG4 and DEG5 had far more Dex-AE contents than the rest DEGs, these 2 DEG hydrogels would be expected to have the highest level of electrostatic molecular interaction between the positive-charged amine group in Dex-AE and the negative-charged BSA. Accordingly, hydrogel DEG4 and DEG5 have a strong electrostatic attraction with BSA, and they could hold BSA firmer than the rest of other DEG hydrogels, thus achieving lower initial burst and subsequent sustained BSA release.

3.7.3. Cumulative release at pH 3.0

The pH sensitivity of the Dex-AE/PEGDA hydrogels (Dex-AE DS 2.05) also reflected in their BSA release as shown in Fig. 10. The data show the cumulative release of BSA from Dex-AE/PEGDA hydrogels at pH 3.0. The

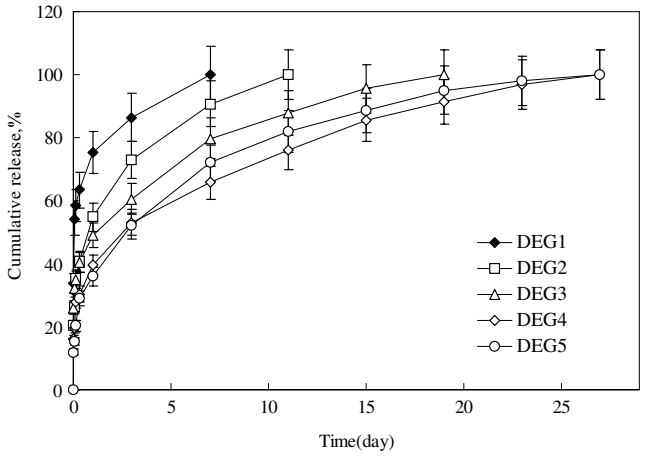


Fig. 10. Cumulative release of BSA from Dex-AE/PEGDA hydrogels at 37 °C and pH 3.0. DS of Dex-AE is 2.05.

BSA release profiles at pH 3.0 were very similar to those at pH 7.4 (Fig. 9), and the main differences were the amounts of BSA released cumulatively over a fixed period as well as the release duration. All hydrogels, except DEG1, have higher amounts of BSA released at pH 3.0 than those at pH 7.4. For instance, at the end of the seventh day, DEG2 had 84.5% BSA released at pH 7.4 but 90.7% released at pH 3.0, DEG3 74.5% and 79.8%, DEG4 59.5% and 66.8%, DEG5 59.7% and 72.3%, respectively. DEG5 had the most BSA released than other DEG hydrogels at pH 3 when comparing with their corresponding ones at pH 7.4, i.e., more than 12.6% BSA released at pH 3.0 than at pH 7.4. For DEG4, all BSA was released at the end of 27 days in pH 3.0 buffer instead of 31 days in pH 7.4.

There are two possible reasons that could contribute to this pH-dependent BSA release. First, due to the protonation of the free amine groups and their electrostatic repulsion effects at a lower pH like 3, the Dex-AE incorporated hydrogel would have a higher swelling ratio than the same hydrogel at a higher pH medium like 7 (Fig. 7), so the hydrogel network cannot retain the BSA as long as it can than at a higher pH medium. Another possible reason might be that the electrostatic interaction between BSA and hydrogel network might be destroyed at a lower pH medium. According to Palacio et al. (2003), BSA is negatively charged when $pH > 4.6$, but becomes positively charged when $pH < 4.6$. Therefore, at pH 3.0, BSA is positively charged. Due to the repulsive force between the positively charged amine groups in Dex-AE and BSA, BSA would be expected to have a faster release at a lower pH (3.0) than at a higher pH (7.4) and this faster release would shorten the sustained period as observed.

Acknowledgments

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References

Abuchowski, A., McCoy, J. R., Palczuk, N. C., Vanes, T., & Davis, F. F. (1977). Effect of covalent attachment of polyethylene-glycol on immunogenicity and circulating life of bovine liver catalase. *Journal of Biological Chemistry, 252*(11), 3582–3586.

Azzam, T., Eliyahu, H., Makovitzki, A., & Domb, A. J. (2003). Dextran-spermine conjugate: An efficient vector for gene delivery. *Macromolecular Symposia, 195*, 247–261.

Bajpai, A. K., & Giri, A. (2003). Water sorption behavior of highly swelling (carboxy methylcellulose-g-polyacrylamide) hydrogels and release of potassium nitrate as agrochemical. *Carbohydrate Polymers, 53*(3), 271–279.

Chen, G. H., & Hoffman, A. S. (1995). Graft-copolymers that exhibit temperature-induced phase-transition over a wide-range of pH. *Nature, 373*(6509), 49–52.

Chen, J., Jo, S., & Park, K. (1995). Polysaccharide hydrogels for protein drug delivery. *Carbohydrate Polymers, 28*(1), 69–76.

Chu, C. C. (2003). Biodegradable hydrogels as drug controlled release vehicles. In D. L. Wise (Ed.), *Biomaterials handbook – Advanced applications of basic sciences, and bioengineering* (pp. 871–909). New York: Marcel Dekker.

Chung, C. W., Kim, H. W., Kim, Y. B., & Rhee, Y. H. (2003). Poly (ethylene glycol)-grafted poly(3-hydroxyundecenoate) networks for enhanced blood compatibility. *International Journal of Biological Macromolecules, 32*(1–2), 17–22.

Franssen, O., Vandervennet, L., Roders, P., & Hennink, W. E. (1999). Degradable dextran hydrogels: Controlled release of a model protein from cylinders and microspheres. *Journal of Controlled Release, 60*(2–3), 211–221.

Greenwald, R. B., Choe, Y. H., McGuire, J., & Conover, C. D. (2003). Effective drug delivery by PEGylated drug conjugates. *Advanced Drug Delivery Reviews, 55*(2), 217–250.

Guo, K., & Chu, C. C. (2005). Synthesis and characterization of novel biodegradable unsaturated poly(ester amide)/poly(ethylene glycol) diacrylate hydrogels. *Journal of Polymer Science Polymer Chemistry*.

Herber, S., Olthuis, W., & Bergveld, P. (2003). A swelling hydrogel-based P_{CO2} sensor. *Sensors and Actuators B: Chemical, 91*(1–3), 378–382.

Hern, D. L., & Hubbell, J. A. (1998). Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. *Journal of Biomedical Materials Research, 39*(2), 266–276.

Hoffman, A. S. (1987). Applications of thermally reversible polymers and hydrogels in therapeutics and diagnostics. *Journal of Controlled Release, 6*(1), 297–305.

Hu, R. G., Zhai, Q. W., He, W. J., Mei, L., & Liu, W. Y. (2002). Bioactivities of ricin retained and its immunoreactivity to anti-ricin polyclonal antibodies alleviated through pegylation. *International Journal of Biochemistry and Cell Biology, 34*(4), 396–402.

Huang, X., & Brazel, C. S. (2001). On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *Journal of Controlled Release, 73*(2–3), 121–136.

Ikada, Y., Jamshidi, K., Tsuji, H., & Hyon, S. H. (1987). Stereocomplex formation between enantiomeric poly(lactides). *Macromolecules, 20*(4), 904–906.

Inoue, T., Chen, G. H., Hoffman, A. S., & Nakamae, K. (1998). A hydrophobically modified bioadhesive polymeric carrier for controlled drug delivery to mucosal surfaces. *Journal of Bioactive and Compatible Polymers, 13*(1), 50–64.

Jeong, B., Kim, S. W., & Bae, Y. H. (2002). Thermosensitive sol-gel reversible hydrogels. *Advanced Drug Delivery Reviews, 54*(1), 37–51.

Kim, I. S., Jeong, Y. I., Kim, D. H., Lee, Y. H., & Kim, S. H. (2001). Albumin release from biodegradable hydrogels composed of dextran and poly (ethylene glycol) macromer. *Archives of Pharmacal Research, 24*(1), 69–73.

Kim, S. H., & Chu, C. C. (2000a). Synthesis and characterization of dextran-methacrylate hydrogel and its structure study by SEM. *Journal of Biomedical Materials Research, 49*(4), 517–527.

Kim, S. H., & Chu, C. C. (2000b). In vitro release behavior of dextran-methacrylate hydrogel using doxorubicin and other model compounds. *Journal of Biomaterials Application, 15*(1), 23–46.

Kim, S. H., Won, C. Y., & Chu, C. C. (1999). Synthesis and characterization of dextran-maleic acid based hydrogel. *Journal of Biomedical Materials Research, 46*(2), 160–170.

Kim, S. J., Yoon, S. G., Lee, Y. M., & Kim, S. I. (2003). Electrical sensitive behavior of poly(vinyl alcohol)/poly (diallyldimethylammonium chloride) IPN hydrogel. *Sensors and Actuators B – Chemical, 88*(3), 286–291.

Koumenis, I. L., Shahrokh, Z., Leong, S., Hsei, V., Deforge, L., & Zapata, G. (2000). Modulating pharmacokinetics of an anti-interleukin-8 $F(ab')_2$ by amine-specific PEGylation with preserved bioactivity. *International Journal of Pharmaceutics, 198*(l), 83–95.

Li, S. K., & D'Emanuele, A. (2001). On-off transport through a thermoresponsive hydrogel composite membrane. *Journal of Controlled Release, 75*(1–2), 55–67.

Liu, Z. S., & Calvert, P. (2000). Multilayer hydrogels as muscle-like actuators. *Advanced Materials, 12*(4), 288–291.

Mamada, A., Tanaka, T., Kungwatchakun, D., & Irie, M. (1990). Photoinduced phase transition of gels. *Macromolecules, 23*(5), 1517–1519.

Massia, S. P., Stark, J., & Letbetter, D. S. (2000). Surface-immobilized dextran limits cell adhesion and spreading. *Biomaterials, 21*(22), 2253–2261.

Molina, I., Li, S., Martinez, M. B., & Vert, M. (2001). Protein release from physically crosslinked hydrogels of the PLA/PEO/PLA triblock copolymer-type. *Biomaterials, 22*(4), 363–369.

Muslim, T., Morimoto, M., Saimoto, H., Okamoto, Y., Minami, S., & Shigemasa, Y. (2001). Synthesis and bioactivities of poly(ethylene glycol)-chitosan hybrids. *Carbohydrate Polymers, 46*(4), 323–330.

Nakamae, K., Miyata, T., & Hoffman, A. S. (1994). Formation of poly(glucosyloxyethyl methacrylate)–concanavalin-A complex and its glucose sensitivity. *Journal of Biomaterials Science – Polymer Edition, 6*(1), 79–90.

Palacio, L., Ho, C. C., Prádanos, P., Hernández, A., & Zydney, A. L. (2003). Fouling with protein mixtures in microfiltration: BSA–lysozyme and BSA–pepsin. *Journal of Membrane Science, 222*(1–2), 41–51.

Peppas, N. A., & Klier, J. (1991). Controlled release by using poly (methacrylic acid–g-ethylene glycol) hydrogels. *Journal of Controlled Release, 16*(1–2), 203–214.

Ratner, B. D., & Hoffman, A. S. (1976). Synthetic hydrogels for biomedical applications. In J. D. Andrade (Ed.), *Hydrogels for medical and Related Applications* (pp. 1–36). Washington, DC: American Chemical Society.

Stenekes, R. J. H, Talsma, H., & Hennink, W. E. (2000). Formation of dextran hydrogels by crystallization. *Biomaterials, 22*(13), 1891–1898.

Wang, P., Tan, K. L., Kang, E. T., & Neoh, K. G. (2002). Plasma-induced immobilization of poly(ethylene glycol) onto poly(vinylidene fluoride) microporous membrane. *Journal of Membrane Science, 195*(1), 103–114.

Won, C. Y., & Chu, C. C. (1998). Dextran–estrone conjugate: Synthesis and in vitro release study. *Carbohydrate Polymers, 36*(4), 327–334.

Won, C. Y., & Chu, C. C. (2000). Polysaccharides as drug carriers. In Donald L. Wise (Ed.), *Biomaterials and engineering handbook* (pp. 356–371). New York: Marcel Dekker.

Wu, D. W., Zhang, X. Z., & Chu, C. C. (2003). Synthesis, characterization and drug release from three-arm poly(epsilon-caprolactone) maleic acid/poly(ethylene glycol) diacrylate hydrogels. *Journal of Biomaterials Science – Polymer Edition, 14*(8), 777–802.

Zhang, M., Li, X. H., Dong, Y. D., Zhao, N. M., & Zhang, X. F. (2002). Properties and biocompatibility of chitosan films modified by blending with PEG. *Biomaterials, 23*(13), 2641–2648.

Zhang, X. Z., & Chu, C. C. (2003). Synthesis of temperature sensitive PNIPAAm cryogels in organic solvent with improved properties. *Journal of Materials Chemistry, 13*(10), 2457–2464.

Zhang, X. Z., & Chu, C. C. (2004a). Preparation of thermosensitive PNIPAAm hydrogels with superfast response. *Chemical Communications*(3), 350–351.

Zhang, X. Z., & Chu, C. C. (2004b). A responsive poly(*N*-isopropylacrylamide)/poly(ethylene glycol) diacrylate hydrogel microsphere. *Colloid and Polymer Science, 282*(12), 1415–1420.

Zhang, X. Z., Lewis, P. J., & Chu, C. C. (2005). Fabrication and characterization of a smart drug delivery system: Microsphere in hydrogel. *Biomaterials, 26*(16), 3299–3309.

Zhang, X. Z., Sun, G. M., & Chu, C. C. (2004). Temperature sensitive dendrite-shaped PNIPAAm/Dex-AI hybrid hydrogel particles: Formulation and properties. *European Polymer Journal, 40*(9), 2251–2257.

Zhang, X. Z., Sun, G. M., Wu, D. Q., & Chu, C. C. (2004). Synthesis and characterization of partially biodegradable and thermosensitive hydrogel. *Journal of Materials Science – Materials in Medicine, 15*(8), 865–875.

Zhang, X. Z., Wu, D. W., & Chu, C. C. (2004). Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex-MA/PNIPAAm hydrogels. *Biomaterials, 25*(19), 4719–4730.

Zhang, Y. L., & Chu, C. C. (2001). Biodegradable dextran-polylactide hydrogel network and its controlled release of albumin. *Journal of Biomedical Materials Research, 54*(1), 1–11.

Zhang, Y. L., & Chu, C. C. (2002a). Biodegradable dextran-polyactide hydrogel networks: Their swelling, morphology and the controlled release of indomethacin. *Journal of Biomedical Materials Research, 59*(2), 318–328.

Zhang, Y. L., & Chu, C. C. (2002b). Biodegradation of hydrophilic-hydrophobic hydrogels and its effect on albumin release. *Journal of Materials Science: Materials in Medicine, 13*(7), 667–676.

Zhang, Y. L., & Chu, C. C. (2002c). In vitro release behavior of insulin from biodegradable hybrid hydrogel networks of polysaccharide and synthetic biodegradable polyester. *Journal of Biomaterials Application, 16*(4), 305–325.

Zhang, Y. L., & Chu, C. C. (2002d). The effect of molecular weight of biodegradable hydrogel precursors on the in vitro Indomethacin release from dextran and poly(D,L) lactic acid hybrid hydrogel. *Journal of Bioactive and Compatible Polymers, 17*(2), 65–85.

Zhang, Y. L., Won, C. Y., & Chu, C. C. (2000). Synthesis and characterization of biodegradable hydrophobic–hydrophilic hydrogel networks with a controlled swelling property. *Journal of Polymer Science Polymer Chemistry, 38*(13), 2392–2404.