Modeling the Swelling Pressure of Degrading Hydroxyethylmethacrylate-Grafted Dextran Hydrogels

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ABSTRACT: Degrading hydroxyethylmethacrylate-grafted dextran (dex-HEMA) hydrogels generate a relatively sudden increase in osmotic pressure upon degradation into dextran solutions. This phenomenon is currently being examined as a possible means of developing a pulsatile drug-delivery system. Here a mathematical model based on scaling concepts is presented to describe this sudden increase in swelling pressure and to provide a framework for the rational design of pulsatile delivery systems based on this phenomena. The model provides a good fit to the swelling pressures measured for dex-HEMA gel/free dextran mixtures that simulate degrading dex-HEMA gels. © 2004 Wiley Periodicals, Inc. J Polym Sci Part B: Polym Phys 42: 3397–3404, 2004
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INTRODUCTION

The sudden release of a drug from a pharmaceutical device after a certain lag time is of interest for many types of indications. A number of approaches have been investigated to achieve this goal. Such strategies include stimuli-regulated drug-delivery systems and time-controlled drug-delivery systems.[1–3] In a stimulus-regulated system, a physiological stimulus triggers the release of a drug (e.g., release of insulin due to an increased glucose concentration). In a time-controlled system, drug release is only governed by, for example, the degradation of the device. In comparison with stimuli-regulated systems, time-controlled systems could be more universal.

We are currently investigating an alternative time-controlled pulsatile drug-delivery system. It is based on microcapsules that are designed to explode and consequently release their drug load at specific times after administration. To obtain exploding microparticles, we propose a design incorporating a degradable hydrogel, containing the drug of interest, which is surrounded by a semipermeable membrane (Fig. 1). As the hydrogel degrades into a polymer solution, the swelling pressure gradually increases inside the capsule. At a critical swelling pressure, the membrane ruptures, and the drug content is released.

We started to investigate this approach with a degradable dextran-based hydrogel.[4–6] The gel consists of dextran chains that have been crosslinked with hydroxyethylmethacrylate (HEMA) oligomers (Scheme 1).

The HEMA groups are linked to the dextran via carbonate linkages, which are hydrolyzable.

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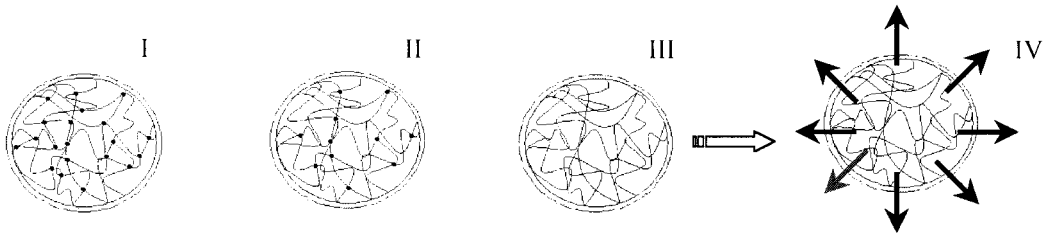
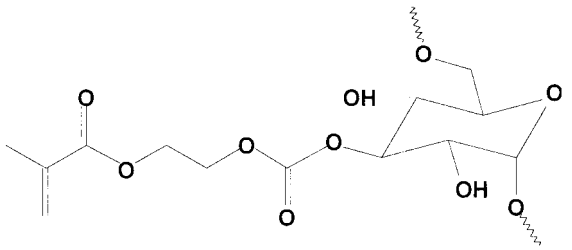


Figure 1. Schematic representation of a gel particle surrounded by a semipermeable membrane. (I) Before degradation, the polymer chains are connected into a three-dimensional network by chemical crosslinks (circles). (II) The gels described in this article degrade as a result of the hydrolysis of the crosslinks. As degradation proceeds, the crosslink density decreases, and free polymer chains are produced. (III) At the end of the degradation process, a polymer solution is produced. (IV) When the swelling pressure exceeds the tensile strength of the membrane, the membrane ruptures, and drug molecules are released.

Upon hydrolysis, the HEMA oligomers are liberated. The gel thus gradually converts into a dextran polymer solution. We have recently reported that the swelling pressure of degrading hydroxyethylmethacrylate-grafted dextran (dex-HEMA) gels only moderately increases during degradation. However, near the end of the degradation process, when the dex-HEMA gels turn into dextran and HEMA solutions, there is a relatively rapid and substantial increase in the swelling pressure (Fig. 2), which is very attractive for realizing the concept illustrated in Figure 1.[4] The sudden increase in the swelling pressure of the degrading dex-HEMA gels results from a sudden increase in the osmotic pressure.[5] Furthermore, we have compared the swelling pressure of degrading dex-HEMA gels and dex-HEMA gels swollen in dextran solutions. The latter system, containing controlled amounts of free (unattached) dextran chains, closely mimics the osmotic behavior of partially degraded dex-HEMA gels.[5]



Scheme 1. Chemical structure of the monomer in dex-HEMA, that is, glucopyranose substituted with HEMA. As dex-HEMA gels are degraded by the hydrolysis of the HEMA crosslinks, free dextran chains are produced.

The aim of this work is to propose a mathematical model that describes the variation of the swelling pressure of dex-HEMA hydrogels during the degradation process. Such a model, which could predict both the time at which the increase in osmotic pressure starts and the maximal swelling pressure that can be obtained, would be very useful in the design of such pressure-driven drug-delivery systems.

MODEL DEVELOPMENT

The dex-HEMA gel system has been fully characterized during degradation, and the model is based on the results, as illustrated in Figure 2.[5] Free dextran chains are not significantly liberated for a prolonged period but, once a specific time has passed, undergo an increase in their rate of liberation. This rate of production of free dextran chains is mirrored by the increase in the swelling pressure of the system. It has been further observed that the shear modulus of the gel undergoes a continuous exponential decline during the whole degradation timeframe.[5]

The model thus assumes that during the initial stages of degradation, the number of elastically effective chains in the network is continually being reduced, and at some critical time, the network is no longer complete. The model further assumes that the polymer volume fraction remains constant during the degradation of the dex-HEMA gels. This assumption is reasonable because in our experiments the dex-HEMA gels were not allowed to swell during degradation. Also, in the microparticles that we would like to

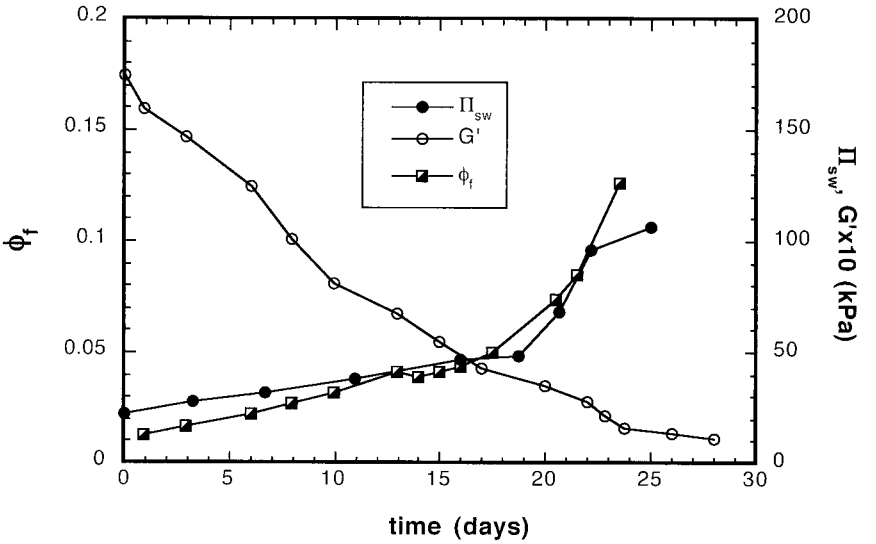


Figure 2. Variation of the shear modulus (G') and swelling pressure (Π_{sw}) of degrading dex-HEMA hydrogels ($\phi = 0.126$) with time. Also shown is the production of free dextran in the degrading dex-HEMA hydrogels as a function of time.

design (Fig. 1), the dex-HEMA volume fraction will remain constant during degradation because a nonflexible semipermeable membrane (that does not permit free dextran chains to leave) will surround the degrading hydrogel core. The system under consideration is thought to consist of free dextran chains and gelled dex-HEMA. Finally, the model assumes that the swelling pressure exhibited by the system after the gel network has been eliminated is governed by the volume fraction of free dextran chains in solution.

The swelling of a neutral polymer network can be described as the additive effect of two opposing pressures: the osmotic pressure generated by the interaction of the polymer chains with the solvent (Π_{os}), which tends to push the chains apart, and the resistance to this pressure produced by the crosslinks forming the network (Π_{el}). An imbalance in these pressures will cause the gel to swell or shrink. With the Flory–Rehner model, the change in the swelling pressure can be written as follows:[7]

$$\Pi = \Pi_{os} + \Pi_{el} \quad (1)$$

The elastic pressure (Π_{el}) resisting term can be described as follows:[8,9]

$$\Pi_{el} = -k_B T\left(\frac{f-2}{f}\right)V_1 N_{e,t}\phi^{1/3} \quad (2)$$

where k_B is the Boltzmann constant, T is the temperature, f describes the average effective functionality of the network junctions, V_1 is the molar volume of the solvent, ϕ is the volume fraction of the polymer in the system, and $N_{e,t}$ is the concentration of effective elastic chains at any given time.

As the crosslink point is a carbonate linkage, it is reasonable to assume first-order degradation kinetics of the bond.[10] Therefore, the change in the elastic resisting pressure as a function of time can be written as follows:

$$\Pi_{el} = -k_B T\left(\frac{f_0-2}{f_0}\right)V_1 N_{e.0}\phi^{1/3}\exp(-k_{app}t) \quad (3)$$

where f_0 is the initial average effective functionality of the network junctions, k_{app} is the overall degradation rate constant (which includes the constant for the kinetics of the reduction in the average functionality and that of the reduction of the overall concentration of effective chains), and $N_{e,0}$ is the initial concentration of effective chains in the network.

The osmotic pressure is now considered. For this term, scaling principles are used. For the situation under consideration, the ultimate dextran solution is at a concentration above its critical overlap concentration.[11] Under these conditions, the individual chains become indistinguish-

able, and the osmotic pressure of the solution no longer depends on the molecular weight. Because this is the same situation for the original gel, the functional form of the osmotic pressure is the same. The osmotic pressure, for semidilute polymer solutions in good solvents, is given by[12]

$$\Pi = A\phi^n \tag{4}$$

where A and n are constants. In the original derivation, n was 9/4,[12] but subsequent experimental work has found that a value of $n = 2.33$ more closely fits the data for the system under study.[4] For clarity, the constant A will be defined as A_g for the gel situation and as A_s for the polymer solution situation.

Combining eqs 3 and 4 for the gel case, and noting that at $t = 0$ there is no change in the swelling pressure of the gel, we can obtain

$$A_g\phi^{2.33} = k_BT\left(\frac{f_0 - 2}{f_0}\right)V_1N_{e.0}\phi^{1/3} \tag{5}$$

Thus, the model expression for the change in the swelling pressure of the gel during degradation (i.e., the increase in the swelling pressure above that of its initial condition) to the gel point can be written as follows:

$$\Pi_{sw} = A_g\phi_g^{2.33}[1 - \exp(-k_{app}t)] \tag{6}$$

where A_g represents the scaling constant for the gel and ϕ_g is the volume fraction of the networked chains, which is obtained by

$$\phi_g = \phi_T - \phi_{sol} \tag{7}$$

where ϕ_T is the total dextran volume fraction present and ϕ_{sol} represents the initial sol fraction. Equation 5 is expected to hold until the number of crosslinks is reduced to the point at which the gel no longer encompasses the whole volume. This is the gel point, and subsequent crosslink degradation produces a mixture consisting of free dextran chains and dextran gel portions (dex-HEMA gel/free dextran mixture).

During this initial stage of degradation, free dextran chains are being produced in the system. The rate of production of free dextran chains is directly proportional to the rate of degradation of the carbonate bonds. As the degradation of the carbonate linkages has been demonstrated to be a first-order process, the volume fraction of free dextran chains in the system (ϕ_f) at any time t below the gel point can be expressed as follows:

$$\phi_f = \phi_{sol}\exp(k_1t) \tag{8}$$

where k_1 is a first-order degradation rate constant.

The osmotic swelling pressure after the time at which the gel point is reached (t_c) is assumed to be governed by the fraction of free dextran chains in the dex-HEMA gel/free dextran mixture. The fraction of free dextran chains increases with time as the remaining dex-HEMA gel portions degrade. Again, the rate of production of free dextran chains will be directly proportional to the rate of degradation of the carbonate bonds and will be a first-order process. Thus, the rate of generation of free dextran chains beyond the gel point can be expressed as follows:

$$\phi_f = \phi_c\exp[k_2(t - t_c)] \tag{9}$$

where ϕ_c is the volume fraction of free dextran chains at the gel point and k_2 is the first-order rate constant for chain liberation above the gel point. This volume fraction can be expressed as follows:

$$\phi_c = (1 - p)\phi_g + \phi_{sol} \tag{10}$$

where p is the fraction of chains remaining connected to the network at the gel point. Once the gel has degraded to the point at which it is below its gel point (i.e., it is no longer a volume encompassing gel), the pressure exerted is assumed to be dominated by the contributions of the free dextran chains and is thus given by the osmotic pressure of the free dextran solution:

$$\Pi_{sw} = A_s\phi_f^{2.33} \tag{11}$$

in which A_s is the scaling constant for the polymer solution condition.

To use this model (i.e., to fit the data to eqs 6 and 11), we need to know A_s, A_g, p, t_c, k_1, and k_2. A_s can be determined from the fitting of eq 4 to experimental data describing the concentration dependence of the osmotic pressure of dextran solutions. A_g can be obtained from osmotic deswelling experiments of the initially prepared gels.[4] k_1 and k_{app} can be assumed to be equal to the degradation rate of carbonate bonds in the gel, and these values have been determined from

previous work on the change in the shear modulus with time.[4] k_2 is considered to be equal to the degradation rate of carbonate bonds in dex-HEMA solutions and is estimated to be 0.18 days^{-1} from Van Dijk-Wolthuis et al.[10]

t_c and p still need to be determined. t_c is calculated as the intersection point between gel-dominated swelling behavior and solution-dominated swelling behavior. Thus, t_c can be given by expressions describing the swelling pressure (eqs 6 and 11) to yield $t_{c,sw}$:

$$t_{c,sw} = \frac{-1}{k_1} \ln\left(1 - \frac{A_s}{A_g}\left(\frac{\phi_c}{\phi_g}\right)^{2.33}\right) \quad (12)$$

t_c can also be calculated by the consideration of the intercept of the equations describing the production of free dextran chains (eqs 8 and 9) to yield t_{c,ϕ_f}:

$$t_{c,\phi_f} = \frac{1}{k_1} \ln\left(\frac{\phi_c}{\phi_{sol}}\right) \quad (13)$$

Now ϕ_c can be obtained by the equating of eqs 12 and 13:

$$\phi_c - \frac{A_s}{A_g}\frac{\phi_c^{3.33}}{\phi_g^{2.33}} = \phi_{sol} \quad (14)$$

Equation 14 must be solved numerically. t_c is then calculated with this value of ϕ_c and either eq 12 or 13. p is calculated with the value of ϕ_c and eq 10.

It is also important to be able to predict the time to the maximum swelling pressure (t_{max}). t_{max} can be calculated with eq 9 solved for the time required to reach ϕ_T:

$$t_{max} = \frac{1}{k_2} \ln\left(\frac{\phi_T}{\phi_c}\right) + t_c \quad (15)$$

Thus, from some preliminary measurements of gel and solution properties, a prediction of the swelling behavior of the degrading dex-HEMA gels is possible. This model was applied to the experimentally obtained swelling pressures of dex-HEMA gels containing increasing amounts of free dextran chains. As explained earlier, dex-HEMA gel/free dextran mixtures simulate degrading dex-HEMA gels.

EXPERIMENTAL

Preparation of Dex-HEMA Hydrogels

Dex-HEMA hydrogels containing free dextran chains were prepared by the radical polymerization of aqueous dex-HEMA/dextran solutions. Two dex-HEMA gels were prepared, one with a dextran volume fraction of 0.126 and the other with a volume fraction of 0.175. The solution was prepared by the dissolution of dex-HEMA (degree of HEMA substitution = 2.9 HEMA groups/100 glucopyranose units; synthesized and characterized as described elsewhere[13]) 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′*-tetramethylene ethylenediamine (TEMED; 20% v/v in deoxygenated PB, pH adjusted to 8.5 with hydrochloric acid) and potassium persulfate (KPS; 50 mg/mL in deoxygenated PB). Adding a 50-μL TEMED solution (per gram of the hydrogel), followed by stirring with a 90-μL KPS solution (per gram of the hydrogel), started the gelation. The dex-HEMA gel/free dextran mixtures were made in a swelling pressure device (described later). On average, gelation required 1.5 h and was performed at 4 °C to inhibit dex-HEMA hydrolysis.

Swelling Pressure Measurements

The swelling pressure of the dex-HEMA gel/free dextran mixtures was measured in an in-house-fabricated membrane osmometer.[4] The device consisted of a transducer (AB High Performance, Honeywell) and a gel chamber and a buffer chamber separated by a semipermeable membrane (Medicell; weight-average molecular weight cutoff = 12,000–14,000 g/mol). The cylindrical space between the semipermeable membrane and the transducer membrane was the gel chamber, which has an internal volume of about 4.2 mL. The dex-HEMA gel/free dextran mixtures were prepared in this compartment. The membrane separating the two chambers was permeable to the solvent and small molecules (e.g., buffer components) but impermeable to macromolecules such as dextran. To prevent the rupture of the membrane, it was supported by a porous Bekipor frame, which was further supported by a Teflon perforated cylinder. To prevent the volume expansion that would occur from water flowing from the buffer chamber into the gel chamber, the gel chamber was sealed. The sealing occurred in two

steps to prevent pressure overload due to the closing of the chamber. The whole device was thermostated, and measurements were performed at 4 °C to prevent the hydrolysis of dex-HEMA. Stable values for the swelling pressure were always reached within 4 h after the measurements were started.

Low-molecular-weight products present in the gel after gelation (e.g., TEMED, KPS, and hydrogen chloride) could contribute to the swelling pressure as measured in the device. To determine their influence on the swelling pressure measurements, we prepared dextran solutions containing concentrations of TEMED, KPS, and hydrogen chloride equivalent to those used in the gelation process. These solutions were immersed in the swelling pressure device, and their osmotic pressure was measured. We found that these low-molecular-weight compounds influenced the osmotic pressure of the dextran solutions registered 4 h after the experiment was started. Therefore, the swelling pressure values obtained for the dex-HEMA gel/free dextran mixtures were corrected for their influence.

RESULTS

The first-order rate constant for the decrease in Π_{el} (k_1) was calculated from curve fits to the reduction in the elastic modulus of the gels during degradation.[4] These values were used here. A_s was obtained from the fitting of eq 4 to data demonstrating the osmotic pressure dependence on the concentration of dextran solutions (Fig. 3).

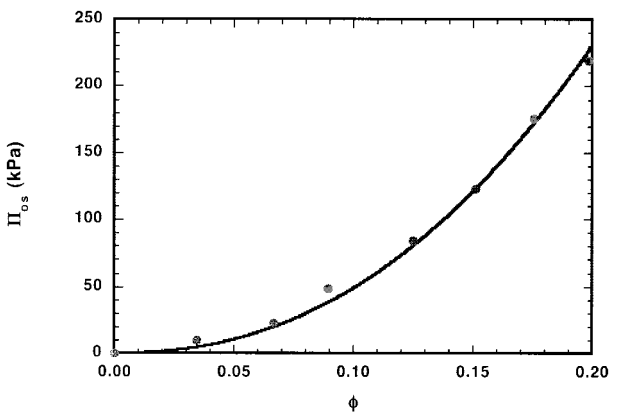


Figure 3. Variation of Π_{os} with ϕ for a dextran solution at 4 °C. The line through the data points shows the least-squares fit to eq 4.

Table 1. Constants Used in the Model Calculations

Dextran in a Pregel Solution (w/w %)	ϕ_T	ϕ_{sol}	k_1 (day^{-1})	A_g (kPa)
25	0.126	0.013	0.08	5562
30	0.175	0.015	0.07	3231

The fitted value thus obtained was 9867 ± 154 kPa, with a correlation coefficient of 0.992. A_g values obtained from osmotic deswelling experiments reported previously were used here. The values for the parameters used in the model are given in Table 1.

With the values listed in Table 1, eq 14 was solved to obtain ϕ_c. This value was used to calculate t_c, which was subsequently used to calculate t_{max} and p. These numbers were then put into eqs 6 and 11 to obtain model predictions. The results can be viewed in Figure 4. The calculated values for p, t_c, and t_{max} are listed in Table 2.

Figure 4 shows that the model provides a reasonable agreement with the data. The model consistently provides a good estimate of the time at which the swelling pressure begins to increase rapidly. Furthermore, the swelling pressure ultimately reaches the osmotic pressure of a dextran solution of the same dextran volume fraction as the initial gel. The p values obtained are close to that predicted by the percolation theory for trifunctional networks (p

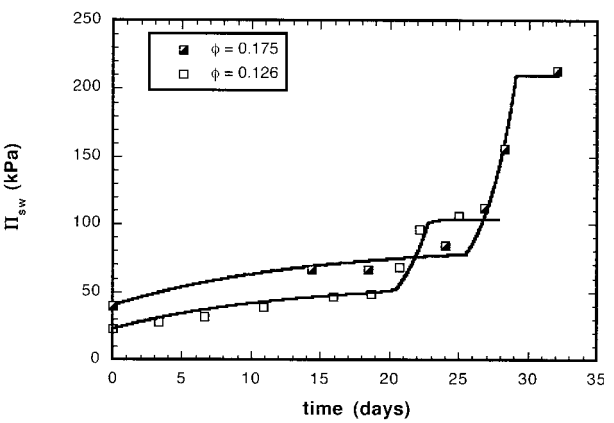


Figure 4. Model fit to the swelling pressure (Π_{sw}) data of degrading dex-HEMA gels of two different initial dextran volume fractions and the same degree of HEMA substitution. The solid lines represent the model predictions.

Table 2. t_c, t_{max}, and p

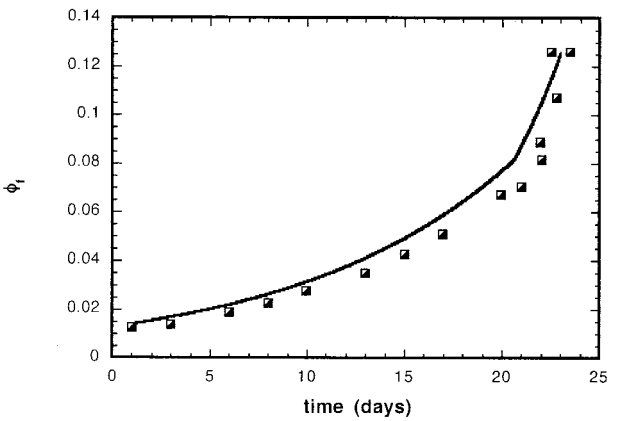
ϕ	t_c (days)	t_{max} (days)	p
0.126	20.5	22.9	0.40
0.175	25.5	29.1	0.52

$= 0.5)$[14] and increase as the polymer volume fraction of the gel increases.

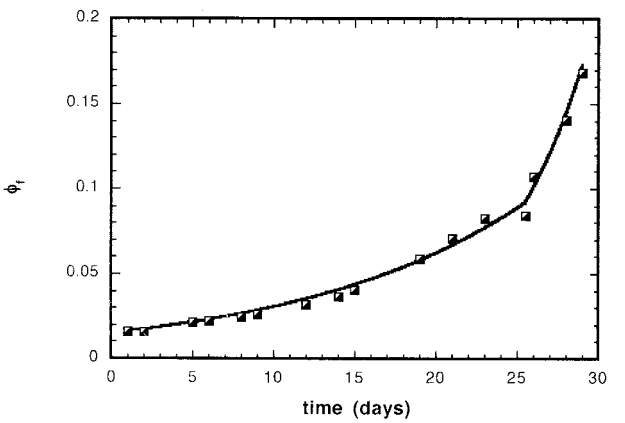
An examination of Table 1 indicates that A_g significantly differs from A_s (being 9867 kPa), and this indicates marked differences between the thermodynamic properties of the crosslinked polymer and the corresponding polymer solution. The difference in the A values can be attributed to the decreased degrees of freedom of the chains once crosslinked. This in turn can be examined in terms of the interaction parameter (χ). The value of A decreases as χ increases. Indeed, it has been established that χ for gels is greater than that for polymer solutions of equivalent polymer fractions.[15,16] This increase in χ may be due to the reduction in the configurational entropy imposed on the polymer–solvent system by the existence of the crosslinks and by differences in the average polymer–solvent interaction energies at the junctions and at the polymer chain interiors.[16] However, a theory capable of describing these effects for the prediction of χ has not yet been developed.

The model can also be used to predict the volume fraction of free dextran present in the degrading dex-HEMA gels with time by use of eqs 8 and 9. These two equations were used to predict the volume fraction of free dextran in the degrading dex-HEMA gels, and the results are given in Figure 5. The model slightly overestimates the volume fraction of free dextran in the $\phi = 0.126$ gel but captures the overall trend reasonably well [Fig. 5(A)]. The agreement is better for the $\phi = 0.175$ gels [Fig. 5(B)]. Thus, the model is consistent with the experimental results, providing further confirmation of the applicability of the approach taken.

The sensitivity of the model to uncertainty in the measurements of the necessary parameters is now considered. The important parameters estimated from the model are t_c and t_{max}. As t_{max} is dependent on t_c, the discussion is focused on the influence of errors in measurement on the predicted values for t_c. t_c is dependent on A_s, A_g, k_1, ϕ_g, and ϕ_{sol}. All other parameters being held constant, an error in the determination of A_g of ± 600 kPa results in a variation in the estimation of t_c of ± 0.3 days. Similarly, an error in the determination of A_s of ± 600 kPa results in a variation in the estimation of t_c of 0.5 days. An error in the estimation of ϕ_g or ϕ_{sol} of 10% results in a variation in the estimation of t_c of ± 1.0 days, whereas an error in the measurement of k_1 of 10% would result in a variation in the estimation of t_c of ± 2.2 days. Thus, it can be concluded that the model predictions are relatively unaffected by errors in the estimate of A_s and A_g. t_c is most affected by an error in the measurement of k_1. A similar analysis shows that t_{max} is most affected by errors in the measurement of k_2. Errors in these rate constants significantly reduce the predictive ability of the model.



(A)



(B)

Figure 5. Model fit to the data of ϕ_f in degrading dex-HEMA gels: (A) $\phi = 0.126$ and (B) $\phi = 0.175$. The solid lines represent the model predictions.

CONCLUSIONS

It has previously been found that the sol–gel transition in degrading dex-HEMA hydrogels is accompanied by a sudden change in the osmotic pressure.[4,5] This change in pressure is being examined as a means of producing a pulsatile drug-delivery system. To understand this process more fully, and with a view toward rationally designing these proposed drug-delivery systems, we have developed a model of the swelling pressure increase with time. The model is based on the Flory–Rehner concept of swelling being a balance between the osmotic pressure of the gel/water system and the elastic resisting pressure of the gel. The model has provided a good fit to swelling pressure data obtained for dex-HEMA gel/free dextran mixtures simulating degrading dex-HEMA gels.

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REFERENCES AND NOTES

1. Medlicott, N. J.; Tucker, I. G. Adv Drug Delivery Rev 1999, 38, 139–149.
2. Kost, J.; Langer, R. Adv Drug Delivery Rev 2001, 46, 125–148.
3. Kikuchi, A.; Okano, T. Adv Drug Delivery Rev 2002, 54, 53–77.
4. Stubbe, B. G.; Braeckmans, K.; Horkay, F.; Hennink, W. E.; De Smedt, S. C.; Demeester, J. Macromolecules 2002, 35, 2501–2505.
5. Stubbe, B. G.; Horkay, F.; Amsden, B.; Hennink, W. E.; De Smedt, S. C.; Demeester, J. Biomacromolecules 2003, 4, 691–695.
6. Meyvis, T. K. L.; De Smedt, S. C.; Demeester, J.; Hennink, W. E. Macromolecules 2000, 33, 4717–4725.
7. Flory, P. J. Principles of Polymer Chemistry; Cornell University Press: Ithaca, New York, 1953.
8. Flory, P. J. J Chem Phys 1977, 66, 5720–5729.
9. Treloar, L. R. G. The Physics of Rubber Elasticity; Clarendon: Oxford, 1975.
10. van Dijk-Wolthius, W. N. E.; van Steenbergen, M. J.; Underberg, W. J. M.; Hennink, W. E. J Pharm Sci 1997, 86, 413–417.
11. Amsden, B. Polymer 2002, 43, 1623–1630.
12. de Gennes, P. G. Scaling Concepts in Polymer Physics; Cornell University Press: Ithaca, NY, 1979.
13. van Dyk-Wolthuis, W. N. E.; Hoogeboom, J. A. M.; van Steenbergen, M. J.; Tsang, S. K. Y.; Hennink, W. E. Macromolecules 1997, 30, 4639–4645.
14. Stauffer, D.; Aharony, A. Introduction to Percolation Theory, 2nd ed.; Taylor & Francis: London, 1992.
15. McKenna, G. B.; Horkay, F. Polymer 1994, 35, 5737–5742.
16. Freed, K. F.; Pesci, A. I. Macromolecules 1989, 22, 4048–4050.