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Crosslinked gelatin matrices: release of a random coil macromolecular solute

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Abstract

The purpose of this investigation was to evaluate the effect of matrix crosslinking and solute size on release of a random coil macromolecular solute from crosslinked gelatin matrices. Gelatin hydrogel matrices crosslinked with different molar ratios of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC):ε-amino groups on gelatin (1:1, 4:1, and 10:1) were prepared containing dextran of molecular weights 12, 20, and 77 kDa, and hydrodynamic diameters 54, 74, and 133 Å, respectively. The extent of matrix crosslinking was determined quantitatively and used to calculate the molecular weight between crosslinks (M_c). The M_c parameter and equilibrium swelling ratio (Q_m) were used to calculate an estimated matrix mesh size (ξ). The in vitro release of incorporated dextran was evaluated at 37 °C in PBS at pH 7.4 for approximately 80 h. The one-, four- and 10-fold molar ratios of crosslinking agent EDC yielded 24, 41, and 78% of gelatin matrix crosslinking, respectively. The calculated average matrix mesh size ranged from 338 to 90 Å. The effect of matrix crosslinking varied with solute size, from retarding diffusional release of the dextran to completely entrapping it inside the crosslinked matrices. These results support the threshold concept of solute size relative to matrix mesh size for release of a flexible, random coil macromolecular solute from a hydrogel.

Keywords: Crosslinked gelatin matrices; Matrix mesh size; Physical and chemical crosslinks; Random coil macromolecular solute release; Hindered diffusion; Dextran

1. Introduction

Macromolecular therapeutic agents such as peptides, proteins, and oligonucleotides have been formulated into matrix devices and studied for their controlled delivery (Kwon et al., 1992; Davis, 1974; Sato and Kim, 1984; Rheinhart and Peppas, 1984). Differences, however, in the physicochemical properties of both macromolecules and matrices in these investigations have produced variable determinants that influence release of macromolecules from polymeric matrices. Moreover, some of the macromolecular solutes used in these investigations have fixed conformations while others are flexible polymers. For example, Kwon et al. (1992), studied release of the macromolecular solute, FITC-dextran (17.2 kDa) using an albumin–heparin matrix crosslinked with different concentrations of a crosslinking agent. They observed in early release periods that crosslinking did modulate the release rate of the macromolecular

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solute while at longer release periods, the sustained release was attributed to release of the entangled FITC-dextran. Sato and Kim (1984) studied diffusion of solutes ranging in size from sodium acetate (82 Da) to albumin (69 kDa) from polymer matrices. They demonstrated a correlation of decreasing diffusion coefficient with increases in molecular weight of the solute. Rheinhart and Peppas (1984) studied the release of the macromolecular solute bovine serum albumin (BSA) from polyvinyl alcohol (PVA) hydrogel matrices crosslinked to different extents using glutaraldehyde. They demonstrated a decreasing diffusional release with increasing degree of matrix crosslinking. Blanch and Prausnitz conducted pioneering studies of random coil macromolecules partitioning into pre-swollen gels that provided fundamental information about the pore (or mesh) size distribution in hydrogels (Kremer et al., 1994; Walther et al., 1995). But, few studies are available on the effect of matrix polymer crosslinking on release of an incorporated random coil macromolecular solute from a hydrogel.

Controlled release of drug solute from polymeric formulations of the monolithic or reservoir type depends on the physical structure of the polymeric material and the drug solute size. Often a diffusion screening effect due to crosslinking is described as the amount of the crosslinking agent added during the preparation, or as the crosslinking time (Vandelli et al., 1991). The symbol M_c refers to the molecular weight, and by inference, the distance between chemical crosslinks; M_c is an important structural parameter of the polymer matrix network (Peppas and Moynihan, 1985). This structural parameter (M_c) is usually associated with the degree of hydrogel swelling and may be used with swelling measurements to calculate the average crosslinked matrix mesh size (ξ). The matrix mesh size parameter is a measure of the effective area available for solute diffusion. This area determines the screening effect on solute diffusion.

Water soluble carbodiimide crosslinking agents induce stoichiometric formation of an amide or peptide bond between primary amino and carboxylic acid groups. This reaction involves amino acid residues of lysine and hydroxylysine, and aspartic and glutamic acids on gelatin molecules. Gelatin contains approximately 33 ε-amino groups and approximately 120 carboxylic acid groups on an ideal 100,000 Da molecule composed of 1000 amino acid residues (Veis, 1964). Thus, the zero spacer arm crosslink bridge has the potential to form a highly crosslinked matrix. Moreover, the crosslinking byproduct is a urea derivative, which is water soluble. These crosslinking agents present advantages of known crosslinking sites and crosslink structure, better control of crosslinking, greater potential for quantitative crosslinking evaluation, and less toxicity compared to commonly used aldehyde crosslinking agents, such as formaldehyde and glutaraldehyde.

A quantitative method has been employed to determine the extent of crosslinking in proteinaceous hydrogel matrices (Gilbert and Kim, 1990; Bubnis and Ofner, 1992). This determination involves the use of 2,4,6-trinitrobenzenesulfonic acid (TNBS) (a UV chromophore) which specifically reacts with primary amino groups.

The overall goal of this investigation was to evaluate the effect of crosslinking extent and solute size in gelatin matrices on the release of a random coil macromolecular solute.

2. Materials and methods

2.1. Materials

Model random coil macromolecular solutes: dextran 12 kDa, lot no. 125H1127; dextran 19.5 kDa, lot no. 85H02249; dextran 41 kDa, lot no. 75H0559; dextran 77 kDa, lot no. 75H0904; dextran 167 kDa, lot no. 15H0387; dextran 260 kDa, lot no. 105H0202; 5% (w/v) TNBS solution reagent; premixed phosphate buffered saline (PBS) granules pH 7.4, 0.01 M, containing 0.138 M NaCl and 0.0027 M KCl; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sodium azide were obtained from Sigma Chemical Company (St. Louis, MO, USA). Type B USP granular gelatin, lot no. 10287 from Rousselot (Paris, France), prepared by alkaline treatment of bone was used without further purification. The moisture content of solid granules during storage was approximately 10% (w/w) determined by loss on drying (LOD) at 105 °C. The pH of a 1% solution was 5.8 and isoionic point was 5.2 (Welz and Ofner, 1992). All other chemicals were ACS reagent grade.

2.2. Characterization of model random coil macromolecular dextran

Three molecular weight dextrans of 12, 20, and 77 kDa with relatively low polydispersity indices of 1.6, 2.0, and 1.4, respectively, were primarily used in release studies. The hydrodynamic radii of the three molecular weight dextrans were determined using high performance size exclusion chromatography (HPSEC) (Mwangi, 2001). A third order polynomial calibration curve using Pullulan standards was used to relate elution volume (V_e) to size

$$R_H = -2.4815V_e^3 + 69.943V_e^2 - 666.49V_e + 2207.7 \quad (1)$$

The hydrodynamic radii (R_H) of the three molecular weight dextrans were 27.1, 36.9, and 66.7 Å, respectively.

2.3. Preparation of crosslinked matrix disks

Approximately 650 mg of dextran (11.6% w/w of gelatin) were dissolved in 35 mL of deionized water; 5.6 g of gelatin granules were added to the solution and hydrated for 1 h. The mixture was heated to 55–60 °C to dissolve the gelatin. Three different molar ratios of EDC to ε-NH_2 groups on gelatin of 10:1, 4:1, and 1:1 were prepared by dissolving the crosslinking agent in 9 mL of deionized water, heating to about 45 °C, then adding to the warm gelatin solution. Crosslinking was fast (∼3 s for the solution to set to a gel). The crosslinked and gelled gelatin matrix was cooled to room temperature for 30 min and at 4 °C for an additional 30 min. The gelatin matrix was cut into 13 mm × 4 mm disks. The excess EDC was removed by soaking the disks in 2000 mL deionized water at 4 °C for 2 h. The disks were then dried at 7% relative humidity (RH) to a moisture content of ∼10% determined by LOD prior to conducting the release studies.

2.4. The in vitro release of dextran through crosslinked gelatin matrices

The disks, in five replicates, were weighed and the dimensions measured before conducting the release experiments in 50-mL test tubes containing 30 mL of PBS, pH 7.4, preserved with 0.05% (w/v) sodium azide. Release was carried out at 37 °C in a water bath with rotational shaking at 100 RPM for approximately 80 h. One milliliter aliquots of the release medium were withdrawn at predetermined times for dextran assay. The aliquot volume was replaced with fresh medium.

2.5. Assay of released dextran

The released dextran was assayed using the phenol–sulfuric acid method (Dubois et al., 1956) with slight modification. Briefly, 1 mL of 5% (w/v) phenol solution was added to the 1-mL sample, followed by 5 mL of concentrated sulfuric acid. The mixture was allowed to stand for 1 h. The absorbance of the yellow–orange color that developed was measured at 490 nm using a double beam Perkin Elmer Lambda 6 UV-Vis spectrophotometer. Calibration of dextran in PBS with 0.05% (w/v) sodium azide with concentration ranging from 10 to 120 μg/mL were prepared for each analysis. Blanks were prepared using PBS with 0.05% (w/v) sodium azide.

2.6. Assay of residual matrix dextran after release

After the release experiments, half-disks were accurately weighed, then digested with 10 mL of 6 N HCl acid at 50–60 °C in a water bath until the matrix was dissolved. On cooling, 1 mL aliquots were diluted with 9 mL of PBS preserved with 0.05% (w/v) sodium azide, then assayed for dextran using the above method. The concentration of calibration solutions with dextran ranged from 10 to 120 μg/mL in PBS with 0.05% (w/v) sodium azide and 10% (v/v) of 6 N HCl acid. Calibrations were prepared for each analysis. Blanks were prepared using PBS with 0.05% (w/v) sodium azide and 10% (v/v) of 6 N HCl. Dextran loading was determined from the amount of dextran released and the amount remaining (unreleased) in the matrices at the end of the release experiment.

2.7. Characterization of crosslinked gelatin matrices

2.7.1. Determination of free ε-amino groups, crosslinking extent, and crosslinking percent

Crosslinking extent and percent were calculated after the determination of free, uncrosslinked, ε-amino

groups in crosslinked gelatin matrices using the TNBS assay procedure of Bubnis and Ofner (1992). The assay was conducted after release experiments were completed on matrices loaded with the smallest dextran (12 kDa) at all three levels of crosslinking. After the release studies, the disks were washed in 2000 mL of deionized water at 4 °C for another 72 h to remove any residual dextran, PBS, and sodium azide. Water was changed at 12, 24 and 48 h. The disks were dried for 6 days at 7% relative humidity (RH) before analysis for free ε-amino groups. The moisture content of dried matrices was determined by LOD at 105 °C prior to the determination of free ε-amino groups. The crosslinking extent (X_c) represents the amino groups lost to crosslinking; it is the difference between the original 33 mol of ε-amino groups/g gelatin and the determined free moles of ε-amino groups/g gelatin (Ofner and Bubnis, 1996). This value corresponds to the number of crosslinks on an ideal gelatin molecule of 100,000 Da and 1000 amino acid residues. Expressing X_c as a percent of the original 33 ε-amino groups on gelatin represents the crosslinking percent.

2.7.2. Equilibrium swelling ratio (Q_m)

Two methods were used to measure swelling. The diameter and thickness of the disks were manually measured as a function of time. Equilibrium, or maximum, swelling volume of accurately weighed half-gels was measured with a glass pycnometer by volume displacement of toluene at 37 °C. The equilibrium swelling ratio was calculated using Eq. (2) (Ofner and Bubnis, 1996)

$$Q_m = \frac{V_s}{V_p} = \frac{V_s \rho_p}{W_p} = \frac{1}{\nu_{2m}} \tag{2}$$

where V_s is the volume of the swollen matrix disk; V_p, volume of the unswollen matrix disk; ρ_p, anhydrous density of the matrix polymer (gelatin); W_p, weight of the anhydrous polymer matrix disk. The polymer volume fraction (ν_{2m}) of the swollen matrix disk at equilibrium swelling is the inverse of equilibrium swelling ratio.

2.7.3. Other crosslinking parameters

Molecular weight between crosslinks (Ofner and Bubnis, 1996)

$$M_c = \frac{M}{X_c} = \frac{1}{X_c} \tag{3}$$

M_c is the average molecular weight between crosslinks. M is average molecular weight of gelatin (100,000 g/mol) X_c has the units of moles crosslinked ε-amino groups/g gelatin (M cancels out when units of X_c are converted to moles of gelatin).

Crosslinking density (Ofner and Bubnis, 1996)

$$P_x = X_c \rho_p \tag{4}$$

P_x is the crosslinking density of the dry polymer (moles of crosslinks per unit volume); ρ_p, dry polymer (gelatin) density (1.369 g/mL).

2.7.4. Average matrix mesh size (ξ)

An equation to calculate the mesh size (ξ) in PVA hydrogels was modified for gelatin hydrogels (Gander et al., 1989)

$$\text{Mesh size } (\zeta) = (r^2)^{1/2} Q_m^{1/3} \tag{5}$$

where ξ is the average mesh size (approximate diameter) in Å and $(r^2)^{1/2}$ is the indirectly measured experimental root-mean-square end-to-end chain distance of a linear polymer in solution (used here between crosslinks). The $(r^2)^{1/2}$ is usually expressed in terms of the unperturbed root mean square end-to-end distance, $(r^2)_o^{1/2}$ and an expansion factor, α (Brandrup and Immergut, 1975)

$$(r^2)^{1/2} = (r^2)_o^{1/2} \alpha \tag{6}$$

The unperturbed value, $(r^2)_o^{1/2}$, has been shown in gelatin to be approximately two-times the freely rotating root mean square end-to-end distance, $(r^2)_{of}^{1/2}$ (Veis, 1964). The freely rotating value can be calculated from tabulated values of a polypeptide as (Brandrup and Immergut, 1975)

$$(r^2)_{of}^{1/2} = \left(\frac{M}{M_r}\right)^{1/2} (2.21\ \text{Å}) \tag{7}$$

where M is the polymer molecular weight and M_r is the average molecular weight of the repeating unit amino acid in the chain. The M_c parameter replaces M because mesh size is determined from the matrix polymer segment between crosslinks. Combining Eqs. (5)–(7), and using the relationship between the unperturbed and freely rotating parameters yields

$$\xi = 2\alpha \left(\frac{M_c}{M_r}\right)^{1/2} (2.21\ \text{Å})\, Q_m^{1/3} \tag{8}$$

where M_r is ~100 g/mol for gelatin, and α is estimated at 2.0 based on reports of gelatin conformation in solution similar to our conditions (Veis, 1964).

3. Results and discussion

3.1. Characterization of crosslinked gelatin matrices

The matrices were evaluated in terms of the crosslinking extent (X_c) and percent, molecular weight between crosslinks (M_c), crosslinking density (P_x), equilibrium swelling ratio (Q_m), equilibrium swelling matrix polymer volume fraction (ν_{2m}) and matrix mesh size (ξ).

Crosslinking percents of 24, 41, and 78% were achieved based on the number of ε-amino groups participating in the crosslinking reaction out of the original 33 ε-amino groups/molecule of gelatin that are potentially available for crosslinking. The crosslinking in matrices containing the higher molecular weight dextrans was assumed to be equal to those containing dextran 12 kDa because matrix swelling was approximately the same at each extent of crosslinking with the different solutes (Table 1). As expected, increasing the molar ratio of EDC to gelatin ε-amino groups increased the ε-amino groups participating in the crosslinking and hence increased the extent and percent of crosslinking.

The average molecular weight between crosslinks, the equilibrium swelling ratio, and matrix mesh size all vary inversely with crosslinking. At low crosslinking, values of M_c can be 10,000 Da or more. The M_c value for the most extensively crosslinked matrices in this study was about 4000 Da. The crosslinking density and swollen polymer volume fraction in the swollen matrix vary proportionately with the extent of crosslinking. The crosslinking density of gelatin represents moles of crosslinks/mL of unswollen gelatin matrix polymer. The values in Table 1 of 1.1, 1.9, and 3.5×10^{-4} mol/mL resulted from crosslinker to gelatin ε-amino group molar ratios of 1:1, 4:1, and 10:1, respectively.

Swelling profiles for dextran 12 kDa loaded crosslinked gelatin matrix disks at the three percents of crosslinking are shown in Fig. 1. Swelling profiles for dextran 20 and 77 kDa loaded disks were very similar (data not shown). Equilibrium swelling was

Table 1
Structural characteristics of crosslinked gelatin matrices loaded with dextran

Dextran molecular weight (kDa)	Crosslinking extent (X_c) (mol/g) ($\times 10^5$)	Crosslinking percent[a] (%)	Molecular weight between crosslinks[b] (M_c) (g/mol) ($\times 10^{-3}$)	Crosslinking density[c] (mol/mL) P_x ($\times 10^4$)	Volume of swollen disk (V_s) (mL)	Equilibrium swelling ratio[d] (Q_m) (V_s/V_p)	Swollen matrix polymer volume fraction[d] (ν_{2m})
12	8.0 ± 1.1	24 ± 3	12.5 ± 1.1	1.1	1.66 ± 0.11	35.6 ± 1.8	0.03 ± 0.002
	13.6 ± 0.3	41 ± 1	7.4 ± 0.1	1.9	0.45 ± 0.03	8.7 ± 0.5	0.11 ± 0.02
	25.8 ± 1.7	78 ± 2	3.9 ± 0.3	3.5	0.25 ± 0.03	4.1 ± 0.5	0.23 ± 0.03
20	8.0 ± 1.1	24 ± 3	12.5 ± 1.1	1.1	1.78 ± 0.05	36.1 ± 1.6	0.03 ± 0.002
	13.6 ± 0.3	41 ± 1	7.4 ± 0.1	1.9	0.41 ± 0.05	7.6 ± 0.6	0.13 ± 0.01
	25.8 ± 1.7	78 ± 2	3.9 ± 0.3	3.5	0.26 ± 0.01	4.8 ± 0.5	0.21 ± 0.03
77	8.0 ± 1.1	24 ± 3	12.5 ± 1.1	1.1	1.78 ± 0.11	40.2 ± 1.3	0.02 ± 0.001
	13.6 ± 0.3	41 ± 1	7.4 ± 0.1	1.9	0.39 ± 0.02	7.0 ± 0.3	0.14 ± 0.01
	25.8 ± 1.7	78 ± 2	3.9 ± 0.3	3.5	0.22 ± 0.03	4.3 ± 0.6	0.23 ± 0.03

[a] Based on number of ε-amino groups lost to crosslinking out of original 33 present on an ideal 100,000 Da gelatin molecule composed of 1000 amino acids residues.
[b] Eq. (3).
[c] Eq. (4).
[d] Eq. (2).

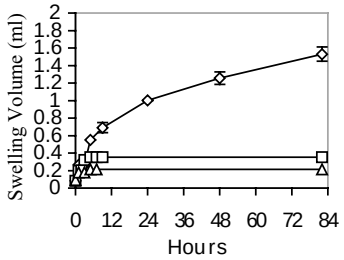

Fig. 1. Swelling profiles for dextran 12 kDa-loaded crosslinked gelatin matrix disks at three percents of matrix crosslinking at 37 °C in PBS at pH 7.4. (◇) 24%, (□) 41%, and (△) 78%. Error bar represents S.D. ($n = 5$).

reached by 5 h for the medium and highest crosslinked matrices, while the lowest crosslinked matrices continued swelling throughout the release experiments, ∼80 h. All matrices were highly swollen with water contents of 77–98% based on the polymer volume fraction values.

Interestingly, the values of M_c and P_x were very similar to those reported for PVA hydrogels crosslinked with glutaraldehyde (Gander et al., 1989). PVA crosslinked matrices with M_c values of 11,600, 7100, and 3900 Da had crosslinking densities of 1.14, 1.9, and 3.88×10^{-4} mol/mL, respectively. However, Q_m values of the PVA matrices were higher (14.84 and 11.00) than those in the present study (8.7–4.1) for the medium and highest extents of matrix crosslinking. On the other hand, the matrix mesh size of 90 Å, for the highest extent of crosslinking in the current study was larger than that reported for other PVA matrices with a similar M_c of 3750 g/mol (Rheinhart and Peppas, 1984). The PVA crosslinked matrices had an estimated matrix mesh size of 63 Å based on the hydrodynamic radius of entrapped BSA.

3.2. Release of dextran from crosslinked gelatin matrices

3.2.1. Effect of macromolecular size on release

Release characteristics of the three sizes of dextran solutes from crosslinked gelatin matrices are shown in Table 2 and Fig. 2a–c. Dextran release from these gelatin hydrogels displayed a rapid initial release rate followed by a gradual increase with time. Fig. 2a shows that virtually all three sizes of dextran solutes were released through matrices with the lowest crosslinking. The dextran hydrodynamic diameters of 54, 74, and 133 Å were well below the mesh sizes of 325–338 Å. Fig. 2b shows that only the 12 kDa dextran was completely released from the intermediate crosslinked matrices, yet all three solutes were smaller than the mesh size of 144–157 Å. There was a prolonged and intermediate release of the medium size dextran reaching 40%, and only a small amount of the large dextran released. Based on this small release, the 77 kDa dextran with a diameter of 133 Å appears

Table 2
Dextran release characteristics from crosslinked gelatin matrices

Dextran molecular weight (kDa)	Crosslinking percent[a] (%)	Matrix disk weight (g ± S.D.)	Dextran loading (% w/w)	Fraction released	Dextran diameter[b] (Å)	Matrix mesh diameter[c] (Å)
12	24 ± 3	0.071 ± 0.003	7.3	0.94	54	325 ± 18
	41 ± 1	0.083 ± 0.005	6.5	0.92	54	157 ± 3
	78 ± 2	0.071 ± 0.006	4.7	0.96	54	90 ± 2
20	24 ± 3	0.084 ± 0.007	8.4	0.95	74	326 ± 18
	41 ± 1	0.084 ± 0.010	8.3	0.40	74	148 ± 2
	78 ± 2	0.074 ± 0.004	5.7	0.66	74	93 ± 1
77	24 ± 3	0.064 ± 0.003	8.8	0.95	133	338 ± 22
	41 ± 1	0.084 ± 0.002	6.5	0.12	133	144 ± 1
	78 ± 2	0.079 ± 0.003	6.6	0.10	133	90 ± 2

[a] Based on number of ε-amino groups lost to crosslinking out of original 33 present on an ideal 100,000 Da gelatin molecule composed of 1000 amino acids residues.
[b] Determined by HPSEC.
[c] Eq. (5).

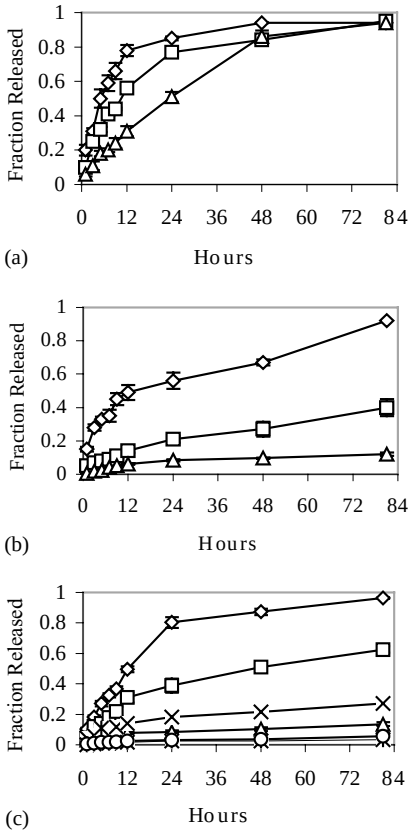

Fig. 2. Effects of macromolecular solute size on release through gelatin matrix disks crosslinked at different crosslinking extents. Release at 37 °C in PBS at pH 7.4. (◇) Dextran 12 kDa, (□) dextran 20 kDa, and (△) dextran 77 kDa. (a) 24%, (b) 41%, and (c) 78% crosslinking. 78% crosslinking includes: (×) dextran 40 kDa, (○) dextran 167 kDa, and (★) dextran 260 kDa. Error bar represents S.D. ($n = 5$).

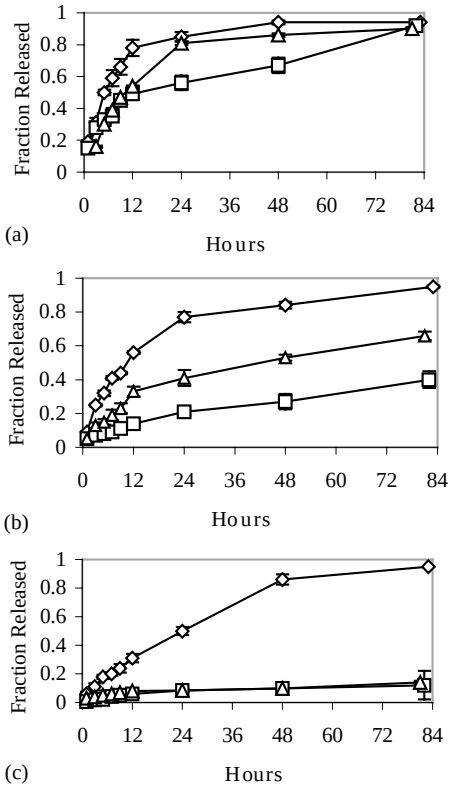

Fig. 3. Effects of crosslinking on the release of three dextran solutes through crosslinked gelatin matrices at 37 °C in PBS at pH 7.4. (◇) 24%, (□) 41%, and (△) 78% of crosslinking. Loaded dextran solutes: (a) dextran 12 kDa, (b) dextran 20 kDa, and (c) dextran 77 kDa. Error bar represents S.D. ($n = 5$).

to be large enough for entrapping entanglement in the 144 Å matrix mesh size. This entrapment is supported by the observation of these matrices remaining opaque throughout the release experiment. The ∼10% fraction released is attributed to the early release of low molecular weight species of dextran present in the sample. Fig. 2c shows the fraction release of dextran with molecular weights of 12, 20, 40, 77, 167, and 260 kDa from the highest crosslinked (78%) matrices. This investigation concentrated on the 12, 20, and 77 kDa solutes because of the <5% release of the dextran 167 and 260 kDa solutes. These large dextran solutes were entrapped, as evidenced by the negligible release and the opaque matrices throughout the release experiments. By 80 h, the fractions released for dextran 12, 20, and 77 kDa were about 96, 66, and 10%, respectively. The 77 kDa dextran solute with hydrodynamic diameter of 133 Å was entrapped in the smaller matrix mesh of 90 Å. These matrices also remained opaque throughout the release experiment.

3.2.2. Effect of crosslinking on macromolecular release

The release of each dextran solute through the matrices of different crosslinking are shown in Fig. 3a–c. Fig. 3a for the 12 kDa solute shows unexpectedly that by 24 h, the 41% crosslinking profile dropped below that of both the 24 and 78% crosslinked matrices. A repeat of the 78% crosslinking produced identical results

(data not shown). Fig. 3b for the 20 kDa solute, shows that the release profile for the medium crosslinking was again lower than that of the highest crosslinking. All medium crosslinked matrices were visibly more rigid and firmer than the more fragile 24 and 78% crosslinked matrices. Fig. 3c shows clearly that the largest dextran solute of 77 kDa with a hydrodynamic diameter of 133 Å was only released from matrices with the lowest crosslinking and mesh size of 338 Å. This solute was effectively entrapped in matrices with the medium and high crosslinking.

It is suggested that the lower than expected release and the more rigid matrices of the medium crosslinked matrices are due to formation of physical crosslinks in addition to the chemical, covalent crosslinks. Physical crosslinks are re-structured triple and/or double helical gelatin chain entanglements similar to those found in native collagen due to hydrogen bonding (Okawa et al., 1997). The phenomenon of mixed physical and chemical crosslinks has been reported (Ross-Murphy, 1997; Lou and Chirila, 1999), and could produce a smaller mesh size to cause the low fraction of dextran released in these matrices. Flory (1953) and Gander et al. (1989) have noted that the matrix network characteristics, as determined by swelling, depend not only on the number of crosslinks (chemical crosslinks) but also on entanglement (physical crosslinks) of the macromolecular chain. However, the suggested physical crosslinks do not appear to influence matrix swelling in this study. The chemical crosslinks could have had a dominant effect and set the limits of matrix swelling. The continuously swelling lowest crosslinked matrices showed no evidence of these physical crosslinks. And at the highest crosslinking, both the high number of covalent crosslinks and excess crosslinking agent byproduct could have hindered formation of the physical crosslinks (Otani et al., 1998).

4. Conclusions

It was reasonably assumed that the macromolecular solute released from the matrices was essentially diffusion driven based on two observations: (1) it took a relatively short time (10 min) for the aqueous release medium to penetrate into the dry matrix disks to induce the glassy-rubbery state transition, and (2) the crosslinked matrices, at all three extents of crosslinking, remained intact without eroding. The macromolecular solute diffusion characteristics were affected by changing the extent of matrix crosslinking. The matrix network was well characterized by parameters calculated from swelling measurements and a crosslinking assay. However, the possibility of physical crosslinks in addition to the chemical crosslinks in the intermediate crosslinked matrices must be considered. The present investigation supports the threshold concept for release of a flexible random coil macromolecular solute such as dextran. Higher crosslinking extents produced matrix mesh sizes below the threshold solute size that resulted in entrapment of the large solute inside the matrices. However, at the lowest extent of crosslinking, the mesh size was greater than the solute size resulting in complete release of the solutes.

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