Swelling Pressure Observations on Degrading Dex-HEMA Hydrogels

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ABSTRACT: The variation of the swelling pressure of dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels is determined as a function of the degradation time. In the first stage of the degradation process a moderate increase in the swelling pressure is observed due to the decrease of the elastic pressure. In this period the cross-link density of the polymer network gradually decreases, but only a small amount of free polymer (dextran) is released. Toward the end of the degradation process, however, a sudden increase in the swelling pressure occurs which is accompanied by the release of a major amount of dextran chains. It is demonstrated that the chemical composition of the network (dex-HEMA content and the number of HEMA groups on the dextran chains) strongly affects the degradation rate of dex-HEMA hydrogels. These observations are important to design degrading hydrogel systems with tailored swelling pressure profile for pulsed drug delivery.

Introduction

Hydrogels are well-suited for biomedical applications because of their tissue compatibility. *Nondegradable* hydrogels have been extensively studied as diffusion-controlled and swelling-controlled drug delivery devices.[1,2] Recently, there is a growing interest to evaluate *biodegradable* hydrogels for drug delivery applications.[3−6] Sustained drug release from a degrading hydrogel is obtained when the initial mesh size of the network is smaller than the size of the drug molecules, since the latter cannot leave the gel before the network has been degraded.[1]

Currently, there is a major interest in pulsed drug delivery in which the pharmaceutical device releases the drug at a preprogrammed time.[7−9] Pulsed drug release can be achieved by creating a *rigid*, semipermeable membrane around the degradable gel particle. The role of the membrane is twofold: (i) allows the transport of small molecules (e.g., water molecules, ions) between the gel and the surrounding solution and (ii) prevents large molecules (e.g., proteins, polymeric degradation products) from leaving the gel. During degradation the gel gradually liquefies, and the swelling pressure Π_{sw} increases. When Π_{sw} exceeds the tensile strength of the membrane, it ruptures,[10] followed by a sudden release of the drug. The combination of different types of degradable coated gel particles could be of practical use in for example "single shot vaccination" in which the initial and subsequent booster release of antigens could be obtained in one single injection. This avoids problems inherent with repeat immunizations and leads to an increased patient compliance.

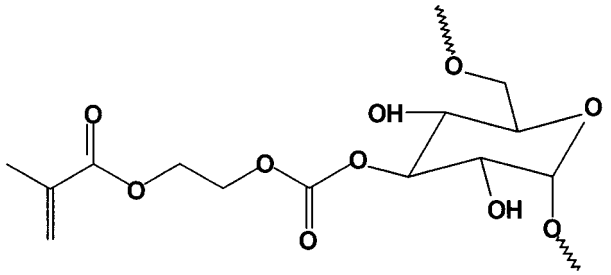

Figure 1. Chemical structure of the monomer in dex-HEMA, i.e., glucopyranose substituted with HEMA.

Development of drug delivery devices with predictable release profiles requires the understanding of the thermodynamic and kinetic properties of the *degrading* hydrogel matrix. In an earlier paper we investigated the kinetics of degradation of dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels.[11,12] In this system degradation is caused by the hydrolysis of the carbonate ester link formed between the methacrylate group and the dextran molecule (Figure 1). The aim of the present study is to investigate the effect of degradation on the swelling pressure of dex-HEMA hydrogels. Although the swelling behavior of polymer networks has been the subject of numerous investigations,[13−19] according to our knowledge the variation of the swelling pressure in degrading gel systems has not been studied previously. Osmotic swelling pressure measurements are performed at different stages of degradation. The variation of the elastic modulus and the amount of free dextran produced in the degradation process are also measured. The results of the osmotic measurements are analyzed in the framework of the scaling theory.

Experimental Section

Dex-HEMA Preparation and Characterization. Dex-HEMA batches were prepared and characterized according to

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a method described elsewhere.[20] Dextran (Fluka, from *Leuconostoc ssp.*) with a molecular weight $M_n = 19\,000$ g/mol was used. The degree of substitution (DS, the number of HEMA groups per 100 glucopyranose residues of dextran) was determined by proton nuclear magnetic resonance spectroscopy (H NMR) in D_2O with a Gemini 300 spectrometer (Varian). The DS of the samples used in the present study were 2.9, 5.0, and 7.5.

Preparation and Degradation of Dex-HEMA Hydrogels. Dex-HEMA gels were made by radical polymerization of aqueous dex-HEMA solutions. The solutions were prepared by dissolving dex-HEMA in a phosphate buffer (PB: 10 mM Na_2HPO_4, 0.02% sodium azide, adjusted with 1 N hydrochloric acid to pH 7.0). The polymerization reagents were *N*,*N*,*N*′,*N*′-tetramethyleneethylenediamine (TEMED; 20% v/v in deoxygenated PB, pH 8.5) and potassium persulfate (KPS; 50 mg/mL in deoxygenated PB). 50 μL of TEMED solution was added to 1 g of polymer solution. After homogenization, 90 μL of KPS solution was subsequently added to the system to initiate gelation. All containers were coated with poly(ethylene glycol) (PEG, 20 000 g/mol; 10% in PB) to reduce adhesion. Gelation required approximately 1 h at room temperature. The hydrogel slabs used in the rheological measurements were made in cylindrical molds (diameter 23 mm, height 2 mm). For the other experiments gels were prepared in 2.5 mL polypropylene syringes (diameter 8.5 mm), from which the heads were sawn. After gelation the gel samples (~0.3 g of gel) were removed from the syringe and cut with a thin wire. Degradation was studied in phosphate buffer (pH = 7) at 37 °C. Throughout this work the dex-HEMA concentration (% w/w) refers to the concentration at which cross-links were introduced.

Osmotic Deswelling. Osmotic deswelling measurements were performed on dex-HEMA gels using a method described by Horkay and Zrinyi.[16] Gel specimens were surrounded by a semipermeable membrane (Medicell dialysis bags, M_w cutoff between 12 000 and 14 000 g/mol). Similar dialysis bags were used in the purification step of the synthesis of dex-HEMA.[20]

After different degradation times gel samples were equilibrated with PEG-solutions at 4 °C. PEG (Merck, $M_w = 20\,000$ g/mol) was dissolved in citrate buffer (9.44 g/L Na_2HPO_4; 10.3 g/L citric acid and 0.2 g/L NaN_3, pH 4.4). The PEG concentration was varied in the range 0−12.5 g/100 mL. It was verified (from rheological measurements) that further degradation of the dex-HEMA gels did not occur during the osmotic deswelling measurements.[12] Equilibrium swelling was attained within 7 days. The reversibility of the swelling process was checked.

At equilibrium the swelling pressure of the gel is equal to the osmotic pressure of the PEG solution. The osmotic pressure of the PEG solution was calculated from the equation[21]

$$\Pi_{PEG} = \left[\frac{1}{M_n} + A_2 c + A_3 c^2\right] cRT \times 10 \qquad (1)$$

where R is the gas constant, T is the absolute temperature, c is the PEG concentration (in g/100 mL), and A_2 and A_3 are the second and third virial coefficient, respectively. According to the data reported by Edmond and Ogston,[22] for PEG (M_n = 20 000 g/mol) $A_2 = 2.59 \times 10^{-5}$ (mol·10^2 mL)/g^2 and $A_3 = 1.35 \times 10^{-6}$ (mol·10^4 mL^2)/g^3.

The dex-HEMA concentration of the gels was calculated using the relationship

$$c = \frac{w_{dex-HEMA}}{w_{dex-HEMA} v_1 + \dfrac{w_e - w_{dex-HEMA}}{\rho}} \times 100 \qquad (2)$$

where w_e is the weight of the dex-HEMA gel, $w_{dex-HEMA}$ is the weight of dex-HEMA determined gravimetrically after drying the gel in a vacuum oven at 50 °C, ρ is the density of the buffer, and v_1 is the specific volume of the dex-HEMA ($v_1 = 0.72$ mL/g).[23] The polymer volume fraction (φ) of the gels was calculated from the concentration of dex-HEMA and v_1.

Mechanical Characterization of Degrading Hydrogels. Rheological measurements were performed using an AR1000-N controlled stress rheometer from TA-Instruments. To avoid slippage, the acrylic top plate was covered by sandpaper (diameter 2 cm). The bottom plate was replaced with a Plexiglas plate with a roughened surface. Measurements were done in oscillation mode at 1 Hz in the linear viscoelastic region of these gels by applying a constant strain of 0.5%. After the measurements the hydrogel slabs were transferred into PB and stored at 37 °C. Further details of this method were reported by Meyvis et al.[24]

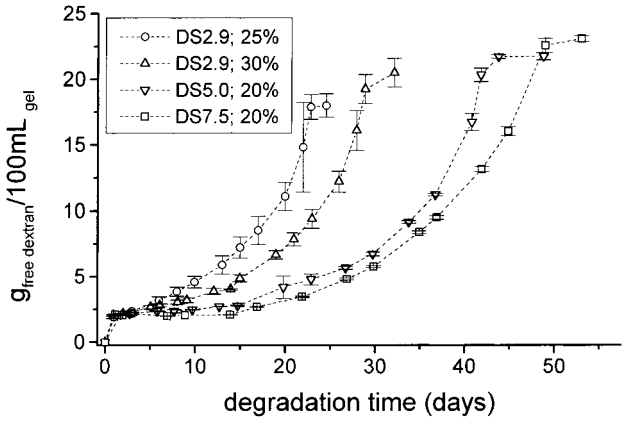

Figure 2. Variation of the amount of free dextran as a function of time in degrading dex-HEMA hydrogels. The data are the average of three independent measurements. The continuous lines were drawn arbitrarily through the data points.

Determination of Free Dextran Chains in dex-HEMA Gels. The concentration of the free dextran in the dex-HEMA hydrogels was determined from a release experiment performed in phosphate buffer at 37 °C. The amount of dextran chains in the solution was measured by gel permeation chromatography (GPC). The system consisted of a high-pressure pump (Waters M510), an injector (Waters U6K), and a differential refractometer (Waters 410). 250 μL of sample was injected, and a flow rate of 0.5 mL/min was applied. The dex-HEMA concentration was calculated from the height of the peak using a calibration curve (concentration between 0 and 2.5 mg/mL) obtained for the corresponding dex-HEMA (DS2.9, DS5.0, and DS7.5). In the concentration range explored here the reproducibility of the GPC measurements is excellent. (The correlation coefficient for linear regression exceeds 0.996 for each standard line.)

Results and Discussion

Figure 2 shows the variation of the concentration of free dextran in different dex-HEMA hydrogels as a function of the degradation time, t. The dextran concentration, calculated from the amount of dextran released from dex-HEMA gels, refers to the initial volume of the (undegraded) gel. In principle, this concentration corresponds to the concentration of free dextran molecules in degrading gel particles surrounded by a rigid semipermeable membrane. It appears in Figure 2 that first the sol fraction (unreacted dex-HEMA chains) leaves the gel. This feature is independent of the degradation process. In the second region (delay region) a relatively small amount of dextran is released. Finally, when the majority of the cross-links are cleaved, the liberation of dextran chains is significantly enhanced. The delay in the release of dextran is expected since a chain can only become free when all cross-links connecting it to the network are cleaved.

Figure 3 shows the elastic modulus G' as a function of the degradation time for the dex-HEMA gels studied in Figure 2. This quantity exhibits a continuous decrease during the degradation process. The decrease of

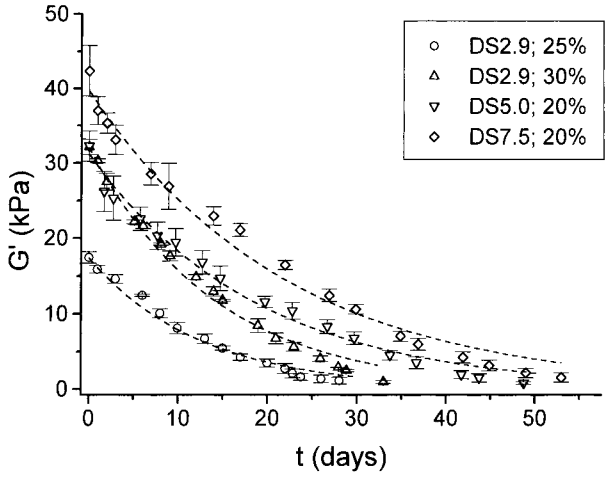

Figure 3. Variation of the elastic modulus as a functuion of time in degrading dex-HEMA hydrogels. The data are the average of three independent measurements. The dashed curves show the least-squares fits to eq 3.

Table 1. Fitting Parameters to Elastic Modulus (G') Data (Eq 3)

gel sample	$G'(0)$/kPa	k_{app}/day^{-1}
DS 2.9; 25%	17.5 ± 0.7	0.079 ± 0.003
DS 2.9; 30%	32.2 ± 1.0	0.071 ± 0.002
DS 5.0; 20%	32.3 ± 2.0	0.056 ± 0.002
DS 7.5; 20%	42.4 ± 3.4	0.046 ± 0.002

G' is significantly slower in gels having higher dex-HEMA concentration or higher DS. It was found previously that both the initial dex-HEMA concentration and DS affect the cross-link density of these networks.[24] An apparent rate constant can be determined from the decrease of G' by using the following relationship

$$G'(t) = G'(0)\exp(-k_{app}t) \quad (3)$$

where $G(0)'$ is the modulus of the undegraded gel and k_{app} is a constant. The values displayed in Table 1 indicate that the variation of the modulus in these highly swollen gels can be reasonably described by a pseudo-first-order hydrolysis kinetics, and k_{app} decreases with increasing cross-link density. The latter result implies that degradation is slower in densely cross-linked gels.

To reveal the effect of degradation on the thermodynamic properties, we measured the swelling pressure Π_{sw} at different stages of degradation. The swelling pressure Π_{sw} of a nonionic gel can be described as the sum of two terms:[15] an osmotic pressure Π_{osm} that expands the network and an elastic pressure Π_{el} that acts against expansion

$$\Pi_{sw} = \Pi_{osm} + \Pi_{el} \quad (4)$$

Figure 4 shows the variation of the swelling pressure as a function of the polymer volume fraction for different *undegraded* dex-HEMA hydrogels. The continuous curves in Figure 4 are the least-squares fits of the swelling pressure data to the equation:[16]

$$\Pi_{sw} = A\varphi^n - A\varphi_e^{n-1/3}\varphi^{1/3} \quad (5)$$

where A is a constant that depends on the particular polymer−solvent system and φ_e and φ are the volume fraction of the polymer in equilibrium with pure buffer and PEG solutions, respectively. For the exponent n scaling theory[25] predicts $n = 2.31$ (good solvent condition) and $n = 3.0$ (Θ-solvent condition). The values of A and n obtained from the fits to eq 5 are listed in Table 2. As expected, A depends on the chemical composition of the network. The value of n is close to that predicted for good solvent condition.

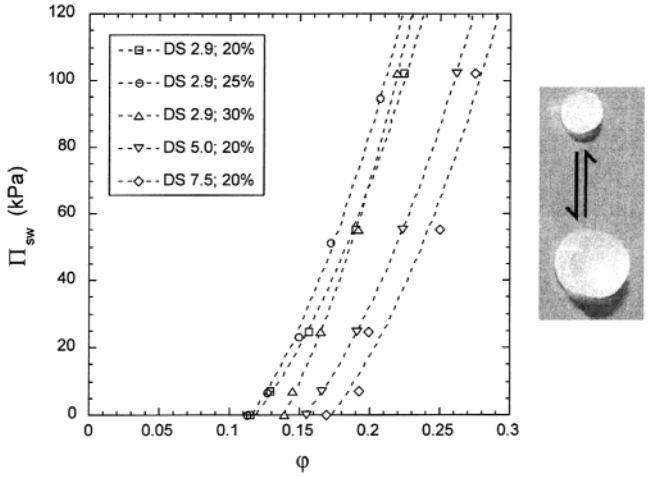

Figure 4. Plot of the swelling pressure vs polymer volume fraction for dex-HEMA gels before degradation. The legend shows the DS and the dex-HEMA concentration of the gels. Each measuring point is the mean value of measurements on three hydrogels, which were independently prepared. The dashed curves show the least-squares fits to eq 5. The image at the top shows a dex-HEMA gel shrunken in a PEG-solution while the image on the bottom shows the corresponding fully swollen dex-HEMA gel.

Table 2. Parameters Obtained from the Fit of the Swelling Pressure Data to Eq 5

sample	degradation time (days)	volume fraction[a]	A (kPa)	n	R^b
DS 7.5; 20%	0	0.1150	4554	2.35	0.999
DS 5.0; 20%	0	0.1391	5767	2.33	0.999
DS 2.9; 20%	0	0.1547	3587	2.33	0.999
DS 2.9; 30%	0	0.1688	3231	2.36	0.981
DS 2.9; 25%	0	0.1128	5562	2.36	0.998
DS 2.9; 25%	3	0.0919	5563	2.34	0.997
DS 2.9; 25%	6	0.0745	5554	2.35	0.997
DS 2.9; 25%	10	0.0598	5487	2.34	0.997
DS 2.9; 25%	15	0.0493	5337	2.30	0.997
DS 2.9; 25%	30	< 0.01	7920	2.33	0.993

[a] Volume fraction at equilibrium swelling. [b] Correlation coefficient.

The effect of degradation on the swelling pressure was studied on the sample having the shortest degradation time (dex-HEMA DS2.9; 25%). In Figure 5 is plotted the swelling pressure as a function of the polymer volume fraction measured at different stages of degradation (after 3, 6, 10, 15, and 30 days). The Π_{sw} vs φ curves are gradually shifted to the left as the gel degrades.

The parameters obtained from the fits to eq 4 at different degradation times (Table 2, Figure 6) indicate that neither A nor n varies notably in the first 15 days of degradation. It can also be seen that after 30 days (i.e., when the network is completely liquified) the value of A significantly increases.

We note that similar deviation between the values of A obtained for cross-linked and un-cross-linked polymers has been reported for other polymer/solvent systems.[26,27] It seems to be a general phenomena probably caused by the effect of cross-linking on the entropy of mixing that is not considered in the existing theories.

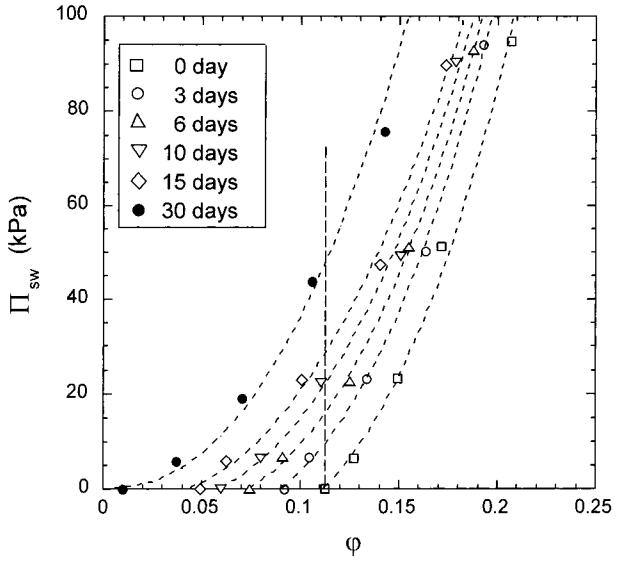

Figure 5. Swelling pressure of DS 2.9 dex-HEMA hydrogels deswollen in PEG solutions. The dex-HEMA concentration at cross-linking was 25% (w/w). Before deswelling the hydrogels were degraded during 0, 3, 6, 10, 15, and 30 days. Each data point is the mean value of measurements on three hydrogels. The dashed curves through the data points are the least-squares fits to eq 5. The vertical line shows the increase in Π_{sw} at $\varphi = 0.112$.

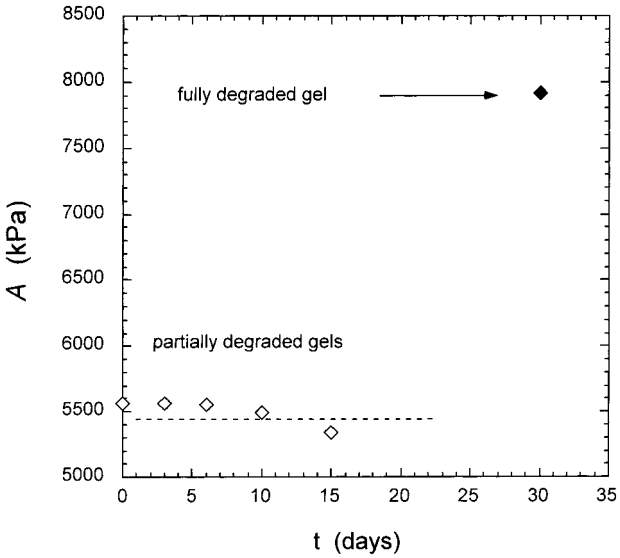

Figure 6. Variation of *A* as a function of degradation time *t*: open symbols, partially degraded gels; filled symbol, fully degraded gel.

The dashed line in Figure 5 shows the situation that occurs when the gel is surrounded by a rigid semipermeable membrane. During degradation Π_{sw} increases from 0 kPa (swelling pressure of the fully swollen undegraded gel) to 49 kPa (swelling pressure of the totally degraded dex-HEMA gel). The latter is the hydrostatic pressure required to maintain the initial concentration ($\varphi = 0.112$) of the gel during the degradation process.

We note that in general the entrapped drug is expected contribute to the osmotic response. This effect depends on the drug load and the properties of the particular system (e.g., molecular weight of the drug and its interaction with the polymer matrix).

Conclusions

Osmotic deswelling measurements were performed on degrading dex-HEMA hydrogels to estimate the swelling pressure developed during network degradation. It is found that the degradation rate strongly depends on the initial dex-HEMA concentration and DS. The variation of the swelling pressure at each stage of degradation is satisfactorily described by the equation $\Pi_{sw} = A(\varphi^n - \varphi_{e,t}{}^{n-1/3}\varphi^{1/3})$, where $\varphi_{e,t}$ is the concentration of the fully swollen gel at degradation time t and A and n are constants. In the earlier phase of the degradation process the swelling pressure gradually increases. This is caused by the decrease of the elastic pressure. Toward the end of the degradation process a pronounced increase in the swelling pressure is observed. This pressure change is accompanied by a sudden increase in the amount of dextran released from the gel.

In drug delivery systems the osmotic pressure can cause the rupture of the coat surrounding the hydrogel particle. Consequently, the knowledge of the variation of the swelling pressure during the degradation process is essential to design hydrogel systems that have a Π_{sw} profile tailored for pulsed delivery of drugs.

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