Biodegradation of hydrophilic–hydrophobic hydrogels and its effect on albumin release

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The objective of this study was to examine the effects of composition ratio of a new class of bicomponent biodegradable hydrogels and the molecular weights of the constituents on the hydrolytic degradability of the hydrogels and their release of bovine serum albumin (BSA). Biodegradable hydrogels were prepared from dextran derivative of allyl isocyanate (dex-AI) and poly (D,L) lactide diacrylate macromer (PDLLAM) over a wide range of dex-AI to PDLLAM composition ratio. The results obtained indicated that the hydrolytic degradation of these biodegradable hydrogels could be controlled by adjusting the composition ratio of dex-AI to PDLLAM or by changing their molecular weights. Along with the hydrogel degradation, water content of the hydrogels changed, and 3D porous network structure was observed. Generally, as the PDLLAM composition in the hydrogels increased, the rate of weight loss increased due to the hydrolytic degradation of the PDLLAM. The increase in molecular weights of either dex-AI or PDLLAM would decrease the degradation rate of the dex-AI/PDLLAM hydrogels. BSA release data correlated well with the hydrogel degradation profiles, suggesting that the extent and rate of BSA release would be mainly controled by hydrogel degradation. As the PDLLAM composition in the hydrogel increased, the extent and rate of BSA release also increased. An increase in the molecular weights of the hydrogel constituents, however, led to a decrease in BSA release.

Introduction

Hydrogels are a class of biomaterials that receive increasing commercial attention [1, 2]. Hydrogel-based controlled drug delivery is among the current topics of intense interest [3–6]. By using suitable polymeric materials to construct hydrogel delivery systems, predictable release profiles of bioactive agents could be achieved over a period of time.

The performance of hydrogels depends on the characteristics of the constituent polymers, such as degradability, hydrophobicity, molecular weight, etc. [7]. The susceptibility of constituent polymers toward hydrolytic degradation is an important parameter, because hydrogels with various degrees of sensitivities toward hydrolytic degradation can be used for different drug release characteristics, from short-term to long-term release. Such characteristics are the essential requirement for the use of hydrogels as the controled delivery systems of large biomolecules like proteins. Therefore, the overall aim of this study was to determine the hydrolytic degradation property of our newly synthesized dextran-polylactide hydrogel network, the material factors that could affect the degradability of the hydrogels, and the effect of hydrolytic degradation on the albumin release.

The synthesis of this new type of biodegradable hydrogel using hydrophilic enzymatic degradable dextran and hydrophobic hydrolytic degradable poly (D,L) lactide (PDLLA) was described in our previous studies [8, 9]. We discussed the potential use of such hydrogels as a novel polymeric drug delivery system and the advantages of achieving a wide range of controlled swelling, biodegradability and hydrophilicity to hydrophobicity. In this paper, we report our findings of (1) the hydrolytic degradation property of the dextran-PDLLA based hydrogels, and (2) the effect of polymer molecular weight (MW) and the composition ratio of dextran to polylactide on the hydrolytic degradability of the hydrogels and on the release of the incorporated albumin *in vitro*.

Experimental

Materials

Dextran (MW 43 000 and 70 000) was purchased from Sigma Chemical Company (St Louis, MO). PDLLA of MW 740 and 7300 was supplied by Boehringer Ingelheim Company (Milwaukee, WI) and Purac Biochem Company (Gorinchem, Netherlands), respectively. Fluorescein isothiocyanate labeled bovine serum albumin (BSA) of MW 69 000 was purchased from Sigma Chemical Company. Dimethyl formamide (DMF),

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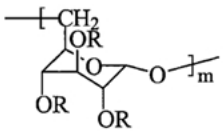
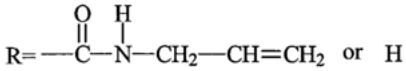
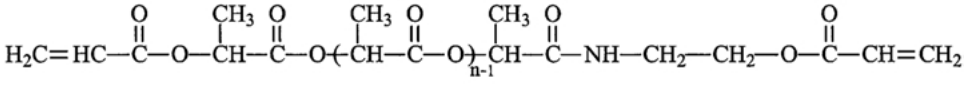




Dex-AI



PDLLAM

Figure 1 Chemical structure of dex-AI and PDLLAM.

allyl isocyanate (AI), and 2,2-dimethoxy 2-phenyl acetophenone were purchased from Aldrich Chemical Company (Milwaukee, WI). Ultrapure water was used for making phosphate buffer solutions (PBS, 0.1 M, pH 7.4).

Preparation of BSA impregnated hydrogels

Both PDLLA and dextran were chemically modified to incorporate unsaturated groups for the subsequent UV-induced network formation. In brief, PDLLA diacrylate macromer (PDLLAM) was synthesized by introducing vinyl groups at both chain ends of PDLLA. Dextran derivative of allyl isocyanate (dex-AI) was synthesized by incorporating of AI into the pendant hydroxyl groups in dextran. The dex-AI used in this study had a degree of substitution 6 (DS, the number of crosslinkable AI group per 100 anhydroglucose units). The detailed procedures of synthesizing these two hydrogel precursors were reported in our previous studies [8, 9]. Fig. 1 illustrates the chemical structure of PDLLAM and dex-AI.

PDLLAM and dex-AI were dissolved in DMF to prepare precursor solutions of a concentration 50% w/v. The range of dex-AI to PDLLAM composition ratios was from 100/0 (100% dex-AI), 80/20, 50/50, 20/80 to 0/100 (100% PDLLAM). 2,2-dimethoxy 2-phenyl acetophenone (2.5% w/v) was used as a photoinitiator. BSA of concentration 2.5% (w/w based on polymer precursors) was added to the solution and stirred for uniform distribution. The solution was then transferred to a Teflon plate and converted to hydrogel form by exposure to a long wavelength UV light (365 nm, 8 watt) for 3 h. The diameter of the resulting hydrogel disk was 8 mm and its thickness was 0.8 mm.

Three dex-AI/PDLLAM hydrogel systems shown in Table I (dex-AI/PDLLAM-**1**, -**2** and -**3**) were synthesized and they had different MWs of dex-AI and PDLLAM. These three hydrogel systems were used for the studies of hydrogel degradation and BSA release.

In vitro biodegradation of the hydrogel

The hydrolytic degradation of the dex-AI/PDLLAM hydrogels was evaluated by their weight loss at 37 °C in PBS. The PBS was replaced completely at three-day intervals. The course of hydrogel degradation was followed gravimetrically until the loss of structural integrity of the hydrogel introduced handling errors. Both the weight change and water content of the hydrogels were measured as hydrolytic degradation proceeded. For each measurement, three replicates were analyzed.

Weight loss measurement

An exactly weighed dry hydrogel (e.g. 35 mg) was immersed into 15 ml PBS at 37 °C. At various intervals (up to 50 days), the residual hydrogel was removed and dried under vacuum at room temperature till constant weight. After weighing, the dry hydrogel was placed into PBS again as before for further degradation-induced weight loss measurement. The weight loss was calculated according to the following equation: percentage weight remaining (of initial weight) $= (W_t'/W_0) \times 100\%$, where W_0 was the initial $(t = 0)$ dry weight of hydrogel, and W_t' was the dry weight of the same hydrogel after incubation at time t.

Water content measurement

Individual dry hydrogel of known weight (e.g. 35 mg) was allowed to swell in 15 ml PBS. At predetermined intervals (up to 56 days), the hydrogel was retrieved from the medium and the wet weight was recorded after drying off excess water on the hydrogel surface. The water content was calculated according to the following equation [10]:

$$\text{water content } (\%) = (W_t - W_0)/W_t \times 100\%$$

where W_0 was the initial $(t = 0)$ dry weight of hydrogel,

TABLE I Composition and molecular weights of dex-AI/PDLLAM hydrogels and their constituents

Hydrogel systems	Dextran MW	PDLLA MW	Sample series (dex-AI/PDLLAM w/w)				
Dex-AI/PDLLAM-1	43 000	740	100/0	80/20	50/50	20/80	0/100
Dex-AI/PDLLAM-2	70 000	740	100/0	80/20	50/50	20/80	0/100
Dex-AI/PDLLAM-3	70 000	7300	100/0	80/20	50/50	20/80	0/100

and W_t was the wet weight of the same hydrogel in swollen state after incubation at time t.

In vitro BSA release studies

About 35 mg BSA-containing dex-AI/PDLLAM hydrogel was placed in 15 ml PBS and incubated at 37 °C. At predetermined intervals, 2 ml of medium was withdrawn and the same volume of fresh PBS was added back to the flask in order to keep the total volume constant. BSA release from the hydrogel was determined by measuring fluorescent intensity of the withdrawn solution at 490 nm using a Perkin Elmer Lambda 2 UV/VIS spectrometer (Norwalk, CT). Cumulative release of BSA was used to depict its release profile as a function of hydrogel composition and time.

Hydrogel morphology

The changes in the surface morphology of 50/50 dex-AI/PDLLAM hydrogels of different MW after 50 days *in vitro* degradation were evaluated by scanning electron microscopy (SEM, Hitachi S4500). Specimens were freeze-dried according to our previously published method to retain their structure in the swollen state and then sputter-coated with gold prior to SEM examination [11].

Result

In vitro biodegradation

An example of the *in vitro* biodegradation of the dex-AI/PDLLAM hydrogels in terms of their weight losses as functions of immersion time and composition ratio of the hydrogels' constituents is shown in Fig. 2. Generally, we observed a two- to three-stage weight loss profile of the hydrogels, depending on the composition ratio of dex-AI to PDLLAM: an initial rapid loss of weight (1st stage) followed by a slower weight loss (2nd stage). For those dex-AI/PDLLAM hydrogels of high PDLLAM composition, there was an accelerated weight loss thereafter (3rd stage). The distribution and severity of these two to three stages depended mainly on the composition ratio but less on MW of dex-AI and PDLLAM (Figs. 3 and 4).

Although the initial rapid weight loss was observed for all hydrogels, irrespective of their composition ratios, the 0/100 dex-AI/PDLLAM hydrogels always had the smallest initial weight loss. For example, after three-day immersion in PBS, the weight losses of the 0/100, 20/80, 50/50, 80/20 and 100/0 dex-AI/PDLLAM-**1** hydrogels were 4%, 12%, 19%, 20% and 17%, respectively. The duration of the slower weight loss phase (2nd stage) became shorter as the PDLLAM composition in the hydrogel increased, e.g. 36, 24 and 17 days for the 50/50, 20/80 and 0/100 dex-AI/PDLLAM-**1** hydrogels, respectively. The slope of this slow weight-loss stage increased from 0.74 for 50/50, 1.25 for 20/80 to 1.95 for 0/100 dex-AI/PDLLAM-**1** hydrogel. Finally, the last stage of rapid weight loss of the hydrogels became apparent when the PDLLAM composition was greater than or equal to 50%. We did not observe this last phase of accelerated weight loss in 100/0 and 80/20 dex-AI/PDLLAM-**1** hydrogels during the 50-day study period.

Fig. 3 revealed that, at the same composition ratio, an increase in MW of dex-AI did not significantly alter the profiles of the three-stage weight loss of the hydrogels. For example, the weight-loss profiles of the dex-AI/PDLLAM-**1** and -**2** hydrogels were very similar at both 100/0 and 50/50 dex-AI to PDLLAM ratio. An increase in the MW of PDLLAM, however, resulted in a profound change in weight-loss profiles, particularly at the second and third stages. For example, the slope for the slow

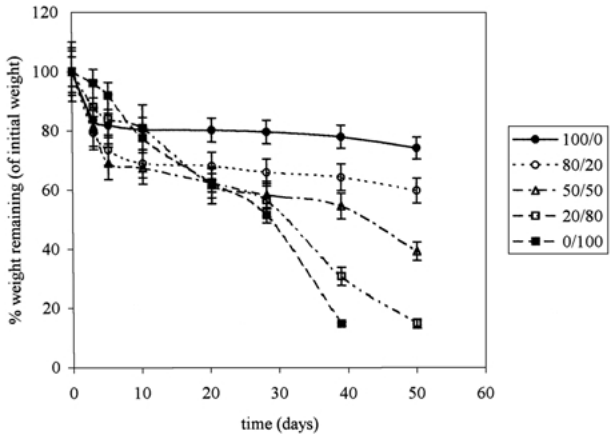


Figure 2 Weight loss profiles of dex-AI/PDLLAM-**1** hydrogels in pH 7.4 PBS at 37 °C.

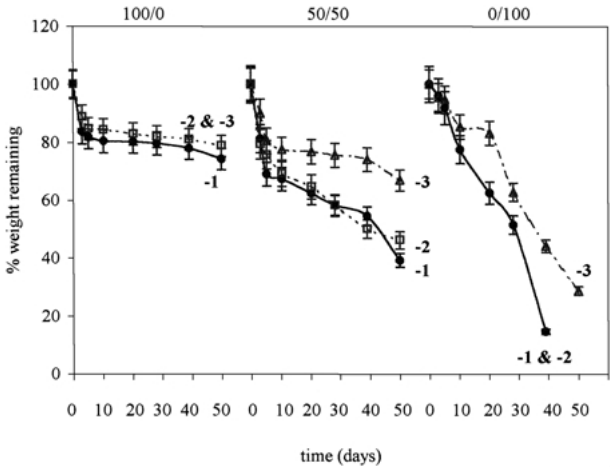


Figure 3 The effect of molecular weight of hydrogel components on the distribution of various stages of weight loss of dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels as a function of immersion time in pH 7.4 PBS at 37 °C.

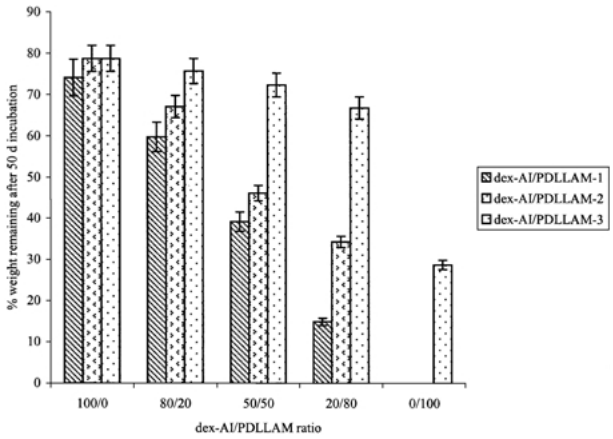


Figure 4 The effect of molecular weight of hydrogel components on the total weight loss of dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels after 50-day incubation in pH 7.4 PBS at 37 °C.

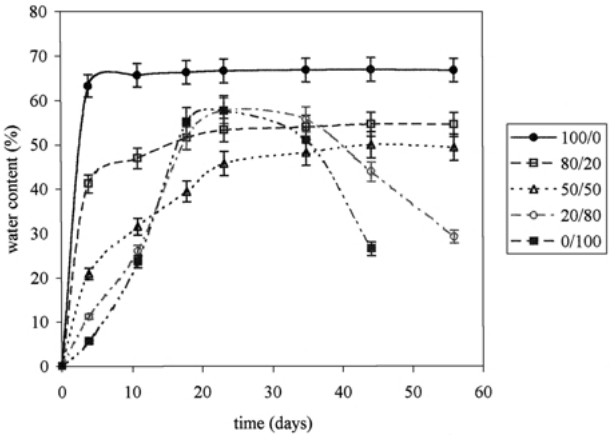


Figure 5 Water content of dex-AI/PDLLAM-**1** hydrogels in pH 7.4 PBS at 37 °C.

weight-loss stage decreased from 1.95 for 0/100 dex-AI/PDLLAM-**1** hydrogel to 0.72 for 0/100 dex-AI/PDLLAM-**3** hydrogel, an indication of slower weight loss rate due to a higher MW of PDLLAM.

The total amounts of weight loss of the dex-AI/PDLLAM hydrogels after 50-day immersion were also composition and MW dependent as shown in Fig. 4. At the end of 50-day study period, the total weight losses of the 100/0, 80/20, 50/50, 20/80 and 0/100 dex-AI/PDLLAM-**1** hydrogels were 26%, 40%, 61%, 85% and 100%, respectively. An increase in the MW of dex-AI (i.e. dex-AI/PDLLAM-**1** vs. dex-AI/PDLLAM-**2**) had a statistically insignificant effect on the total weight loss of all dex-AI-containing hydrogels, e.g. the total weight losses of the 100/0 dex-AI/PDLLAM-**1** and -**2** hydrogels were 21% and 26%, respectively, an indication of a slight reduction in the degradation rate of the dex-AI/PDLLAM hydrogel as a result of an increase in the MW of dex-AI.

The change in the MW of PDLLAM, however, resulted in a large impact on the total weight loss of all PDLLAM-containing hydrogels. For example, only 14% of its initial weight of the 0/100 dex-AI/PDLLAM-**1** hydrogel (low MW of PDLLAM) remained at the end of 40 days and the hydrogel disappeared after 50 days incubation, i.e. degraded completely. In contrast, the remaining weights of the 0/100 dex-AI/PDLLAM-**3** hydrogel (higher MW of PDLLAM) were 45% and 29% after 40 and 50 days incubation, respectively. In addition, it appeared that the MW effect became more apparent as the PDLLAM composition increased, i.e. the total weight losses of the 80/20 dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels were 40%, 33% and 24%, respectively, while those of the 20/80 dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels were 85%, 66% and 33%, respectively.

Water contents

The water contents of the dex-AI/PDLLAM-**1** hydrogels as a function of hydrogel composition ratio and immersion time are shown in Fig. 5. It revealed that the change in water content with immersion time depended on the amount of PDLLAM in the hydrogel. The water uptake of the dex-AI/PDLLAM hydrogels in the initial period (a few days) of study decreased as the hydrophobic PDLLAM composition in the hydrogel increased. After the initial period, the hydrogels having high dex-AI composition reached to a relatively stable water content; however, those hydrogels having high PDLLAM composition showed a maximum water content pattern. For example, a maximum water content pattern was observed for the 0/100 and 20/80 dex-AI/PDLLAM-**1** hydrogels, while the 100/0, 80/20 and 50/50 dex-AI/PDLLAM-**1** hydrogels did not show such a maximum water content pattern during the eight-week study period. The water content profiles of the dex-AI/PDLLAM-**2** and -**3** hydrogel systems showed similar behavior as the dex-AI/PDLLAM-**1** hydrogel system.

SEM observation of degraded hydrogels

Fig. 6 shows the representative SEM micrographs of the 50/50 dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels after 50 days *in vitro* degradation. The images revealed that the dex-AI/PDLLAM hydrogel showed porous network structure upon incubation in aqueous medium. The formation of pores inside these hydrogels was due to both the swelling of the hydrophilic dex-AI component and the hydrolytic degradation of the PDLLAM component [12]. The MW of the hydrogel components appeared to have some effect on the porous morphology. A very loose and more open 3D network structure with thin pore walls and large number of pores was formed in the 50/50 dex-AI/PDLLAM-**1** hydrogel (Fig. 6(A)). As the MWs of either dex-AI or/and PDLLAM increased, this three-dimensional network structure became more compact and ''solid'' (thicker pore walls and less pores) as shown in the SEM images of the 50/50 dex-AI/PDLLAM-**2** and dex-AI/PDLLAM-**3** hydrogels (Fig. 6(B) and (C)).

In vitro BSA release

The release of BSA from the dex-AI/PDLLAM hydrogels was composition ratio and MW dependent. As the PDLLAM to dex-AI composition ratio increased, the extent and rate of the BSA release also increased. An increase in the MW of dex-AI, however, slightly decreased the BSA release extent, while an increase in the MW of PDLLAM resulted in a large reduction in the BSA release extent.

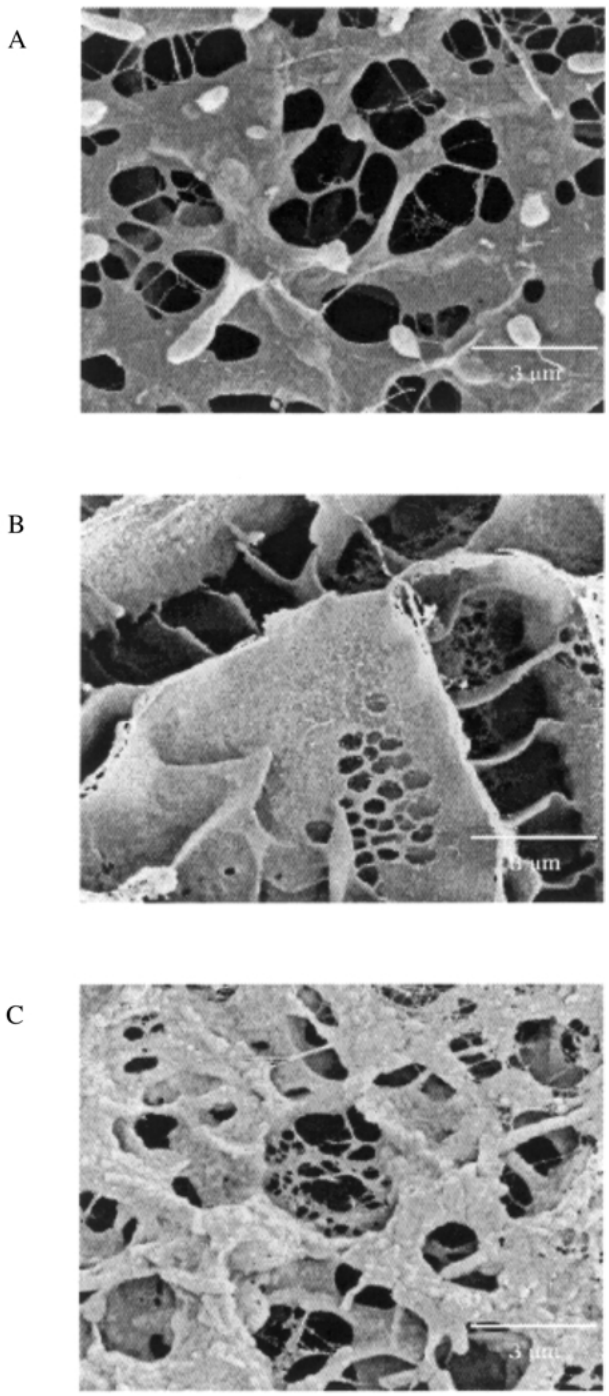


Figure 6 Scanning electron micrographic images of 50/50 dex-AI/PDLLAM hydrogels after 50-day immersion in pH 7.4 PBS at 37 °C, (A) 50/50 dex-AI/PDLLAM-**1**, (B) 50/50 dex-AI/PDLLAM-**2**, (C) 50/50 dex-AI/PDLLAM-**3**.

Fig. 7 summarizes the release profiles of BSA from the dex-AI/PDLLAM-**1** hydrogels as a function of composition ratio and incubation time. In brief, a burst release of BSA was observed in the first two days of immersion, followed by a sustained release up to 58-day study period. The extent and rate of the sustained release of the BSA increased with the PDLLAM composition in the hydrogel.

The dex-AI/PDLLAM-**2** and -**3** hydrogels showed similar composition-dependent BSA release profiles as the dex-AI/PDLLAM-**1** hydrogel. However, the extent of BSA release decreased with an increase in the MW of the hydrogel constituents. As shown in Fig. 8, an increase in the MW of dex-AI seemed to slightly decrease the total

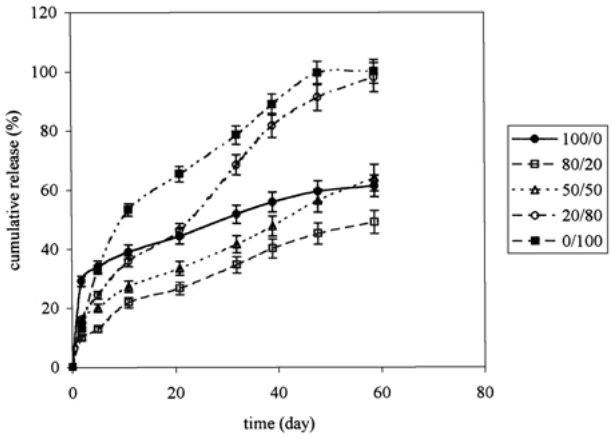


Figure 7 Cumulative release of BSA from dex-AI/PDLLAM-**1** hydrogels in pH 7.4 PBS at 37 °C.

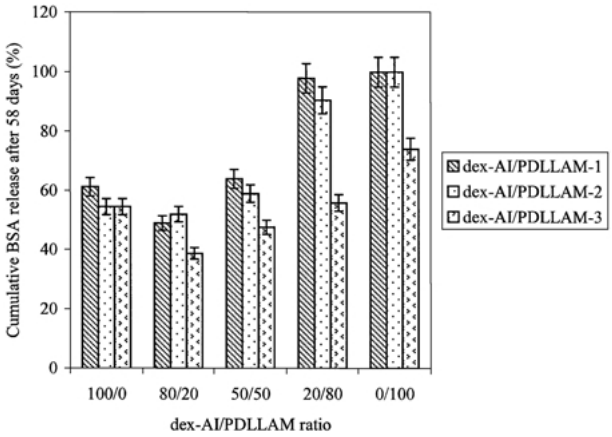


Figure 8 The effect of molecular weight of hydrogel components on the cumulative release of BSA from dex-AI/PDLLAM-**1**, -**2** and -**3** hydrogels after 58-day incubation in pH 7.4 PBS at 37 °C.

amount of BSA released, while an increase in the MW of PDLLAM resulted in an obvious reduction in the release extent of BSA. For example, at the end of 58 days, 64% BSA was released from the 50/50 dex-AI/PDLLAM-**1** hydrogel, followed by 59% for the 50/50 dex-AI/PDLLAM-**2** hydrogel and 47% for the 50/50 dex-AI/PDLLAM-**3** hydrogel.

Discussion

The effect of composition ratio on the degradation of the hydrogels

Generally, the initial rapid weight loss of the dex-AI/PDLLAM hydrogel came from the leakage of uncrosslinked hydrogel components; the hydrolytic degradation of the PDLLAM component contributed to the weight loss thereafter. As the PDLLAM composition in the hydrogel increased, the rate of hydrolytic degradation of the hydrogel increased, consequently, the weight loss rate increased.

The 100/0 dex-AI/PDLLAM hydrogel had the smallest weight loss among all the composition ratios studied. The initial rapid reduction in dry weight of the 100/0 dex-AI/PDLLAM hydrogel came from the leakage of uncrosslinked dex-AI segments through pores and channels that formed upon the swelling of the hydrogel.

After this initial phase, the subsequent reduction in the weight of the 100/0 dex-AI/PDLLAM hydrogel was very small. This is because dextran is non-hydrolytically degradable and can only be degraded by dextranase [13, 14]. The very small weight loss after the initial stage might be attributed to the further diffusion of uncrosslinked dex-AI segment from the bulk. Although the degradation of urethane linkage in the crosslinkers of dex-AI was possible, its effect on weight loss would not be apparent until in a very long immersion due to the relatively hydrolytic stable nature of the urethane linkage in PBS. We expect that the rate of weight loss would increase if a less hydrolytic stable crosslinker would be chosen, which was confirmed by other researchers [15, 16]. For example, van Dijk-Wolthuis [15] found that the dissolution of hydroxyethyl methacrylate-derivatized dextran (dex-HEMA) hydrogel was slower than HEMA-oligolactate-derivatized dextran (dex-lactateHEMA) hydrogel because of the existence of lactate esters in the crosslinkers of dex-lactateHEMA. Chakravarthy and Smith [16] dextran microspheres crosslinked by cyanogen bromide (CNBr) were degraded to soluble product in neutral buffer via their CNBr crosslinker. The degradation rate increased linearly with the ratios of CNBr to dextran. In contrast, they found that epichlorohydrin-crosslinked dextran microspheres were resistant to hydrolysis and only degraded enzymatically (dextranase), because epichlorohydrin could not be degraded in buffer.

The observed triphasic degradation profile in those dex-AI/PDLLAM hydrogels having a larger proportion of PDLLAM was attributed to the initial leakage of uncrosslinked PDLLAM and dex-AI located near the surface as well as the time-dependent formation of porous 3D network structure (from an initial compact structure to a loose 3D network structure in a late stage). Due to the initial compact network structure of the 0/100 dex-AI/PDLLAM hydrogels and the hydrophobic nature of the PDLLAM, the initial weight loss of the 0/100 dex-AI/PDLLAM hydrogel from the leakage of uncrosslinked PDLLAM segments was the lowest among all the composition ratios studied. The hydrolysis of the PDLLAM backbone, however, contributed to the weight loss of the hydrogel thereafter. It is well documented that the polylactic acid-based polymer can be hydrolyzed in PBS via random chain scissions of the ester linkages in the polymer backbone [17–20]. At an earlier immersion period, most of the degraded PDLLAM fragments were difficult to diffuse out of the compact hydrogel. This resulted in a relatively slow weight loss as observed. At a later immersion period, more water penetrated into the network due to the gradual formation of a loose 3D network structure, the degradation rate of the 0/100 dex-AI/PDLLAM hydrogel increased. Large amounts of the cleaved PDLLAM fragments could then diffuse out of the porous network relatively easily and an accelerated weight loss was thus observed. If polylactide had a much higher MW ($> 59\,000$) than our PDLLAM ($< 10\,000$), only biphasic weight loss pattern was observed. For example, Sansdrap and Moes [19] observed a biphasic weight loss of lactide (MW $\sim 60\,000$) based microsphere in buffer. Ramchandani *et al*. [20] further suggested that the biphasic degradation of lactide (MW 73 000–91 000) based microsphere came from accelerated degradation. This accelerated degradation was attributed to the acidic degradation products that were too large to diffuse out of the compact core of the high MW polylactide-based microspheres. These entrapped acidic degradation products would lower the pH in their vicinity and lead to acid-autocatalysis degradation as reflected in the second accelerated degradation phase observed by Ramchandani *et al*. [20]. The high acidity (i.e. lower pH) near the core of the hydrolytically unstable aliphatic polyesters in the late stage of their hydrolytic degradation was very recently demonstrated experimentally by Slivka *et al*. [21]. They used laser confocal microscopic technique and found that the interior core of a poly-*p*-dioxanone absorbable fiber indeed had a significantly lower pH than the ones near or on the fiber surface.

In a dex-AI/PDLLAM bicomponent hydrogel, its initial weight loss came from the leakage of the uncrosslinked dex-AI and PDLLAM segments. These segments could diffuse out of the hydrogel through water-filled pores and channels formed mainly from the swelling of the hydrophilic dex-AI segments. After this initial weight loss, additional weight loss occurred when the degraded PDLLAM fragments were small enough to be water-soluble and to be able to diffuse out of the hydrogel network. In the bicomponent hydrogels having high dex-AI composition, large amounts of pores could form in the hydrogel network during early-medium immersion stage. These pores and water channels would facilitate the migration of the degraded short PDLLAM fragments out of the hydrogel network easily, and hence the accelerated weight loss in the late stage was not observed, i.e. the lack of a triphasic degradation pattern.

On the contrary, after the initial weight loss, the bicomponent hydrogels having high PDLLAM composition would make the degraded PDLLAM fragments difficult to diffuse out because of the relatively compact network structure of the hydrogels, i.e. a slower degradation phase was followed and observed. This relatively slower degradation phase would eventually lead to the formation of a more open 3D porous hydrogel network and large amounts of PDLLAM fragments could diffuse out of the hydrogel thereafter. Hence, in the late stage of immersion, a significant weight reduction was observed in those bicomponent hydrogels having high PDLLAM composition.

The slope of the slower weight loss phase (2nd stage) became steeper and the duration of this slow weight loss phase became shorter as the PDLLAM content in the dex-AI/PDLLAM bicomponent hydrogel increased. This clearly indicated that the degradation rate of the dex-AI/PDLLAM hydrogel increased as the PDLLAM composition in the hydrogel increased. Because the hydrolytic degradation rate of the ester linkages (in PDLLAM bacbone) is much faster than the urethane linkages (in dex-AI), as the ester concentration (i.e. the PDLLAM composition) increased, degradation rate of the hydrogel increased. Therefore, the degradation rate of the dex-AI/PDLLAM hydrogel depended mainly on the concentration of the PDLLAM component instead of the dex-AI component. The hydrophilicity of the non-hydrolytic degradable dex-AI did not have much effect on the

degradation rate of the dex-AI/PDLLAM bicomponent hydrogels. Similar conclusions were also reported by others [7, 22]. Friederike *et al*. [22] found that the erosion kinetics of copolymers made of non-degradable polyethylene glycol monomethylether (Me.PEG) and degradable poly (D,L-lactic acid) (PLA) were similar to that of PLA alone. They suggested that the impact of the Me.PEG segment (the hydrophilic component) on the copolymer erosion was not pronounced. In addition, Friederike *et al*. [22] also found that during erosion the weight of dried copolymer samples remained constant for 11 weeks, after which erosion set in rapidly. Similarly, Fan *et al*. [7] showed that, in their study of the hydrolytic degradation property of poly (γ-glutamic acid) hydrogel, the process of the hydrogel degradation could be controlled mainly through the concentration of the ester linkages in the network.

The effect of MW on the degradation of the hydrogel

An increase in the MW of dex-AI slowed down the degradation of the dex-AI/PDLLAM hydrogel. This is because an increase in dex-AI chain length may enhance the formation of physical entanglements among polymer chains and provide a relatively tighter network structure. As a consequence, less water could penetrate into the hydrogel network, i.e. slower degradation rate [23]. This was confirmed by our SEM data, i.e. a tighter 3D network structure formed in the 50/50 dex-AI/PDLLAM-**2** hydrogel (high MW of dex-AI, Fig. 6(B)) than in the 50/50 dex-AI/PDLLAM-**1** hydrogel (low MW of dex-AI, Fig. 6(A)).

An increase in the MW of PDLLAM also slowed down the degradation rate of the hydrogels, particularly those having high PDLLAM composition. Because the degradation rate of the high MW PDLLAM is significantly slower than that of the low MW PDLLAM, and the fragments of high MW PDLLAM would be larger than the ones from low MW PDLLAM, these larger size fragments would be difficult to diffuse out of the hydrogel network. Therefore, hydrogels having high MW PDLLAM (e.g. dex-AI/PDLLAM-**3** hydrogels) would retain higher weight than the ones having low MW PDLLAM (e.g. dex-AI/PDLLAM-**1** hydrogels) (Fig. 4).

The MW effect on the total weight loss of the dex-AI/PDLLAM hydrogel was more significant in those hydrogels having high PDLLAM composition. This is because the total composition is 100%, as the PDLLAM component (the hydrolytic degradable component) increased, the dex-AI component (the hydrolytic stable component) decreased correspondingly, i.e. the hydrogel became more sensitive to hydrolytic degradation. Therefore, hydrogels having high PDLLAM composition would become more sensitive to the change of those factors (e.g., MW) that would affect the hydrolytic degradation rate.

Water content in the hydrogel

The hydrophilicity of the dex-AI component was responsible for the initial rapid water uptake. After this initial stage, the relatively stable water content of the hydrogels having high dex-AI composition came from the stable network structure of the dex-AI in PBS (i.e. 100/0 and 80/20 dex-AI/PDLLAM-**1** hydrogels in Fig. 5). However, for those hydrogels having high PDLLAM composition, the water uptake at an early stage of immersion mainly depended on the swelling-induced porous network structure formed inside the hydrogels. Such swelling-induced porous structure was facilitated by the presence of the hydrophilic dex-AI component. Therefore, hydrogels having higher dex-AI composition would exhibit faster and higher water uptake as observed. After this initial period, the accelerated water content was due to the formation of loose 3D network structure because of the degradation of the PDLLAM. As immersion time increased further, the PDLLAM degradation would destroy the hydrogel structural integrity and water content decreased thereafter. The composition of the degradable PDLLAM in the dex-AI/PDLLAM hydrogel must reach beyond 80% to observe this maximum water content pattern. Similar maximum water content pattern was also observed by van Dijk-Wolthuis [15]. They found that the water content of both dex-HEMA and dex-lactateHEMA hydrogels initially increased as biodegradation proceeded, then decreased during late immersion time (around 15 days). However, Yeh *et al*. [24] found that the degradation of the *N*,*N*-dimethylacrylamide hydrogel via the azo bond cleavage was only characterized by a continuance increase in water content in the hydrogel. They didn't observe the maximum water content pattern possibly due to their short study period (four days).

There was a close correlation between the weight loss and water content profiles as shown in the example of 50/50 dex-AI/PDLLAM hydrogel (Fig. 9). The data illustrated that the change in water content was directly related to the change in weight loss, i.e. as the rate of weight loss increased, the rate of water content change also increased accordingly. For example, both the weight loss and water uptake of the 50/50 dex-AI/PDLLAM-**1** hydrogel were fast initially. Afterward, the rate of weight loss became slower and the rate of increase in water content also became smaller accordingly. Similar correlations between weight loss and water content were also found in hydrogels of other dex-AI to PDLLAM composition ratios. The 0/100 dex-AI/PDLLAM-**1** hydrogel had a unique weight loss to water content correlation at the late stage of immersion (> 28 days). The onset of the accelerated weight loss in the 3rd stage coincided with the accelerated reduction in water content. This suggested that the accelerated weight loss in the 3rd stage started to destroy the structure integrity of the hydrogel and the fragmented hydrogel network could not hold water as much as before, i.e. reduction in water content.

In vitro BSA release

The release of BSA from the dex-AI/PDLLAM hydrogels was characterized by an initial burst release followed by a sustained release phase. By comparing these BSA release profiles with the hydrogel degradation (weight loss) profiles (Fig. 10), it appears that the

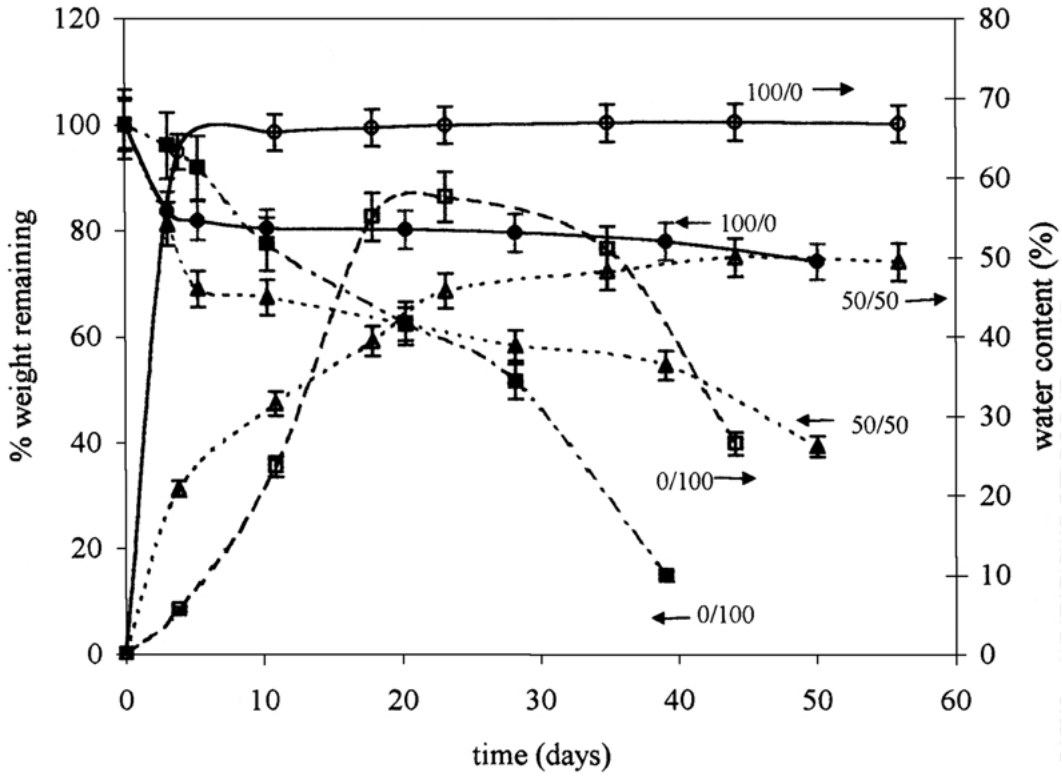


Figure 9 Comparison of the changes in weight loss (solid symbols) with the changes in water content (open symbols) of dex-AI/PDLLAM-**1** hydrogels as a function immersion time in pH 7.4 PBS at 37 °C.

degradation profiles correlated well with the BSA release profiles over the entire study period. For example, the initial rapid weight loss correlated with a fast release of BSA, then both the rates of weight loss and BSA release became slower thereafter. As the hydrogel became completely degraded, all the remaining BSA would be released, i.e. the 0/100 dex-AI/PDLLAM-**1** hydrogel disappeared at about 50-day immersion period and the release of BSA reached 100% at the same incubation period.

The mechanisms of BSA release from the dex-AI/PDLLAM hydrogels were investigated in detail in our

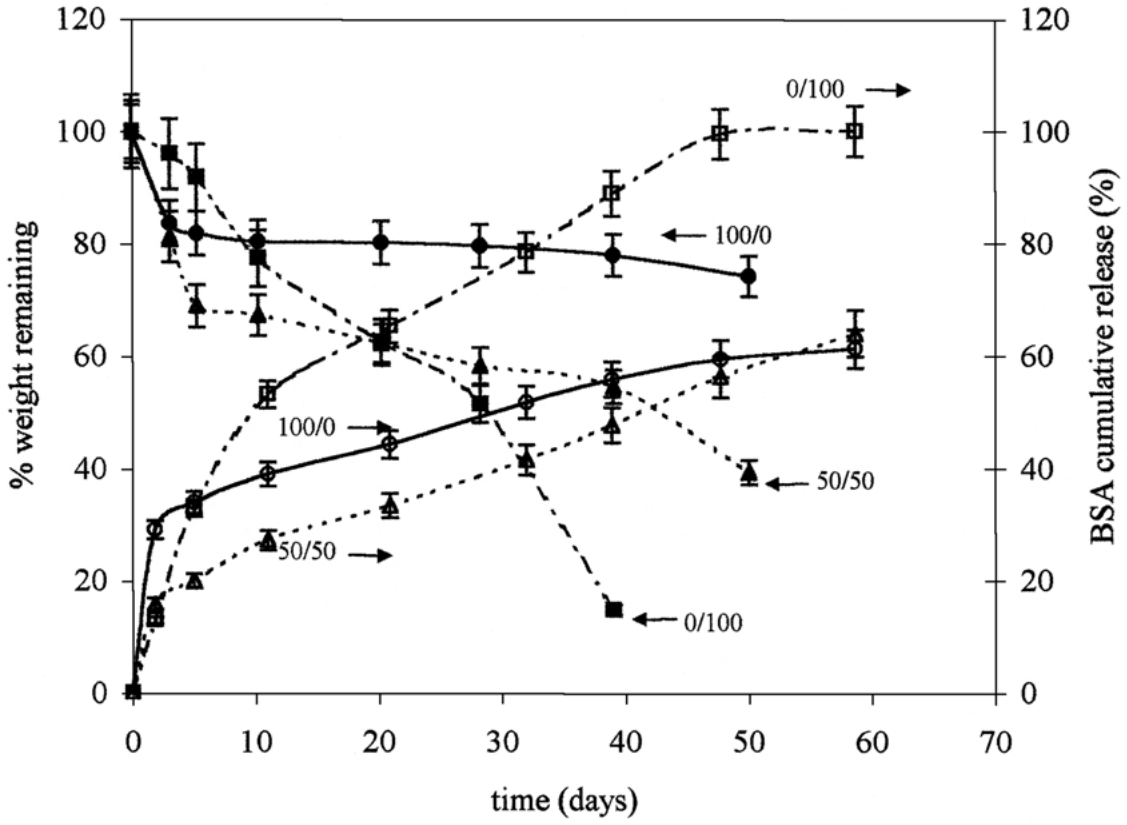


Figure 10 Comparison of weight-loss profiles of (solid symbols) dex-AI/PDLLAM-**1** hydrogels and their BSA cumulative release (open symbols).

previous study [25]. In brief, the BSA release was controlled by the formation of both swelling-induced porous structure in the early stage and a loose 3D network structure in the medium-late stage via PDLLAM hydrolytic degradation. The initial swelling-induced pore formation and permeability was controled by the dex-AI component. The degradation of the PDLLAM component (weight loss) further modified the swelling-induced porosity and permeability of the hydrogel, i.e. enlarged the pore size and transferred to a more open and loose 3D network structures gradually. These progressive changes in the physical and morphological properties of the hydrogel controlled the ability of BSA (large size protein) to diffuse out of the hydrogel network and, thus, its release rate and extent. An increase in the PDLLAM composition in dex-AI/PDLLAM hydrogels would enhance the rate and extent of forming such a more open and loose 3D network structure, which inevitably would enhance BSA release.

The release profiles of BSA was also MW-dependent as shown in Fig. 8. An increase in MW of dex-AI resulted in a slight reduction in the total amounts of BSA released during 58 days. This might be due to the formation of a relatively tighter network structure resulting from longer chain entanglement. On the other hand, an increase in the MW of PDLLAM exhibited a significant reduction in the total amounts of BSA released from the hydrogels. This is because the higher the MW of PDLLAM was, the slower the hydrolytic degradation of the hydrogel became (Fig. 3), i.e. a slower formation of a more open and loose three-dimensional network structure which led to a smaller total amount of BSA released. This MW-dependent BSA release was also reported by Song *et al*. [26]. They found that BSA release from microparticles of poly (lactic-*co*-glycolic acid) (PLGA) decreased with an increase in the MW of PLGA. For example, an increase in the MW of PLGA from 58 K to 102 K resulted in a 35% reduction in the total amounts of BSA released during 20-day immersion period. Similarly, an increase in the MW of PDLLAM in our 0/100 dex-AI/PDLLAM hydrogels from 740 to 7300 resulted in a 26% reduction in the total amounts of BSA released during 58 days period. We thus expect that the rate and extent of forming a more open and loose network structure via biodegradation of hydrogel components would play a major role in the controlled release of large MW drugs.

Conclusions

This study shows that a wide range of hydrolytic degradation profiles of dex-AI/PDLLAM hydrogels could be achieved via dex-AI to PDLLAM composition ratio as well as the MW of dex-AI and PDLLAM. Degradation of these hydrogels mainly resulted from the hydrolysis of the ester bonds on PDLLAM backbone. Generally, as the PDLLAM composition increased, the rate of weight loss due to the hydrolytic degradation of the PDLLAM increased. The increase in MW of either dex-AI or PDLLAM would decrease the degradation rate of the hydrogel mainly because of the formation of relatively compact network structure or slower polymer degradation rate. The water content in the hydrogel was directly related to the rate and extent of formation of porous network structure in the hydrogel, which, in turn, depended on the composition ratio and weight loss of the hydrogel.

The release of BSA from the dex-AI/PDLLAM hydrogels was correlated well with the water content and degradation profiles of the hydrogels. Such correlations suggest that the BSA release was controlled by the formation of both swelling-induced porous structure in the initial stage and a more open and loose 3D network structure in the middle–late stage via PDLLAM hydrolytic degradation. The extent and rate of BSA release increased as the PDLLAM composition increased, and decreased as the MWs of the hydrogel precursors increased. Therefore, the release profiles of albumin from the dex-AI/PDLLAM biodegradable hydrogels can be tailored to specific needs by choosing hydrogels with a proper composition ratio and MW.

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