Sorption of Lysozyme by HEMA Copolymer Hydrogels

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SYNOPSIS

Sorption of lysozyme by 2-hydroxyethyl methacrylate (HEMA) copolymer hydrogels was studied as a function of pH and gel composition. Three types of HEMA gels were synthesized: neutral (HEMA), acidic (HEMA + acrylic acid), and basic (HEMA + dimethylaminoethyl methacrylate). Each gel was synthesized at four initial volume fractions to obtain different equilibrium swelling ratios and microstructures. Sorption as a function of time was measured for each gel at pH 7, 7.5, and 8. The rate of uptake by the acidic gels was more rapid than that by the neutral gels: To sorb 90% of the protein required only 1 h for the acidic gels but 15 days for neutral gels. Lysozyme did not adsorb or partition into the basic gels. The fractional approach to equilibrium was most rapid for the more swollen gels, and the effect of pH was small. The results reported here may be useful for rational design of new biomaterials where it is desirable to know the relative magnitude of the effects of composition, synthesis, and pH on protein sorption. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Sorption of tear proteins is an important contributing factor to the fouling of contact lenses. Previous studies, both *in vitro* and *in vivo*, have shown that significant amounts of protein are sorbed by commercial soft contact lenses.[1-8] Contact lenses with a high water content and ionic character (Federal Drug Administration Group IV classification) sorb more protein than do other classes of soft contact lenses.[5,6,9,10] Protein fouling of contact lenses not only reduces the optical quality of the lens, but also may contribute to allergic and inflammatory reactions in the eye.[11,12] Moreover, a film of adsorbed protein on the surface of the lens may encourage bacterial adhesion and subsequent infection of the eye.[13-16]

Adsorption of proteins to polymer surfaces has been the subject of considerable investigation because the presence of a film of protein can modify the biocompatibility of the polymer surface. In these studies, it is generally assumed that the protein adsorbs in multilayers on the surface of the polymeric material and does not diffuse into the material itself.

The soft contact lens is a hydrogel and therefore contains a relatively large amount of water. Therefore, sufficiently small solutes diffuse into the gel. The permeability and partitioning of small solutes such as metabolites and preservatives into contact lenses has been studied by several authors.[17-19] In the contact lens field, it was first generally accepted that proteins adsorb in multiple layers to the surface of the lens. Several investigators provided direct or indirect evidence, however, that the smallest proteins present in tears are able to diffuse into a gel matrix, depending on the gel microstructure.[20-22] Refojo and Leong showed that proteins diffuse farther into a gel after a given amount of time if the water content of the gel is high.[21]

High water content is desirable for contact lenses because of increased oxygen permeability and ease of fit.[7,23] Water content, which is directly related to the degree of swelling, may be increased dramatically by copolymerizing an ionizable monomer (such as methacrylic acid) with the principal, neutral component of the hydrogel (such as 2-hydroxyethyl methacrylate [HEMA]). The amount of protein sorbed (*in vivo*) increases with the anionic character

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of the polymer.[24] The water content of a hydrogel may also be raised by reducing the crosslink density or by polymerizing the gel in the presence of a diluent. The extent to which the water content can be increased is limited in practice because the resulting gel must meet specifications concerning optical properties and mechanical strength.

High water content lenses require more rigorous cleaning regimens because they sorb more protein, especially if the matrix is loose enough to permit proteins to diffuse into the gel and if the lens is designed for extended wear, which allows more time for proteins to diffuse into the gel. A potential problem exists in the use of protease enzymes as cleaning agents because the protease may not be able to diffuse into all the regions where smaller proteins, such as lysozyme, can enter; some sections in the gel may never be "cleaned" because the proteases never reach those sections.

The composition of the protein film on the surface of the gel is a complex function of factors such as surface roughness, ionic character, pH, tear composition, and disease state of the patient. Nevertheless, studies indicate that lysozyme is usually the most prevalent protein in the adsorbed layer.[1-3,25] This is not surprising, because lysozyme is a small protein, positively charged at physiologic pH,[26] while ionic lenses are usually negatively charged. Further, lysozyme is the most abundant protein in human tears, constituting one-third of the total protein content.[27]

The sorption of protein by soft contact lens materials was investigated by several authors.[5,6,9,10,12,28,29] Most of the studies, however, were on patient-worn lenses, where the individual effects influencing sorption are difficult to discern and the exact chemical composition and synthesis procedure are unknown. Gachon et al. studied the adsorption of less than a monolayer of lysozyme onto commercial polyvinylpyrrolidone (VP)/methyl methacrylate (MMA) lenses as a function of concentration and pH.[28] Refojo and Leong studied the penetration of lysozyme into poly-HEMA gels synthesized in solution and in bulk.[21]

This work reports systematic studies of the sorption of lysozyme as a function of time, pH, and preparation conditions by hydrogels that we have synthesized. We chose poly-HEMA, the first and still one of the most common soft contact lens materials. We also copolymerized HEMA with dimethylaminoethyl methacrylate (DMA) or acrylic acid (AA) to prepare ionic lenses with basic or acidic character, respectively. We varied the ratio of monomers to diluent in our solution polymerization to obtain different gel microstructures and water contents. Our experiments differ from those of Gachon et al. in that we place our gels into a solution of lysozyme within the range of physiologic concentrations.[28] We observed the effect of pH, ionic character of the gel, and diluent concentration at polymerization on the kinetics of lysozyme uptake under approximate physiologic conditions. To our knowledge, a systematic investigation of this type has not previously been carried out on a "family" of copolymer hydrogels. Our results may be useful for rational design of new biomaterials where it is desirable to know the relative magnitude of the effects of composition, synthesis, and pH on protein sorption.

MATERIALS

2-Hydroxyethyl methacrylate (HEMA), acrylic acid (AA), and sodium azide were purchased from Kodak. Ethanox 330 [1,3,5-trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene] was obtained from Ethyl Co. Azoisobutyronitrile (AIBN), dimethylaminoethyl methacrylate (DMA), and ethylene glycol dimethacrylate (EGDMA) were purchased from Polysciences. AIBN was purified by recrystallization from ethanol as described by Chou.[30] Butanol, hydrochloric acid, sodium hydroxide, and sodium phosphates were purchased from Fisher Scientific and used as received. Hen-egg-white lysozyme, grade I (lot 39F8213), was obtained from Sigma. Distilled water was filtered (0.2 μm) and deionized (17.9 MΩ-cm resistivity) with a Barnstead Nanopure II unit. Monomers were purified by vacuum distillation as described by Chou.[30] The monomer to be distilled was stirred and heated in a round-bottom flask. Ethanox 330, an antioxidant, was added at a concentration of 1 mg/mL to inhibit polymerization during distillation; however, a few grams of monomer was always polymerized by the end of the distillation. The vapor was condensed using cold tap water in the outer shell of the condenser and collected using a distributor to feed to one of four receiving round-bottom flasks. The top and bottom fractions (approximately 15% each) of the distillate were discarded.

The gels were made by free-radical polymerization in butanol with EGDMA as the crosslinker. Gels were synthesized from solutions at four different volumetric fractions of monomer: 0.29, 0.38, 0.44, and 0.77. These volume fractions (% V) are the same volume fractions used in the study of swelling equi-

libria by Baker et al.[31] Polybasic gels were synthesized with 30 mol % DMA (on a diluent-free basis), and polyacidic gels were synthesized with 30 mol % AA. The appropriate volumes or masses of HEMA, EGDMA, butanol, AIBN, and comonomer were mixed and degassed for 30 min under vacuum (20 in Hg). The monomer solution was then quickly injected into flat, square molds using a syringe. The molds consisted of a sandwich of two square, silanized-glass plates separated by a 0.48 mm-thick Teflon spacer and held together with standard 1 in. binder clips. The plates were silanized by immersion for 5 min in 2% dichlorodimethylsilane in toluene. The molds containing the monomer solution were then placed in plastic bags and immersed in an aqueous constant-temperature bath at 60°C. The reaction was allowed to proceed for 24 h. The molds were then removed from the bags and opened. Disks 1 cm in diameter were cut immediately from the flat sheets of gel and placed in excess butanol, which was replenished periodically up to 2 weeks, to extract any soluble fraction. The gels were then placed on sheets of aluminum foil (HEMA and HEMA/DMA) or wax paper (HEMA/AA) and allowed to dry at ambient conditions. The HEMA/AA gels were dried upon wax paper because these gels adhered strongly to aluminum foil. After drying to constant mass at ambient conditions, the gels were dried overnight under a 25 in Hg vacuum.

SORPTION EXPERIMENTS

The dried gels were first swollen to equilibrium in 20 mL scintillation vials containing sodium phosphate buffer (0.1 *M* ionic strength; pH 7.0, 7.5, or 8) at 36.4°C in a Blue M temperature bath with gentle agitation. The pH range from 7.0 to 8.0 was chosen to span most of the range of human-tear pH reported in the literature. Sodium azide (0.001 *M*) was added as an antibacterial agent, as the time required to reach swelling equilibrium was on the order of several weeks. The buffer pH's were chosen to span the maximum range of tear pH as reported in the literature.[32-35] Swelling equilibria were determined gravimetrically, as in previous work.[31] Two gels were placed in each labeled vial.

After the swelling of the gels had reached equilibrium, the gels from each scintillation vial were weighed and the diameter and height measured with calipers.† Because of the large swelling ratio of the polyacidic gels, disks approximately 0.84 cm in diameter (approximately the diameter of the neutral gels) were cut from the original disks and weighed and the diameter measured with calipers. The gel disks were then carefully placed on edge in a 2 mL crimp-top, autosampler vial, and the vial was filled with approximately 1 mL of solution containing 0.1 *M* ionic strength sodium phosphate buffer, 0.001 *M* sodium azide, and 2 mg/mL lysozyme at the same pH. The concentration of lysozyme was set at 2 mg/mL to correspond to a typical physiologic concentration in human tears.[27,37-40] The polyacidic gels were too fragile to be inserted in an autosampler vial and were instead placed in 1.7 mL polypropylene microcentrifuge tubes. The exact mass of solution added to each vial was determined by weighing.

To avoid transfer of protein from the surface of the gel to other surfaces such as spatulas and blotting tissues, we did not touch the gel during the sorption experiment. For the HEMA and HEMA/DMA gels, the vial was loaded immediately after adding the lysozyme solution onto the autosampler of a Hewlett-Packard 1090 high-performance liquid chromatography system, and the concentration of lysozyme was determined using cation-exchange chromatography with a 50 × 7.8 mm Bio-Rad HRLC® MA7S cation exchange column with UV detection by a diode-array detector at 280 nm. A gradient of 0–60% of 1 *M* NaCl in 20 m*M* bis-Tris buffer (pH 7.5) was used as the eluant. The injection volume was 25 μL; using this injection volume, we were able to measure lysozyme concentrations down to the order of 17 μg/mL. The autosampler was used to replace the volume withdrawn for injection with fresh buffer of the appropriate pH; thus, the volume of solution in each vial remained constant. Care was taken that the position of the gel in the autosampler vial did not block the path of the injection needle of the autosampler; when necessary, the position of the gel was adjusted using a syringe needle poked through the crimp top. For the HEMA/AA gels, 0.4 mL of solution was withdrawn from the microcentrifuge tube and ejected into a vial for autosampling. Immediately after injection into the HPLC, the remaining 0.375 mL was returned to the microcentrifuge tube; thus, the volume decreased by 25 μL per injection.

Lysozyme concentrations were determined by comparing the peak area for a given sample against a calibration curve defined by peak areas of solutions of known concentration. The concentration of lysozyme in the solution was measured periodically using the above procedures to determine the

† Swelling of hydrogels is isotropic.[36]

time course of sorption and to monitor the attainment of equilibrium. After each measurement, the vials were returned to the temperature bath and agitated. The total time required for each measurement was approximately 4 min. The concentration of the stock solution for each of the three pH's was also monitored as a control. No statistically significant changes in the chromatogram or the lysozyme peak area were observed for the stock solutions during the experiment. The injection-to-injection variability of the lysozyme peak area was approximately 0.4%.

In many protocols for studying the adsorption of proteins onto biomaterials, proteins are radiolabeled. One can thus detect easily the adsorption of minute quantities of protein, and one can determine binding and rate constants in a straightforward manner. As we were interested in observing the effect of a wide range of conditions (36 conditions were studied, each in triplicate) on the uptake from a solution containing a relatively high concentration of protein (2 g/L) compared to many other studies, we chose to use a solute-depletion protocol with absorption of light at 280 nm to determine the concentration of protein in the solution. We thus avoided all disadvantages (cost, safety, bureaucracy) regarding the use of radiolabeled proteins.

Because we do not measure directly the protein in or on the gel phase, we verified that the adsorption of lysozyme onto the walls of the vials used in the experiment was negligible. For each combination of gel and pH, we monitored the concentration in a vial/centrifuge tube which contained no gel, but was treated identically to vials containing gels. For each control, the first sample was taken immediately after the vial was loaded into the autosampler. The concentration of protein in the control vials changed less than 8 μg/mL over the course of the experiment.

RESULTS

Table I gives swelling equilibria and hydration of the various gels studied. Swelling equilibria were measured in 0.1 M sodium phosphate buffers with 0.001 M sodium azide at 36.4°C. The swelling ratio is defined as the ratio of the weight of the swollen gel at equilibrium to its dry weight. The hydration is defined as the mass fraction of water in the swollen gel. Both the swelling ratio and the hydration are measures of the water content of the hydrogel; in the hydrogel literature, it is customary to report the swelling ratio; in the contact lens literature, it is customary to report the hydration.

The effect of %V and pH on the swelling equilibria of the neutral poly-HEMA gels is small, as expected. The swelling ratio is between 1.6 and 1.8, corresponding to a hydration of 0.38 to 0.46. The swelling ratio decreases slightly with %V because the polymer strands are more entangled when less diluent is present at polymerization. Similarly, the effect of %V and pH is small for the poly-basic DMA gels. The swelling ratio for the DMA gels is between 2 and 3.5, corresponding to hydrations of 0.5 to 0.7. The swelling ratio decreases slightly with %V. The swelling ratio decreases slightly with pH because DMA (a weak base) becomes less ionized as pH increases.

In contrast, the HEMA/AA gels swell considerably more; the lowest swelling ratio measured was approximately 8.4, corresponding to a hydration of 0.88. The swelling ratio decreases with %V and increases slightly with pH because AA is a weak acid and becomes more ionized at alkaline pH. For example, the swelling ratio of a 29%V HEMA/AA gel in pH 8 buffer is approximately 23, whereas the swelling ratio of a 77%V HEMA/AA gel in pH 7 buffer is approximately 8.4.

Figure 1 presents experimental results for the sorption of lysozyme by neutral poly-HEMA gels of varying %V. The experiments were conducted at pH 7.5 and at 36.4°C. The points of the same geometry represent all the data from experiments using gels of the same %V. The mass of protein sorbed by the gel is normalized with the mass of protein sorbed at equilibrium; this quantity is called the "normalized sorption."

The rate of sorption by the gels synthesized at 29%V is more rapid than that by the gels synthesized at higher %V. In general, the rate of sorption decreases with %V, although the data for 38 and 44%V gels are scattered. We are not surprised by the scatter in the data; standard deviations on the order of 10% are commonly reported in the literature.[6,7,9,10] The rate of sorption by the neutral gels is slow for the 77%V gels; it takes 9–10 days to attain 90% equilibrium sorption.

Figure 2 presents experimental results for the sorption of lysozyme by neutral poly-HEMA gels at pH 7, 7.5, and 8. The gels were synthesized at 77%V, and the buffer was sodium phosphate. pH has virtually no effect on the sorption of lysozyme by the neutral gels, as expected, because there are no electrostatic interactions between protein and gel.

Table I Swelling Ratio and Hydration for Poly-HEMA Copolymer Hydrogels

% *V*	pH	Swelling Ratio	Hydration
Poly-HEMA			
29	7	1.8 ± 0.2	0.44 ± 0.06
29	7.5	2.03 ± 0.04	0.051 ± 0.01
29	8	1.8 ± 0.1	0.46 ± 0.03
38	7	1.7 ± 0.3	0.40 ± 0.1
38	7.5	1.82 ± 0.4	0.45 ± 0.01
38	8	1.6 ± 0.1	0.39 ± 0.05
44	7	1.66 ± 0.06	0.40 ± 0.02
44	7.5	1.8 ± 0.1	0.43 ± 0.04
44	8	1.73 ± 0.05	0.43 ± 0.02
77	7	1.50 ± 0.05	0.33 ± 0.02
77	7.5	1.65 ± 0.01	0.393 ± 0.005
77	8	1.64 ± 0.03	0.39 ± 0.01
Poly-HEMA/30% DMA			
29	7	3.3 ± 0.6	0.69 ± 0.06
29	7.5	2.06 ± 0.09	0.51 ± 0.02
29	8	2.5 ± 0.3	0.60 ± 0.04
38	7	3.65 ± 0.07	0.726 ± 0.005
38	7.5	3.2 ± 0.1	0.69 ± 0.01
38	8	1.8 ± 0.1	0.43 ± 0.05
44	7	3.2 ± 0.1	0.69 ± 0.01
44	7.5	2.94 ± 0.05	0.660 ± 0.005
44	8	2.0 ± 0.1	0.50 ± 0.03
77	7	2.32 ± 0.03	0.570 ± 0.005
77	7.5	2.15 ± 0.06	0.53 ± 0.01
77	8	1.65 ± 0.01	0.393 ± 0.005
Poly-HEMA/30% AA			
29	7	22.5 ± 0.3	0.9555 ± 0.0006
29	7.5	24. ± 1.	0.958 ± 0.005
29	8	23.1 ± 0.5	0.957 ± 0.001
38	7	16.98 ± 0.03	0.94111 ± 0.00009
38	7.5	17.7 ± 0.2	0.9434 ± 0.0005
38	8	18.1 ± 0.8	0.945 ± 0.002
44	7	14.5 ± 0.4	0.931 ± 0.001
44	7.5	15.6 ± 0.2	0.9357 ± 0.0007
44	8	14.8 ± 0.2	0.9326 ± 0.0008
77	7	8.4 ± 0.3	0.881 ± 0.004
77	7.5	8.7 ± 0.5	0.886 ± 0.006
77	8	8.8 ± 0.3	0.886 ± 0.005

% *V*: 100 times the total monomer volume fraction at synthesis; HEMA: 2-hydroxyethyl methacrylate; DMA: dimethylaminoethyl methacrylate; AA: acrylic acid.

Figure 3 presents experimental results for the sorption of lysozyme by the weakly acidic poly-HEMA/AA gels of varying % *V* at pH 7.5 in sodium phosphate buffer. Sorption by the 77% *V* gels is slower than that by the 29% *V* gels. The rate of sorption by the weakly acidic gels is much faster (1 h) than that by the neutral gels (days), probably because of the favorable electrostatic interactions between the protein and the polymer gel. Between pH 7 and 8, the net protein is positively charged, and the gel is negatively charged. The net protein charge decreases 0.7 units as the pH is changed from 7 to 8.

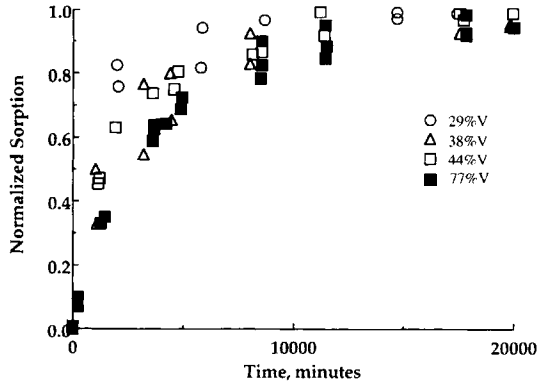

Figure 1 Effect of %*V* on sorption of lysozyme by poly-HEMA hydrogels (0.5%*C*) in 0.1*M* ionic strength sodium phosphate buffer at pH 7.5. The temperature was set at 36.4°C, and the solution contained 0.001*M* sodium azide as an antibacterial. Open circles denote the data for 29%*V*; open triangles, the data for 38%*V*; open squares, the data for 44%*V*; and filled squares, the data for 77%*V*. The uptake of lysozyme was most rapid for the 29%*V* gel.

Figure 4 presents experimental results for the effect of pH on the sorption of lysozyme by the weakly acidic poly-HEMA/AA gels synthesized at 77%*V*. It is difficult to discern the effect of pH; however, it appears that the rate of sorption may decrease slightly between pH 7 and 8.

We could not measure any sorption of lysozyme by the HEMA/DMA gels. Apparently, the electrostatic repulsion between the positively charged solute and the positively charged gel prevents any measurable sorption of the protein by the gel.

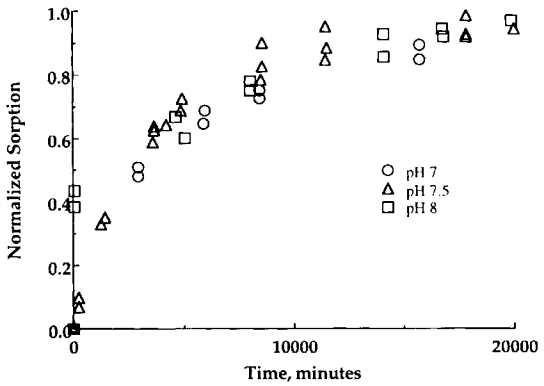

Figure 2 Effect of pH on sorption of lysozyme by poly-HEMA hydrogels (0.5%*C*, 77%*V*) in 0.1*M* ionic strength sodium phosphate buffer. The temperature was set at 36.4°C, and the solution contained 0.001*M* sodium azide as an antibacterial. Open circles denote data at pH 7; open triangles, the data at pH 7.5; and open squares, the data at pH 8. There is little effect of pH on uptake.

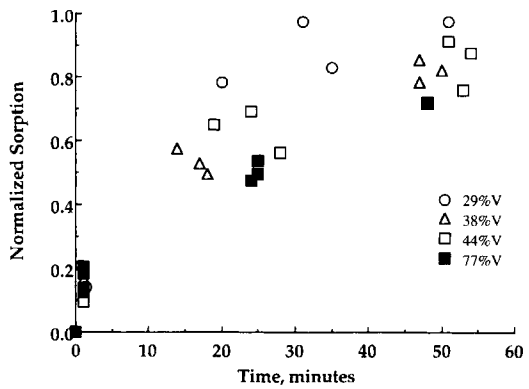

Figure 3 Effect of %*V* on sorption of lysozyme by poly-HEMA/AA hydrogels (0.5%*C*, 30% AA) in 0.1*M* ionic strength sodium phosphate buffer at pH 7.5. The temperature was set at 36.4°C, and the solution contained 0.001*M* sodium azide as an antibacterial. Open circles denote the data for 29%*V*; open triangles, the data for 38%*V*; open squares, the data for 44%*V*, and filled squares, the data for 77%*V*. The sorption of lysozyme was much more rapid than for the neutral poly-HEMA gels (data in Fig. 1). The uptake of lysozyme was most rapid for the 29%*V* gel, as in Figure 1.

DISCUSSION

In the literature, sorption of lysozyme by contact lens materials is usually reported as a surface coverage, but lysozyme may also be able to penetrate the matrix and adsorb onto the polymer strands inside the matrix. The mode of sorption (surface adsorption, partitioning, or both) has important clin-

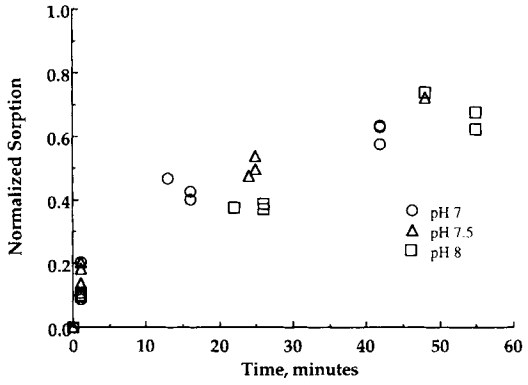

Figure 4 Effect of pH on sorption of lysozyme by poly-HEMA/AA hydrogels (0.5%*C*, 30% AA, 77%*V*) in 0.1*M* ionic strength sodium phosphate buffer. The temperature was set at 36.4°C, and the solution contained 0.001*M* sodium azide as an antibacterial. Open circles denote data at pH 7; open triangles, the data at pH 7.5; and open squares, the data at pH 8. There is little effect of pH on uptake.

ical ramifications for cleansing regimens of contact lenses.[7] For example, if proteins diffuse into the gel, the cleansing agent must also be able to penetrate the matrix, and the treatment time will be greater than that for surface cleansing alone. Lysozyme is the smallest abundant protein in tears and also the most prevalent protein found in deposits on clinically worn lenses.[3,20,25] Lysozyme is also one of the smallest of all the proteins; the proteases used in cleaning formulations, such as papain, are slightly larger.[41]

Based on analysis of our data and those existing in the literature, it appears that the primary mode of sorption of lysozyme by poly-HEMA copolymer gels is by adsorption, which may or may not be confined to the surface of the gel. For our experimental data, we calculated partition coefficients (defined as the ratio of protein concentration in the gel to that in solution) between 80 and 400, as all but a few percent of the initial protein is sorbed by the gels. Using methods outlined in Ref. 42, we calculated partition coefficients for lysozyme in the HEMA/AA gels between 2 and 8, neglecting size-exclusion effects. In these calculations, we used the cell model for polyelectrolyte solutions to calculate the partitioning due only to attractive electrostatic effects. Because the effect of size exclusion is to reduce the partition coefficient and because the experimental partition coefficients are orders of magnitude greater than the calculated values, we believe sorption of proteins is a combination of partitioning and adsorption.

We estimated the time necessary for 90% of the protein to be taken up by the gels by diffusion of the protein into the gel. We assumed the diffusivity of lysozyme to be on the order of that in pure water, $11.8 \cdot 10^{-7}$ cm^2/s.[41] Using results published by Crank for diffusion into a disk, we estimated that it would take 24 h for 90% of the protein to be taken up by diffusion.[43] If, however, the diffusivity is on the order of $6 \cdot 10^{-8}$ cm^2/s, as reported for lysozyme diffusing into PVP/MMA (hydration: 0.7) by Cassiani-Ingoni et al., it would take on the order of 20 days for 90% of the lysozyme to be taken up.[44]

Experimentally, the time scale for sorption of 90% of the protein is on the order of 1 h for the HEMA/AA gels, much more rapid than the shortest estimate of 24 h. The experimental time scale for sorption of 90% of the lysozyme by the poly-HEMA gels ranges from about 4 to 9 days, which might indicate that the proteins diffuse into the matrix. The extent to which penetration of the protein into the gel contributes to the amount of protein sorbed is the subject of some debate in the literature. It was first generally accepted that all protein deposition occurred at the surface of the gel. For example, Refojo and Holly reported that their staining studies of hydrogels coated with lysozyme, albumin, and gammaglobulin gave no evidence of penetration of lysozyme into solution-polymerized HEMA.[12] However, studies by Royce et al. (poly-HEMA/MMA), Gachon et al. (poly-VP/MMA), Cassiani-Ingoni et al. (poly-VP/MMA), and Refojo and Leong (poly-HEMA) indicate that lysozyme is able to diffuse into a hydrogel contact lens.[21,22,28,44] Refojo and Leong reported that lysozyme penetrated a solution-polymerized poly-HEMA hydrogel (hydration: 0.41) to a depth of 0.25 mm after 9 days, a time-frame consistent with our data for the neutral poly-HEMA gels.[21]

Expressing our data in terms of surface coverages, we found that for a given gel chemistry the surface coverage was virtually independent of $\%V$ and pH for the neutral poly-HEMA gels and slightly dependent on these parameters for the acidic poly-HEMA/AA gels, within the accuracy of our experimental method. To calculate the surface coverage, we calculated the surface area of a cylinder of diameter and height measured experimentally with calipers. The mass of protein on the gel was taken to be the difference between the initial mass of protein in the solution and the mass of protein in solution at equilibrium (when the protein concentration in the solution no longer changed). Calculated coverages were on the order of 600–750 μg/cm^2, much greater than that required to form a monolayer. Taking the diameter of lysozyme to be 30 Å, we calculated that a monolayer would have a surface coverage of approximately 0.270 μg/cm^2. Our coverages are in general agreement with the results of Minarik and Rapp and Mirejovsky et al., who placed commercial lenses in contact with an artificial tear solution.[5,6] Mirejovsky et al. reported that they could extract on the order of 0.5 mg of lysozyme per high water content, ionic lens (DuraSoft 3, Vistamarc, and Acuvue lenses) after 1 day in an artificial tear solution containing 1.9 mg/mL hen-egg-white lysozyme. Minarik and Rapp reported that they extracted 0.3 mg of lysozyme from high water content, ionic lenses after 24 h of incubation in an artificial tear solution containing 2 mg/mL lysozyme. Kita et al. also reported a surface coverage for lysozyme on poly-HEMA; they reported a value of 0.23 μg/cm^2 but did not cite the conditions of their study.[29]

There are several possible explanations for the high surface coverage observed experimentally. Ly-

sozyme is a self-associating protein, and, therefore, multilayer adsorption is plausible. Lysozyme may also penetrate the network and adsorb onto the polymer strands. Thus, the surface area accessible to the protein might be much greater than that calculated. Most of the surface of the gel disks is in contact with the glass plates of the mold during polymerization. If the surface has a morphology different from that in the interior of the gel, the protein might also be more able to diffuse into the surface layers of the matrix.

It is likely that the relatively rapid adsorption of lysozyme onto the acidic poly-HEMA/AA gels is a result of the strong electrostatic attraction between the charged polymer and the protein and the high water content of the gel. Between pH 7 and 8, acrylic acid should be fully charged, as its nominal pK is 4.3. In this pH range, lysozyme has a net charge on the order of +7.[26] Gachon et al. reported similar kinetics for lysozyme adsorbing onto poly-VP/MMA.[28] They studied the adsorption of less than a monolayer of lysozyme and found that equilibrium was attained in 1 h. Gachon et al. also observed that the affinity of lysozyme for the poly-VP/MMA lenses increases as the layer of protein on the surface increases. The adsorption onto poly-HEMA/AA may be further enhanced by the apparent nonelectrostatic affinity between lysozyme and poly-HEMA, as shown by our experiments with poly-HEMA. Although the uptake by our gels is more rapid than our estimates for diffusion into the matrix, it is possible that the protein may be penetrating the network and adsorbing onto the polymer strands. Refojo and Leong reported that lysozyme was able to penetrate on the order of 1–2 mm into poly(glyceryl methacrylate) (GMA) hydrogels in 1 h.[21] The poly-GMA gels had a water content of 84.8%; the poly-HEMA/AA gels have a water content greater than 88% at all conditions studied.

The much slower uptake of lysozyme by neutral poly-HEMA probably follows from a combination of penetration and adsorption coupled with slow denaturation. Mannucci et al. reported that lysozyme does not denature immediately upon adsorption onto commercial lenses.[45] After adsorption, proteins begin to denature slowly, as observed by Castillo et al., and the denaturated layer may serve as a site for additional deposition.[46,47]

Several authors have reported that binding of a protein to a polymer material is stronger as the pH approaches the isoelectric pH of the protein.[28,48,49] For example, Gachon et al. reported that the apparent affinity of lysozyme for poly-VP/MMA increased slightly from pH 7.2 to pH 8.[28] Examining our experimental data, we found virtually no effect of pH on surface coverages of lysozyme onto neutral poly-HEMA gels. For poly-HEMA/AA, however, approximately 15% more lysozyme was bound at pH 8 than at pH 7. However, our data show little effect of pH on sorption kinetics.

The effect of $\%V$ on the final surface coverage of lysozyme on neutral poly-HEMA is virtually negligible; this is not surprising given that the swelling ratios are not much different for the gels made at different $\%V$. The effect of $\%V$ on the swelling ratio for poly-HEMA/AA is strong, even in a 0.1M ionic-strength buffer. For example, the swelling ratio of a 29%V poly-HEMA/AA gel is 22.4 and that for a 77%V gel is 8.8 (about 2.5 times lower). However, the amount of lysozyme adsorbed per cm^2 on the 77%V gel is only about 7% higher than that on the 29%V gel. This slightly higher adsorption corresponds rather well to the difference in hydration; the hydration of the 29%V gel is 8% higher than that of the 77%V gel.

CONCLUSIONS

We studied the sorption of lysozyme by neutral poly-HEMA, basic poly-HEMA/DMA, and acidic poly-HEMA/AA gels as a function of pH and the fraction of monomer at synthesis, $\%V$. The water contents of the gels ranged from 33% to nearly 96%. We observed the following effects:

- Lysozyme did not adsorb or partition into the poly-HEMA/DMA gels between pH 7.2 and pH 8, even though these gels have a higher water content than that of the poly-HEMA gels. We believe that electrostatic repulsion between the positively charged protein and the positively charged groups on the gel made sorption impossible.
- The kinetics of sorption for the acidic poly-HEMA/AA gels are much faster than those for the neutral poly-HEMA gels; 1 h was required for 90% of the protein to be sorbed by the acidic gels; about 15 days was required for the neutral gels.
- The fractional approach to equilibrium was most rapid for gels synthesized at low $\%V$, which were also the gels with higher water contents.
- In the range $7 \leq \text{pH} \leq 8$, there is little effect of pH on the rate of sorption by the poly-HEMA

copolymer gels, but the equilibrium affinity of poly-HEMA/AA for the protein is slightly higher at pH 8 than at pH 7.

The mechanism of sorption of lysozyme by poly-HEMA copolymer hydrogels appears to be a combination of surface adsorption and adsorption onto the polymer strands in the interior of the gel. We base this conclusion on studies of lysozyme diffusion into hydrogels by other authors as well as on our experimental and theoretical evidence.

In summary, we found that the effects of pH and $\%V$ on uptake kinetics are minor compared to the effect of incorporating an ionic comonomer into the gel. This finding may be important for developing lenses for extended wear. The contact lens is immersed in a tear fluid where the lysozyme level is maintained over time, rather than depleted as in our experiment. This will inevitably lead to more protein adsorption. As the presence of acidic groups in the gel greatly accelerates the uptake of lysozyme, it may be fruitful to investigate other means of modifying gel chemistry to obtain high water content. A step in this direction was taken by Wang, who synthesized gels containing 90% water from HEMA to which galactose had been covalently linked.[50]

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