Polymer Matrix Controlled Release Systems: Influence of Polymer Carrier and Temperature on Water Uptake and Protein Release

NORMAN F. SHEPPARD, JR.,[†] M. YVETTE MADRID, and ROBERT LANGER*,[‡]

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

SYNOPSIS

The release of bovine serum albumin (BSA) from two types of ethylene-co-vinyl acetate (EVAc) polymer matrices was studied over the temperature range 4–50°C. Protein release and weight change of the matrices were evaluated *in vitro*. The copolymers were characterized using differential scanning calorimetry (DSC) and thermomechanical analysis (TMA). During release, the devices initially exhibit a rapid increase in weight to a maximum, followed by a more gradual decrease for the duration of the release. The time to the maximum weight and the magnitude of the maximum weight gain are temperature dependent. These effects are related to the temperature-dependent diffusivity of the BSA and elastic modulus of the EVAc. The DSC and TMA reveal melting of the crystalline phase of the polymer. The corresponding loss of mechanical integrity of the polymer leads to anomalous weight gains at these temperatures. The observed swelling and release is explained by a model in which the osmotic pressure of the protein within the pore network causes elastic deformation of the polymer matrix.

INTRODUCTION

Polymeric matrix systems show particular promise for the delivery of high-molecular-weight drugs such as polypeptides.[1] These systems are composed of a polymeric carrier in which particles of a drug, typically a few hundred microns in diameter, are dispersed. With the device immersed in fluid, the drug is slowly released by a mechanism in which drug particles at the surface of the device dissolve, causing channels to form through which fluid may further penetrate.[2,3] Dissolved drug diffuses out of the device through the tortuous network of channels so formed. The polymer carrier is typically considered as a stationary support, yet recent work in our laboratory has demonstrated that matrix systems swell, and the swelling depends on the elastic modulus of the polymer.[4] The purpose of this study was to characterize the swelling and release behavior from matrices of two polymers of differing composition, as a function of temperature. The polymers used in the experiments described below, copolymers of ethylene and vinyl acetate, are approved for use in humans by the U.S. Food and Drug Administration, and have been the subject of considerable study for controlled release applications. Work to date has looked primarily at pharmacological applications of ethylene-co-vinyl acetate (EVAc) copolymer matrix controlled release systems, over a limited temperature range.[1] Examining the behavior of systems of EVAc copolymers–model proteins [in this study, bovine serum albumin (BSA) was used] over a much wider temperature range than has been previously examined should improve the understanding of the physics of the release process. Temperature provides a convenient means of varying the elastic modulus of the polymer, and as will be shown below, significant changes accompany melting of the crystallites in these semicrystalline polymers. Insights gained

* To whom correspondence should be addressed.

[†] Now with the Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21218.

[‡] Additional affiliations: Harvard-MIT Division of Health Sciences and Technology, and Whitaker College of Health Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, and Department of Surgery, Children's Hospital, Boston, MA 02115.

Journal of Applied Polymer Science, Vol. 46, 19–26 (1992)
 CCC 0021-8995/92/010019-08$04.00

from two copolymers differing in vinyl acetate content (33 versus 40%) should be applicable to other polymer systems, including degradable ones. The results of these temperature studies could be important in nonmedical applications of the controlled release of proteins such as pesticides and consumer products, where the range of use temperatures is considerably greater than in physiological applications.

EXPERIMENTAL

Matrix Preparation

The matrix devices used in this release study were formed by incorporating bovine serum albumin (Sigma Chemical Co., St. Louis, MO) into poly(ethylene-co-vinyl acetate) (DuPont Co., Wilmington, DE) polymers using a solvent casting technique.[5] The two copolymers used in the study were DuPont Elvax 40P, 40% vinyl acetate by weight, and DuPont Elvax 150, containing 33% vinyl acetate, based on the manufacturer's specifications. Both polymers were washed with water and extracted with acetone as described previously.[5] The devices were fabricated with a 50% loading by weight of albumin, sieved to contain particle sizes in the range of 150–250 μm.[6]

Protein Release and Matrix Swelling

Release experiments were carried out in 0.1*M* potassium phosphate buffer adjusted to pH 7.4 with sodium hydroxide, at temperatures ranging from 4 to 50°C. Disks of 1.2 cm diameter were punched from sheets of EVAc–BSA matrix and weighed. Thicknesses (approximately 1 mm) were determined to a precision of 0.1 mm with micrometer calipers. These were placed into 10 mL of buffer, and at each time point removed and placed into fresh buffer. The intervals between time points were chosen so as to maintain infinite sink conditions. The concentration of BSA in the release media was determined spectrophotometrically (Perkin-Elmer 553 UV/visible spectrophotometer) by measuring the absorbance at 220 and/or 280 nm. To quantify the swelling behavior of the devices, the weight change at each time point was measured by carefully blotting dry the polymers on absorbent paper and weighing them in a weighing bottle.

Polymer Characterization

Differential scanning calorimetry (DSC) was done using a Mettler TA3000 System DSC, at a heating rate of 10°C per minute, using 10-mg samples in sealed aluminum pans. A Perkin-Elmer TMA 7 thermomechanical analyzer was used to characterize the deformation of the polymers under a nonoscillating compressive load as a function of temperature.

RESULTS

Figures 1–3 summarize the results of the protein release and weight change measurements.

Release

Panel (a) of each figure illustrates the fraction of incorporated protein released versus the square root of time. Figure 1 exhibits the results at 4, 23, and

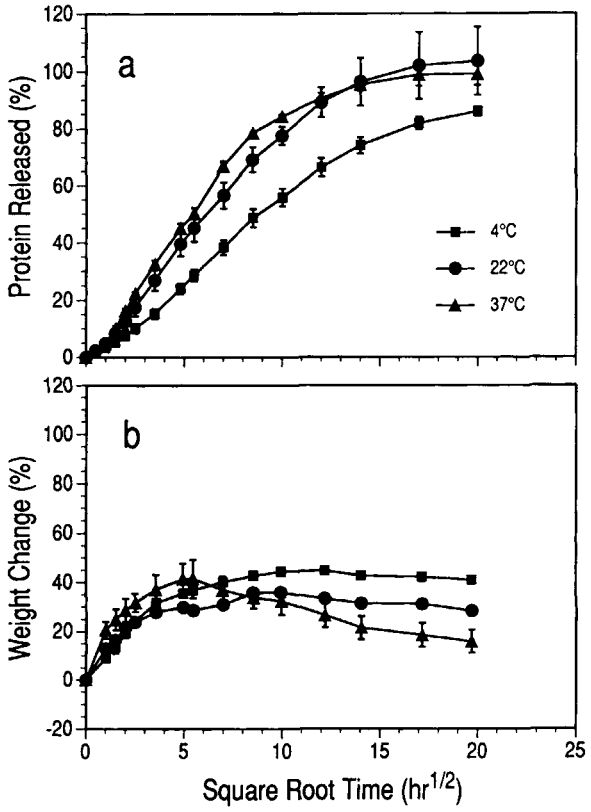

Figure 1 Protein release and weight change for 40% vinyl acetate copolymer at temperatures of 4, 22, and 37°C. Release curves are averages of eight samples, weight change is the average of three of the eight. Where standard deviations are not shown, value is smaller than the sized of the data point.

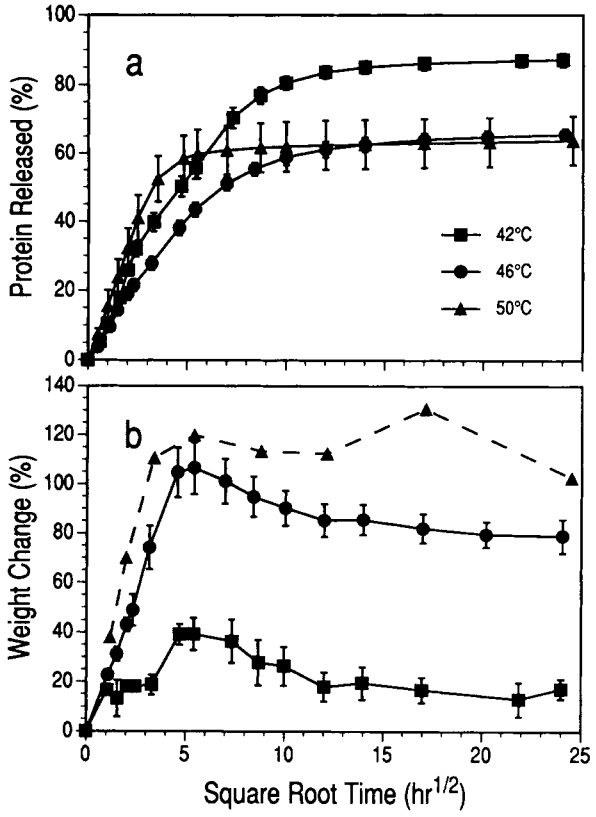

Figure 2 Protein release and weight change for 40% vinyl acetate copolymer at temperatures of 42, 46, and 50°C. Release curves are averages of five samples. Weight change is the average of four different samples at 42 and 46°C. Where standard deviations are not shown, values are smaller than the data point. For 50°C weight change, each point represents a different device, which was discarded after measurement.

37°C for devices fabricated with the 40% vinyl acetate copolymer. The release curves exhibit the sigmoidal shape characteristic of matrix release devices.[5] With increasing temperature, the rate at which the protein is released increases, but the shapes of the curves remain the same. The fraction of protein released approaches 100% at long times, as would be expected given the 50% protein loading used in this experiment (i.e., at this high loading, there will be considerable contact between adjacent protein particles in the matrix, so that the channels formed as protein dissolution occurs should be continuous[7]). The data in Figure 2 represent measurements of the same copolymer matrix, at higher temperatures of 42, 46, and 50°C. The initial rate of protein release, proportional to the slope of the release curve, continues to increase with increasing temperature, but the fraction released falls short of 100%. At 46 and 50°C only 60% of the incorporated protein is released from the devices. Figure 3 presents the results for the 33% vinyl acetate copolymer matrix, at temperatures of 4, 22, 37, and 50°C. The results are similar to those observed in Figures 1 and 2, with again only 60% of the protein released at 50°.

Weight Change

The weight change of the devices as a function of time and temperature are presented in panel (b) of Figures 1–3. The weight change has been normalized to the dry weight of the device, as expressed by

$$\text{Weight change at time } t\ (\%) = 100 \times \left(\frac{\text{Weight } (t)}{\text{Weight } (0)} - 1\right) \quad (1)$$

The weight change of the 40% vinyl acetate copolymer matrices, shown in Figures 1 and 2, is partic-

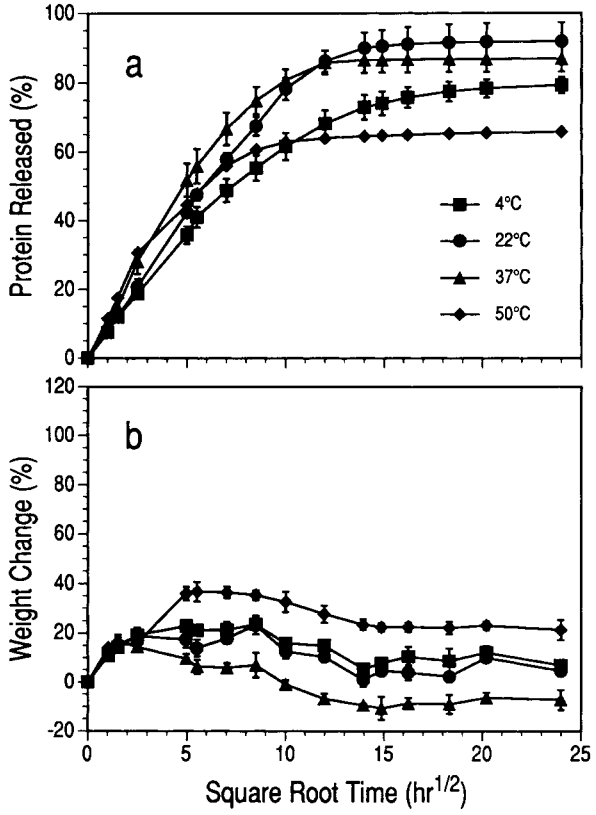

Figure 3 Protein release and weight change for 30% vinyl acetate copolymer at temperatures of 4, 22, 37, and 50°C. Release and weight change curves are averages of the same four samples. Where standard deviations are not shown, values are smaller than the data points.

ularly interesting. Considering first Figure 1, the weight of the devices increases initially to a peak, then decreases slowly over time. This is readily apparent for matrices released at 37°C, which gain an additional 40% of their initial weight after 5 $h^{1/2}$ (= 25 h), then fall to 15% after 20 $h^{1/2}$. At the lower temperatures the weight appears to increase monotonically during release, although careful observation indicates peaks at 35% after 9–10 $h^{1/2}$ at 22°C, and at 45% after 10–12 $h^{1/2}$ at 4°C. In Figure 2 the devices released at 42°C behave similarly to those exposed to 37°C temperature, with a 40% gain after 5 $h^{1/2}$. At temperatures of 46 and 50°C, the weight of the 40% vinyl acetate polymer matrices becomes more than double the dry weight. This causes considerable deformation of the matrices and is quite apparent visually. This large weight gain is not observed with the matrices fabricated with the 33% vinyl acetate copolymer. As a group the trend is similar to the 40% vinyl acetate matrices. After rising to an earlier peak, the normalized weight of the 37°C devices falls below that of the 4 and 22°C devices, which are relatively similar. As for the 40% copolymer, the devices released at 50°C gain the most weight, but the maximum weight gain is only 40%.

Polymer Properties

Figure 4 is a DSC thermogram (heat flow versus temperature) of the two polymers at temperatures from 0 to 100°C. The 40% vinyl acetate copolymer shows two endothermic peaks, one at 46°C, the other at 65°C. The thermogram for the 33% vinyl acetate copolymer shows a very broad melting peak at 50°C, with a shoulder at 65°C, suggestive of another peak

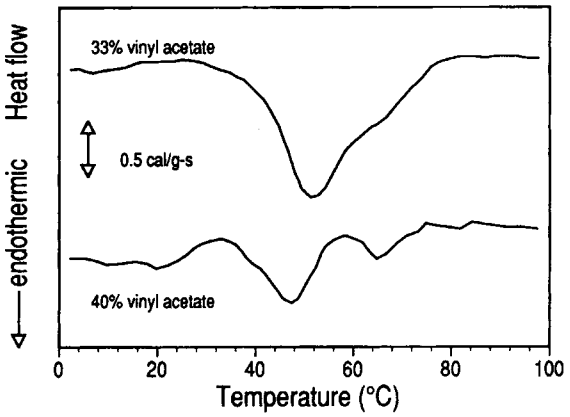

Figure 4 DSC thermograms for 33% vinyl acetate and 40% vinyl acetate copolymers. Scan rate was 10°C/min.

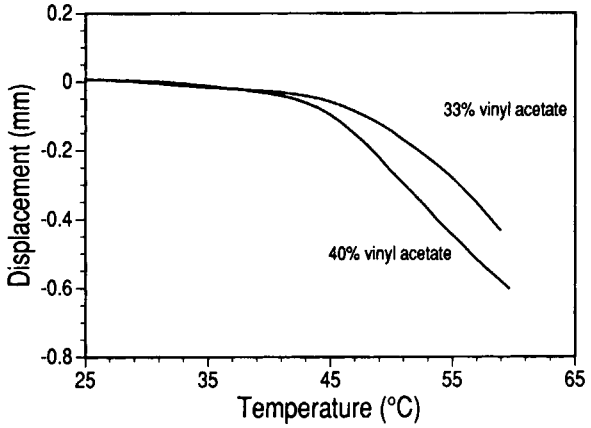

Figure 5 Displacement of thermomechanical analyzer compression probe versus temperature for 33 and 40% vinyl acetate copolymers. Heating rate 10°C per minute.

at this temperature. The 65°C peak appears to be common to both polymers, while the low temperature peak appears to shift up in temperature by 5°C in going from 40 to 33% vinyl acetate. The observed shift in melting point is similar to that observed in dynamic mechanical studies of similar EVAc copolymers.[8]

The effect of the crystalline melting is apparent in the results of the thermomechanical analysis, shown in Figure 5. The figure presents the displacement of the probe into the sample versus temperature, while the temperature is increased at a rate of 10°C per minute and a constant load is applied. The temperature at which the onset of flow occurs, as indicated by the penetration of the probe into the sample, is extrapolated from the linear portions of the displacement versus temperature curve, and is 43.2°C for the Elvax 40P and 50.9°C for the Elvax 150.

DISCUSSION

The following paragraphs address (1) the temperature dependence of the release rate, and its relation to the temperature dependence of the diffusivity of BSA in solution; (2) the incomplete release of protein at high temperatures; (3) the significance of the maximum in the weight change of the device; and (4) the large weight gain of the 40% vinyl acetate devices at high temperatures, and its relation to the loss in mechanical integrity of the polymer. Finally, based on these observations, a model is proposed for the mechanism of swelling and release.

Temperature-Dependent Release Rate

As illustrated in Figures 1–3, the release rate of protein from the devices was temperature dependent. Earlier workers have quantified release rates from polymer matrix systems by calculating an effective diffusion coefficient, D_e, using the solution to Fick's law for diffusion of a solute out of a homogeneous material.[7] At short times the fraction desorbed is proportional to the square root of time, as shown in Eq. (2)[9]:

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_e^t}{\pi L^2}\right)^{1/2} \qquad (2)$$

where M_t is the mass of protein released at time t, M_∞ is the mass released at infinite time, and L is the thickness of the sample. Effective diffusivities describing the BSA release from the 40% vinyl acetate matrices was calculated from the slopes of the release curves in Figures 1 and 2. These effective diffusivities are by necessity estimates but provide a means for examining the temperature dependence of the release rate. First, Eq. (2) provides only an approximate representation of the data; the sigmoidal shape of the curves required that the slope be taken over a time period corresponding to between 10 and 40% release, where the curves are linear. Second, the thickness may be changing during release; the effective diffusivity was calculated using as the value of L the initial thickness of the device. Additionally, diffusivities are concentration dependent, and the concentration of BSA within the pore space ranges from the solubility limit at the dissolution front to approximately zero at the face (infinite sink conditions).

Figure 6 is an Arrhenius plot of D_{eff} determined in this way. For comparison, Figure 6 also includes data from the literature on the diffusivity of BSA in pH 7.4 acetate buffer at a BSA concentration of 2.14 g/100 mL.[10] A number of observations can be made. A comparison of the relative magnitudes of the two diffusivities indicates that the effective diffusivity of the protein in the matrix is roughly a factor of 100 less than that in solution, resulting from the tortuous pore network formed by the protein phase.[7] A linear regression of the experimental data in Figure 6 yields an apparent activation energy for D_{eff} of 23.5 kJ/mol, which corresponds to a temperature sensitivity of 2.9% per °C at 37°C, while the diffusivity of BSA in solution exhibits a 5.3% per °C change.[10] If the polymer carrier were simply a fixed porous matrix, these values should be equal.

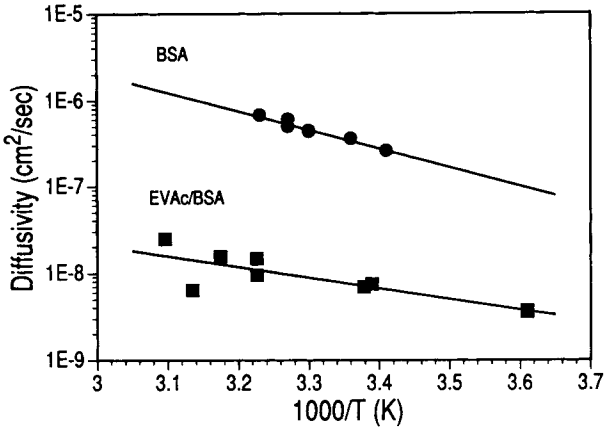

Figure 6 Arrhenius plot of (1) effective diffusivity of protein in the 40% vinyl acetate copolymer matrix during release; and (2) the diffusivity of BSA in solution reported by Wakeham et al. (Ref. 9).

The results suggest that protein release is more complicated than simple Fickian diffusion, and that other factors give rise to the temperature dependence of D_{eff}. These might include porosity changes with temperature, or a convective transport mechanism, and are discussed in more detail below.

Incomplete Protein Release

Two possible reasons for this observation of incomplete protein release at high temperatures [refer to Figs. 2(a), 3(a)] are that (i) denaturation and/or aggregation of the protein renders the protein insoluble or (ii) melting or flow of the polymer phase closes up the pore structure. A simple experiment was conducted to rule out the latter possibility. A device released at 50°C until no further release of protein was observed was then cut with a razor blade into approximately 100 pieces, and the experiment continued. Only an additional few percent of the incorporated protein was subsequently released. This fact, in addition to the TMA results showing that the 33% vinyl acetate polymer maintains its integrity to 51°C, suggests that insolubilization of the protein limits the amount of protein released at these temperatures.

Weight Change

The results of the weight change study revealed that under most conditions, the weight of the matrix device increases to a maximum value during the course of release. The magnitude and time course of this

weight gain depends both on the polymer and the temperature. A physical model of this behavior must include mechanisms by which the matrices can both gain and lose weight. Possible mechanisms for weight gain include the displacement of entrapped protein and void space by the fluid, and an osmotic flow driven by the dissolved protein. Weight loss may arise from physical contraction of the pore space as a result of the relief of residual stress left in the polymer matrix from fabrication.

Addressing first the weight gain, one would expect, in the absence of any deformation of the polymer, an increase in the weight of a matrix device due to the displacement of protein and trapped air in the pore space by water. The specific gravity of the 40% vinyl acetate matrix devices, calculated from the diameter, thickness, and weight of disks punched from a sheet of material, is 0.71 ± 0.04 g/cm^3 (N = 36), while the density of the EVAc copolymer is 0.97.[11] If, during the course of release, the protein and trapped air were simply displaced by water, then one would expect a monotonic increase in the weight of the device to about 140% of the initial value once the protein has been completely dissolved.

Weight loss during release could occur as the matrix contracts during the dissolution of the protein particles. This contraction results as residual stress, created in the matrix during the fabrication process, is relieved. The matrices in this study were cast from a 10% polymer solution in which the powdered protein was suspended. Based on a 50% protein, 50% polymer matrix, the casting solution–suspension contains approximately 80% volatiles. As this solvent is removed during the freeze drying process, the matrix undergoes a volume reduction of roughly a factor of five, leading to residual stress, and observed as curling at the edges of the sheet of matrix. During release, dissolution of the protein particles defining the pore space allows the polymer to relax, reducing the available pore space, and consequently the weight of the device.

The mechanisms described above are sufficient to explain the observed weight change results at temperatures below 37°C. Figure 1 shows that the weight gain of the 40% vinyl acetate matrix released at 4°C increases to a nearly constant value of 45%, consistent with displacement of the pore space by water. At 37°C, the same matrix type exhibits considerable contraction, resulting from the relief of residual stress. The reason stress relaxation and resulting weight loss is not observed at 4°C may be due to the effect of temperature on the rate of relaxation. The Williams–Landel–Ferry (WLF) equation,[12]

$$\log \frac{\tau(T)}{\tau(T_g)} = \frac{17.4(T - T_g)}{51 + T - T_g} \qquad (3)$$

is an empirical relation describing the temperature-dependent relaxation behavior of polymers. Specifically, the ratio of the relaxation time, τ, at a temperature T to that at the glass transition temperature, T_g, depends on the temperature difference $T - T_g$. This law describes the behavior of a large number of polymers and can be used to provide an estimate of the relative relaxation times at 4 and 37°C. Based on a T_g of −20°C,[13] this analysis indicates that the relaxation time at 4°C is approximately 4000 times greater than at 37°C, and therefore the rate at which stress relaxation induced contraction of the matrix occurs will be that much slower as well.

The mechanisms of weight gain and loss described above are insufficient to explain the weight gains above 45% observed with the 40% vinyl acetate matrix at high temperatures, shown in Figure 2. The driving force required for these large weight gains may be the osmotic pressure of the protein in the fluid-filled porous network.[14] In the classical two-compartment determination of osmotic pressure,[15] water is transported across a semipermeable membrane into the compartment containing the high-molecular-weight solute. Pressure increases in this compartment until the activity of water is equal on both sides of the membrane. We would like to suggest that, for short time scales compared to the duration of the release, the polymer matrix acts as a semipermeable membrane. That is, due to the large diffusivity of water relative to the BSA, water diffuses freely in the pore space and establishes a quasi-equilibrium. The resulting osmotic pressure causes an elastic deformation of the polymer matrix. The extent of this deformation, and as a result the mass of fluid in the pore space, will depend on the elastic modulus of the polymer. This explanation is supported by the results of the thermomechanical analysis (Fig. 5). At temperatures of 46 and 50°C, where the maximum weight gain of the 40% vinyl acetate matrices was on the order of 100%, the thermal analyses revealed that melting of the crystalline polymer phase led to loss of mechanical integrity of the polymer at a temperature of 43°C. During release at these temperatures, the polymer matrix may yield to the osmotic pressure generated by the dissolved protein.

Mechanistic Interpretation

An interpretation for the weight change during the release process, based on the mechanisms described above, is illustrated schematically in Figure 7. When the dry matrix is first immersed in fluid, the protein at the surface of the device dissolves. The osmotic pressure of the protein in the fluid-filled region causes the matrix to deform, leading to an increase in the weight of the device. As the protein continues to dissolve, the thickness of the fluid-filled zone increases and the swelling continues. Eventually, the dissolution fronts meet, and the protein is completely dissolved. The water uptake reaches a maximum at this point because the matrix contains the greatest amount of dissolved protein, and therefore the driving force for swelling is greatest. From this time onward the concentration of protein everywhere within the matrix decreases with time, and with the concomitant reduction in the driving force for swelling, the deformation of the matrix will decrease also. An important feature of this model is the identification of the maximum in weight gain with the complete dissolution of the protein phase; experiments to verify this are underway.

The swelling mechanism presented here differs from that of "conventional" swelling-controlled release systems[16] in a number of important ways. Swelling-controlled release systems are generally glassy polymers in which a solute has been dissolved, forming a homogeneous system. Interaction between the solvent and the glassy polymer causes the polymer to swell. The diffusivity of the solute (drug) in the swollen polymer is considerably greater than that in the glassy polymer and is usually large enough so that the release rate depends on the rate at which the solvent can swell the glassy polymer. Because the swelling depends on the interaction between the polymer and solvent, the volume increases monotonically during release. The system described here differs from those swelling systems in that there are three phases: a hydrophobic polymer phase, a solid protein phase, and a dissolved protein phase. The swelling of the device depends on the interaction between the solvent and the dissolved protein. Once the protein diffuses out of the device, the osmotic driving force for swelling is gone, and contraction of the device is observed. To formulate a mathematical description of this process, existing swelling-controlled release models would need to be modified to incorporate a swelling dependence on the dissolved protein concentration, rather than on the solvent concentration in the polymer.

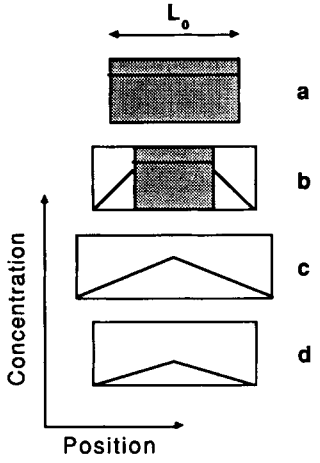

Figure 7 Schematic illustration of release process. Shaded areas represent matrix containing undissolved protein, clear areas represent fluid-filled pore network containing dissolved protein. (a) Matrix before release, loaded with protein at an effective concentration greater than the solubility of protein in water. (b) Early stages of release, with swollen fluid-filled zones at exposed faces, matrix at center. Linear concentration profile shown is for illustration only. (c) Complete dissolution of protein in the matrix, maximum swelling. (d) Late stages of release, swollen zones contract as protein diffuses out.

CONCLUSIONS

The release of a model protein (bovine serum albumin) from ethylene–vinyl acetate copolymer matrices was studied over the temperature range 4–50°C, using two different polymers as the matrix material. During release, the devices initially exhibit an increase in weight to a maximum, followed by a gradual decrease. The time to the maximum weight and the magnitude of the maximum weight gain are temperature dependent and correlate with the decreasing elastic modulus with temperature. The interpretation of these results is that dissolved protein within the matrix provides a driving force for water imbibition. The resulting swelling pressure is balanced by the elastic modulus of the polymer, with the result that the weight change increases as the elastic modulus decreases.

These results and model presented warrant a number of other experiments and analyses. Residual stress remaining in the polymer is postulated to have an effect on the weight loss of the matrix. This could be varied by changing the conditions (temperature, atmosphere) under which the solvent is removed

from the matrix and by changing the molecular weight of the polymer.[13] Variation of the solute released would provide a test of the model, since we would expect that for species with diffusivities much closer to that of water (e.g., salts) the osmotic swelling would be less, since the time to establish an osmotic pressure within the aqueous pore network would be comparable to the time for release. Finally, mathematical descriptions based on this model will enable determination of the importance of these processes on the release kinetics, which might be of particular concern in trying to evaluate the effects of variations in the polymer and fabrication procedure.

The authors wish to acknowledge the assistance of Julie Kim with the experiments and helpful discussions with Mark Saltzman. This work was supported in part by a grant from the National Science Foundation.

REFERENCES

1. R. Langer, *Science,* **249,** 1527 (1990).
2. R. Bawa, R. Siegel, B. Marasca, M. Karel, and R. Langer, *J. Controlled Release,* **1,** 259 (1985).
3. Y. Ogawa, M. Yamamoto, S. Takada, H. Okada, and T. Shimamoto, *Chem. Pharm. Bull.,* **36,** 1502 (1988).
4. T. Hsu, and R. Langer, *Proc. Intl. Conf. Contr. Rel. Bio. Mat.,* **12,** 208 (1985); N. Sheppard, Y. Madrid, and R. Langer, *Proc. Intl. Conf. Contr. Rel. Bio. Mat.,* **15,** 250 (1988).
5. R. Langer, L. Brown, and E. Edelman, *Methods Enzymol.,* **112,** 399 (1985).
6. W. D. Rhine, D. S. T. Hsieh, and R. Langer, *J. Pharm. Sci.,* **69,** 265 (1980).
7. W. M. Saltzman and R. Langer, *Biophys. J.,* **55,** 163 (1989).
8. L. E. Neilsen, *J. Polym. Sci.,* **42,** 357 (1960).
9. J. Crank, *Mathematics of Diffusion,* Clarendon Press, Oxford, 1975.
10. W. A. Wakeham, N. H. Salpadoru, and C. G. Caro, *Atherosclerosis,* **25,** 225 (1976).
11. "Elvax Resins Grade Selection Guide," Industrial Polymers Division, DuPont Co., Wilmington, Delaware, 1989.
12. F. W. Billmeyer, *Textbook of Polymer Science,* Interscience, New York, 1962, p. 196.
13. T. Hsu and R. Langer, *J. Biomed. Mat. Res.,* **19,** 445 (1985).
14. J. Wright, S. K. Chandrasekaran, R. Gale, and D. Swanson, *AICHE Symp. Ser.,* **77,** 62 (1981).
15. G. W. Castellan, *Physical Chemistry,* Addison-Wesley, New York, 1971.
16. R. W. Korsmeyer, S. R. Lustig, and N. A. Peppas, *J. Polym. Sci. Polym. Phys. Ed.,* **24,** 395 (1986); S. K. Singh and L. T. Fan, *Biotech. Prog.,* **2,** 145 (1986); G. C. Sarti, C. Gostoli, G. Riccioli, and R. G. Carbonell, *J. Appl. Polym. Sci.,* **32,** 3627 (1986).

Received September 20, 1991
Accepted October 30, 1991