

Hydrogel Lens Monomer Constituents Modulate Protein Sorption

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PURPOSE. To examine the effect of hydrogel lens monomer constituents on protein sorption.

METHODS. A series of hydroxyethylmethacrylate (HEMA)-based hydrogels with various amounts of methacrylic acid (MAA) or N-vinyl pyrrolidone (NVP) were synthesized. A radiolabel tracer technique was used to measure the amount of protein adsorbed on or penetrating into the hydrogels. Penetration of fluorescence-labeled proteins in the hydrogels was studied by laser scanning confocal microscopy. Single-protein solutions of human serum albumin (HSA) and hen egg lysozyme were studied.

RESULTS. Inclusion of the comonomers MAA or NVP in hydrogels resulted in an increase in water content and also had a strong impact on protein sorption. An increase in the amount of MAA in the poly(HEMA-co-MAA) hydrogels increased lysozyme adsorption and penetration but reduced HSA adsorption. However, the amount of protein adsorbed for both HSA and lysozyme increased with the amount of NVP in the poly(HEMA-co-NVP) hydrogels. In contrast to the marked effect of MAA on protein sorption, in particular, on lysozyme sorption, NVP had little influence on protein sorption. When a hydrogel contains both MAA and NVP, MAA has the dominant effect on protein sorption—in particular, on lysozyme sorption. Furthermore, a large difference was observed in the amount of lysozyme adsorbed on the hydrogels that had similar water contents but little variation in adsorption of HSA.

CONCLUSIONS. Negatively charged carboxyl groups of the MAA constituent may influence lysozyme sorption in two ways: by electrostatic attraction and by increasing the possibility for the small lysozyme molecule to penetrate the hydrogels. Interactions of the surface lactam groups of NVP with proteins may be attributable to the attraction of proteins to NVP. Water content is not a primary factor in determining protein adsorption. It appears that the monomer constituents, such as MAA or NVP, control protein adsorption. (*Invest Ophthalmol Vis Sci.* 2000;41:1687-1695)

In the contact lens industry, copolymerization is extensively used in the synthesis of hydrogel materials. Almost all hydrogel materials are copolymers. By polymerizing various combinations of monomers, the physical and chemical properties of a lens can be modified. By varying the ratios of the same two monomers, materials can be created that have various water contents, refractive indices, hardnesses, mechanical strengths, and oxygen permeabilities.

There are many different hydrogel contact lens materials available that contain 30% to 80% by weight of water and are based on lightly cross-linked (~0.5%–1.0%) combinations of

various monomers including, for example, 2-hydroxyethylmethacrylate (HEMA), N-vinyl pyrrolidone (NVP), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), methyl methacrylate (MMA), cyclohexyl methacrylate, and substituted acrylamides.¹ Of all, poly(HEMA) cross-linked with EGDMA is the basis of many types of soft contact lenses.¹

Water imbibition of hydrogels is a useful property of hydrogel lenses because the greater the water content of a lens, the greater the amount of dissolved oxygen in the lens and, in turn, the greater the amount of atmospheric oxygen that can reach the cornea.² Water imbibition can be defined as the ability of a contact lens to absorb water and swell. This property depends on the ratio of hydrophilic to hydrophobic functional groups in the polymer, as well as on the nature of these groups.²

High-water-content lens materials are commonly obtained by the copolymerization of moderately hydrophilic HEMA with highly hydrophilic, nonionic monomers such as NVP, or with highly hydrophilic, ionic monomers such as MAA.³ Because of its highly polar lactam moiety, NVP is probably the comonomer most often used to increase the ability of a polymer to take up water. The MAA monomer unit is also used to boost the water content in the hydrogel. The ionic functionality of the MAA unit in a buffered saline environment dramatically increases the water content of the resultant hydrogel.⁴

However, hydrogels used in soft contact lenses and containing MAA adsorb considerably more protein than other

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gels⁵⁻⁸ and, moreover, selectively adsorb proteins such as lysozyme. Furthermore, vifilcon A (a terpolymer of HEMA, MAA, and NVP) shows a higher affinity for HSA than hydrogels without NVP.⁹ Therefore, the question of how different comonomer constituents that are used to boost the water content of HEMA-based hydrogels affect the ability of such hydrogels to absorb different proteins was investigated in this study. The results should provide a better understanding of protein deposit formation and assist in development of deposit-minimized hydrogel contact lens materials.

MATERIALS AND METHODS

Materials

HEMA, NVP, and MAA were purchased from Sigma (Sydney, Australia). EGDMA was purchased from Aldrich (Sydney, Australia), and the UV initiator Darocur 1173 (2-hydroxy-2-methyl-propiofenone) was the product of Ciba-Geigy (Basel, Switzerland). Flat molds made of polypropylene (0.5 mm thick and 2 cm in diameter) were used for casting hydrogel discs. HSA and hen egg lysozyme were obtained from Sigma.

Chemical Purification

HEMA was passed down a column of inhibitor-remover packing material (for removing hydroquinone and hydroquinone methyl ether, obtained from Aldrich) and then fractionally distilled under vacuum. After the first 10% was discarded, the fraction boiling at 132°C to 133°C/20 mm Hg was collected. Likewise, MAA was passed through an inhibitor-remover column, then fractionally distilled at 175°C/20 mm Hg. NVP was also fractionally distilled at 62°C/2 mm Hg.

Polymerization of Poly(HEMA)-Based Hydrogels

HEMA with a fixed weight percentage of EGDMA as a cross-linker was polymerized with Darocur 1173 as a UV initiator. Varying amounts of MAA and NVP were added as comonomers to increase the final water content of the swollen hydrogel. The standard formulations for the 10 hydrogels used in this study are shown in Table 1.

Monomers were first mixed according to the ratios in Table 1, before the UV initiator Darocur 1173 was finally

added. The mixtures were gently stirred for approximately 20 minutes. Nitrogen gas was purged through the mixture for 1 minute to remove any dissolved oxygen. The formulation was then cast into flat molds that were clamped shut using a stainless steel clamp assembly designed to hold 10 flat molds at a time. The clamp assembly was sealed into a plastic bag under nitrogen, and the mixtures were cured for 3 hours at 365 nm using lamps of 1.22 mW/cm².

After polymerization was complete, the hydrogel discs were carefully removed from the molds after soaking in MilliQ water (Millipore, Bedford, MA) overnight. The hydrogels were extracted by soaking in ethanol at 37°C overnight, which was exchanged for a 20% ethanol and 80% water mixture for soaking at 37°C overnight and then for MilliQ water to remove any unpolymerized monomers. After extraction, the hydrogel discs were vacuum dried in an oven (37°C) overnight and cooled to room temperature in a desiccator. The dry weight of each hydrogel was measured.

The wet weight and dimensions of the hydrogel discs were determined after soaking hydrogel samples in MilliQ water for 2 weeks. The water on the surface of the hydrogel discs was carefully removed by blotting with lens-cleaning tissue (Olympus, Tokyo, Japan) just before measurements. The equilibrium water content in MilliQ water was determined by the ratio of the weight of water in the hydrogel to the total weight of the hydrogel at hydration equilibrium. The swollen diameters and thicknesses were also measured.

Protein Adsorption and Desorption

The procedures for radioiodination of protein and purification of radioiodinated protein have been described previously.⁹ Adsorption experiments were performed by soaking a hydrogel disc in the single protein solution of HSA or lysozyme for 24 hours at 34°C, the typical surface temperature of a human eye. The concentration of protein solution in phosphate-buffered saline (PBS, pH 7.4) for adsorption was 1 mg/ml. After adsorption, the hydrogel discs were placed gently into PBS for 10 seconds to remove excess solution adhering to the hydrogel discs followed by further static rinsing (without agitation) in 40 ml PBS for 24 hours at room temperature. The radioactivity of the protein remaining on or in the hydrogels was measured. A gamma counter (Cobra II Auto-Gamma Counter; Packard Instruments, Meriden, CT) was used to read the radioactivity of the samples. Samples were measured in triplets.

Laser Scanning Confocal Microscopy

Fluorescein isothiocyanate (FITC) labeling of protein was described previously.^{9,10} The adsorption of FITC-labeled HSA (FITC-HSA) or FITC-labeled lysozyme (FITC-lys) to hydrogel discs was performed at 34°C in polypropylene tubes. A small flat sector cut from the hydrogel discs of each type was soaked in 500 μ l FITC-labeled protein solution for 24 hours. The concentration of the FITC-labeled protein solution for adsorption was 1 mg/ml. After adsorption, each sample was initially rinsed in PBS for 10 seconds followed by 24 hours of static rinsing at room temperature.

The FITC-labeled, protein-absorbed hydrogel sections were mounted on microscopy slides by using an excess of PBS solution containing 50% glycerol. Coverslips were carefully placed on the hydrogel sections without entrapping any air bubbles, and the section was sealed with nail polish. The

TABLE 1. Formulation in Parts by Weight for Making Poly(HEMA)-Based Hydrogels

Hydrogel	HEMA	MAA	NVP
Poly(HEMA)	99.5*	—	—
Poly(HEMA, 1% MAA)	98.5	1	—
Poly(HEMA, 3% MAA)	96.5	3	—
Poly(HEMA, 5% MAA)	94.5	5	—
Poly(HEMA, 10% NVP)	89.5	—	10
Poly(HEMA, 15% NVP)	84.5	—	15
Poly(HEMA, 25% NVP)	74.5	—	25
Poly(HEMA, 0.5% MAA, 5% NVP)	94	0.5	5
Poly(HEMA, 1.5% MAA, 8% NVP)	90	1.5	8
Poly(HEMA, 2.5% MAA, 12% NVP)	85	2.5	12

All the hydrogels were prepared with 0.5% EGDMA. 0.5% UV initiator Darocur 1173 was added to the total monomers and cross-linking mixtures before the polymerization.

* *Parts by weight* are a percentage of total weight of monomer mix, excluding Darocur 1173 initiator.

TABLE 2. Water Content and Swelling of the Poly(HEMA)-Based Hydrogels Equilibrated in PBS (pH 7.4)

Hydrogel	Water Content	Diameter (Swelling)	Thickness (Swelling)
Poly(HEMA)	30.2 ± 1.6	32.4 ± 1.6	44.4 ± 27
Poly(HEMA, 1% MAA)	37.8 ± 2.9	32.9 ± 2.0	50.0 ± 25
Poly(HEMA, 3% MAA)	59.7 ± 1.8	39.8 ± 4.0	54.5 ± 26
Poly(HEMA, 5% MAA)	62.8 ± 2.0	41.5 ± 3.7	61.5 ± 27
Poly(HEMA, 10% NVP)	34.5 ± 1.6	32.9 ± 1.7	44.4 ± 27
Poly(HEMA, 15% NVP)	38.3 ± 2.0	32.9 ± 1.6	50.0 ± 25
Poly(HEMA, 25% NVP)	43.9 ± 3.1	33.8 ± 1.6	58.3 ± 26
Poly(HEMA, 0.5% MAA, 5% NVP)	37.2 ± 2.1	32.9 ± 1.6	37.5 ± 26
Poly(HEMA, 1.5% MAA, 8% NVP)	50.2 ± 3.5	35.5 ± 2.4	44.4 ± 27
Poly(HEMA, 2.5% MAA, 12% NVP)	57.1 ± 2.5	38.3 ± 2.3	50.0 ± 25

All values are the average percentage ± SD of triplicate samples.

hydrogel sections were examined with a laser scanning confocal microscope (LSM-GB 200, with a piezoelectric z -stage with a resolution of $\sim 0.3 \mu\text{m}$; Olympus) using a $\times 40$ objective (numeric aperture, 0.95; S-Plan Apo; Olympus). After a satisfactory observation zone was located in the hydrogel, fast scanning in the x - z plane was performed to locate the hydrogel surface. X - y scans were then made from the hydrogel surface down to a $100\text{-}\mu\text{m}$ depth in the z direction. Each image (1024×768 pixels) representing $160 \times 80 \mu\text{m}$ was saved to disc. The average intensity of each image was measured on an 8-bit scale using application software for the laser scanning confocal microscope (Olympus). Images were captured every $0.1 \mu\text{m}$ from the surface to a depth of $100 \mu\text{m}$. These average intensities, after correction for the background intensity and sharpness of the confocal images, were plotted as a function of depth to obtain the profile of protein penetration in hydrogels. Non-FITC-labeled protein adsorbed in a hydrogel was used for the background intensity.

RESULTS AND DISCUSSION

Effect of MAA

Methacrylic acid (MAA) is one of the most often used, highly hydrophilic ionic monomers in the manufacture of soft contact lenses for increasing hydrogel water content. For example, of the contact lenses previously studied, etafilcon A and vifilcon A, contain MAA, whereas tefilcon lenses contain minute traces of MAA.^{8,9} MAA is also a contaminant in most HEMA preparations and, similar to EGDMA, is almost always present in small amounts, even in a monomer purified by vacuum distillation.¹¹ In neutral or basic environments, MAA can dissociate into methacrylate and hydrogen ions.³ Thus, in isotonic buffered saline solutions, the MAA residues in the poly(HEMA-co-MAA) hydrogels are negatively charged, as was determined in etafilcon A lenses, which consist mainly of HEMA and MAA.⁹

The presence of negatively charged groups had a great impact on hydrogel swelling as well as on protein sorption. The effect of MAA on the swelling of poly(HEMA)-based hydrogels is shown in Table 2, which shows that MAA dramatically increased the water content and swollen dimensions of the resultant hydrogels. Furthermore, MAA had a great impact on protein adsorption and penetration, as shown in Figures 1 through 4.

When protein is assumed to adsorb on the hydrogel surface, Figure 1 shows that HSA was adsorbed less by hydrogels with more MAA content, whereas the amount of lysozyme

adsorption increased with increasing MAA content. The amount of HSA adsorbed was at submonolayer levels (i.e., $<300\text{--}345 \text{ ng/cm}^2$),⁹ whereas lysozyme adsorbed to a far greater extent than its calculated maximum amount for monolayer coverage ($207\text{--}310 \text{ ng/cm}^2$)⁸ for each copolymer of HEMA and MAA.

When bulk absorption of protein into the poly(HEMA-co-MAA) hydrogel matrices was assumed, the apparent concentration of HSA in the water phase of the poly(HEMA-co-MAA)

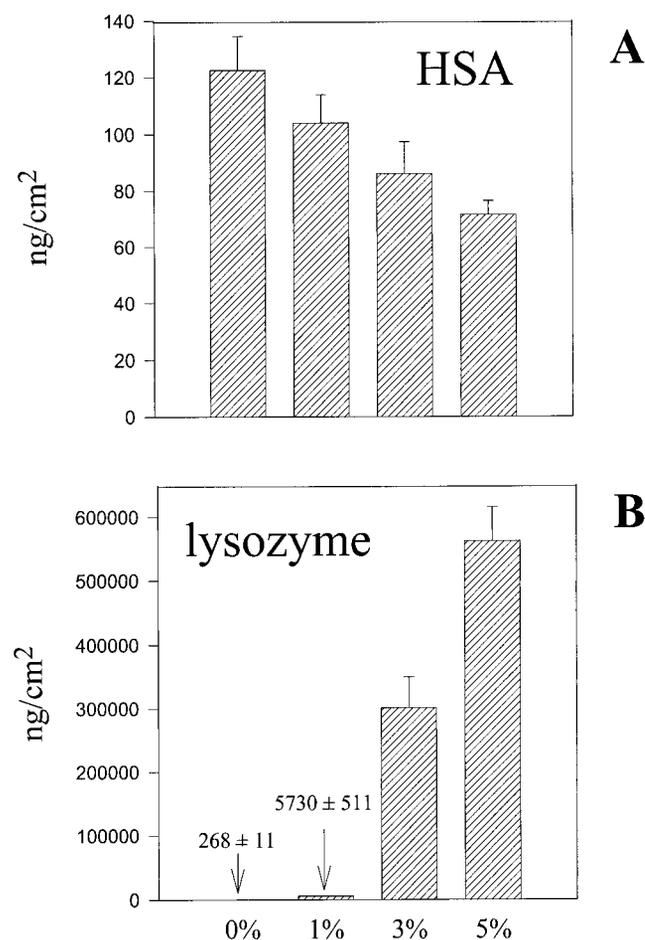


FIGURE 1. Apparent surface concentration (in nanograms per square centimeter) of protein HSA (A) and lysozyme (B) remaining on poly(HEMA-co-MAA) hydrogels as a function of the percentage of MAA in hydrogels. Error bars: SD ($n = 3$).

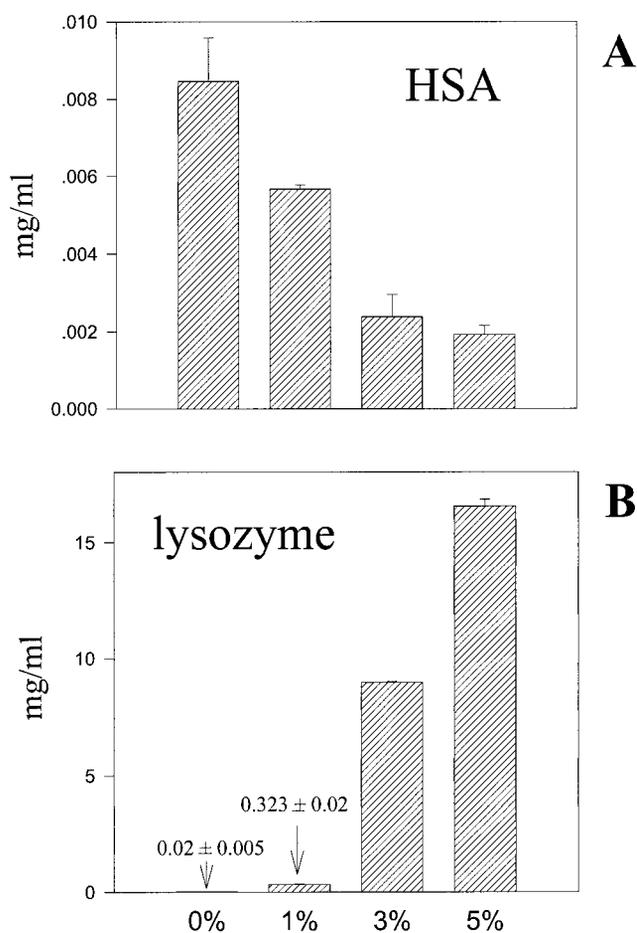


FIGURE 2. Apparent concentration (in milligrams per milliliter) of protein HSA (**A**) and lysozyme (**B**) remaining in the water phase of poly(HEMA-co-MAA) hydrogels as a function of the percentage of MAA in hydrogels. Error bars: SD ($n = 3$).

hydrogels was much smaller than the initial concentration of protein solution (1 mg/ml; Figure 2). However, the apparent concentration of lysozyme in the water phase of the poly(HEMA-co-MAA) hydrogels containing 3% and 5% MAA was much higher than 1 mg/ml. Even with 1% MAA content, this copolymer matrix contained considerable lysozyme, although its apparent concentration in the water phase of the hydrogel was slightly less than 1 mg/ml. These findings indicate that lysozyme has greater affinity for the hydrogel matrix than for the aqueous solution. In fact, penetration of lysozyme into poly(HEMA-co-MAA) hydrogels with 3% or 5% MAA present is theoretically possible, because the protein molecular dimensions of lysozyme ($30 \times 30 \times 45 \text{ \AA}^3$) or HSA (equilateral triangle with sides of 80 Å and an average depth of 30 Å¹³) are less than the effective pore size of poly(HEMA-co-MAA) hydrogels (Table 3). This effective pore size was estimated from the water content of the hydrogels based on the two models developed in our previous study,¹⁰ in which the percentage of MAA was taken into consideration. However, no penetration of HSA into any poly(HEMA-co-MAA) hydrogels is predicted from the models. These observations indicate that the concurrent high degree of concentrated lysozyme in the hydrogels with the presence of high amounts of MAA is most likely due to electrostatic association of lysozyme with hydrogels as well as

the increased porosity resulting from increase in charges. Indeed, penetration by lysozyme but not by HSA into poly(HEMA-co-MAA) hydrogel networks was confirmed with the use of laser scanning confocal microscopy as shown in Figures 3 and 4, respectively. The degree of penetration of lysozyme into hydrogels increases with the increase in the MAA content in hydrogels. This again indicates the roles of charge, present both in hydrogels and proteins, in controlling protein penetration as well as in increasing the porosity of the hydrogels for penetration.

These results strongly support our previous observations^{8,10} that negatively charged groups in hydrogels as well as the net charge on protein have a great impact on the binding of positively charged lysozyme to hydrogels. The binding of lysozyme is a process of both surface adsorption and matrix penetration. This process is controlled by electrostatic attraction between hydrogels and proteins, as well as by increased porosity for penetration as a result of increasing the number of charged groups in hydrogels. The results also substantiate our previous finding¹⁰ that binding of HSA on negatively charged hydrogels is a surface adsorption process. Electrical charge in hydrogels and on proteins does not play a dominant role in HSA adsorption.

Effect of NVP

The advantage of the nonionic monomer NVP is enhanced water imbibition. The presence of the polar lactam group in the pyrrolidone moiety increases the hydrophilicity of the polymer. This increase occurs, because the carbonyl group provides two sites for hydrogen bonding of water. As well, an increasing content of NVP improves the stability of the hydrogels.¹⁴ NVP influences protein adsorption to a degree, although the mechanisms are not clear. For example, in a study of the adsorption of fibrinogen and albumin to polytetrafluoroethylene (PTFE) and polyNVP radiation grafted to PTFE (polyNVP-g-PTFE),¹⁵ fibrinogen adsorption was observed to be much lower on polyNVP-g-PTFE than on PTFE, whereas albumin adsorption to polyNVP-g-PTFE was much greater than to PTFE. An enhanced adsorption of mucin to poly(*N*-vinyl pyrrolidone) grafted silicone contact lenses in comparison with silicone contact lenses was also found.¹⁶ Furthermore, our previous work clearly shows that vifilcon A lenses that contain NVP adsorb more HSA than contact lenses that do not contain NVP—for example, etafilcon A (HEMA, MAA) and tefilcon (HEMA).^{8,9}

TABLE 3. Effective Pore Size of Poly(HEMA), Poly(HEMA-co-MAA) and Poly(HEMA-co-NVP) Hydrogel Networks

	Estimated Effective Pore Size	
	Model 1	Model 2
Poly(HEMA)	13.8	14.4
Poly(HEMA, 1% MAA)	17.3	17.4
Poly(HEMA, 3% MAA)	31.6	27.6
Poly(HEMA, 5% MAA)	34.7	29.3
Poly(HEMA, 10% NVP)	16.3	16.0
Poly(HEMA, 15% NVP)	18.6	18.3
Poly(HEMA, 25% NVP)	22.4	21.4

Estimated from the theoretical Models 1 and 2 developed previously.¹⁰ Data are expressed in Ångstroms.

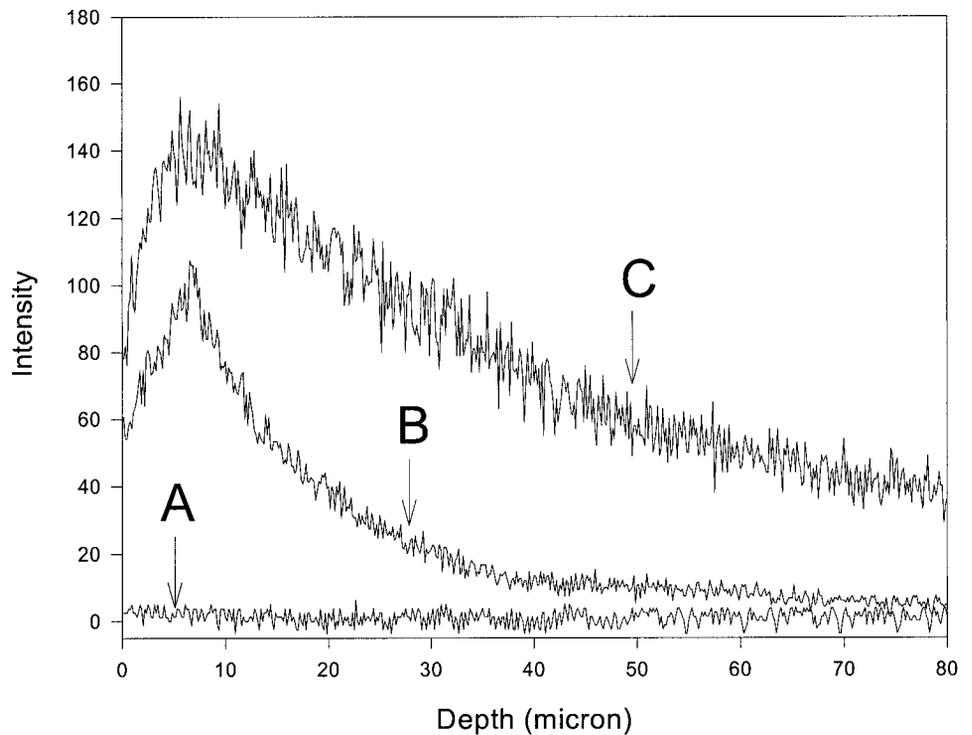


FIGURE 3. Profile of FITC-labeled lysozyme penetration into poly(HEMA-co-MAA) hydrogels containing various percentages of MAA: A, 1%; B, 3%; and C, 5%.

The effect of NVP on protein adsorption onto hydrogels was also observed in this study. Figure 5 shows that for both HSA- and lysozyme-adsorbing surface adsorption, the amount of protein adsorbed increases with the increase in the NVP content of the hydrogels. However, the influence of NVP seems to have a much smaller effect on HSA (Fig. 5A) than on lysozyme adsorption (Fig. 5B). The amount of HSA adsorbed increases only slightly when the NVP content is increased, whereas, lysozyme adsorption, as a result of increasing the NVP content, increases very rapidly. Figure 5 also shows that HSA adsorption on hydrogels does not achieve a monolayer coverage, whereas

lysozyme adsorption on poly(HEMA-co-NVP) hydrogels forms mono- to multilayers when the percentage of NVP increases from 10% to 25%.

It has been suggested in the literature¹⁷⁻¹⁹ and in our previous work¹¹ that protein molecules with strong internal coherence (hard proteins such as lysozyme) favor hydrophobic surfaces, but on hydrophilic surfaces, they adsorb only if they are electrostatically attracted. However, the protein molecules with low internal structural stability (soft proteins such as HSA), adsorb even under the seemingly unfavorable conditions of a hydrophilic, electrostatically repelling surface. The unique

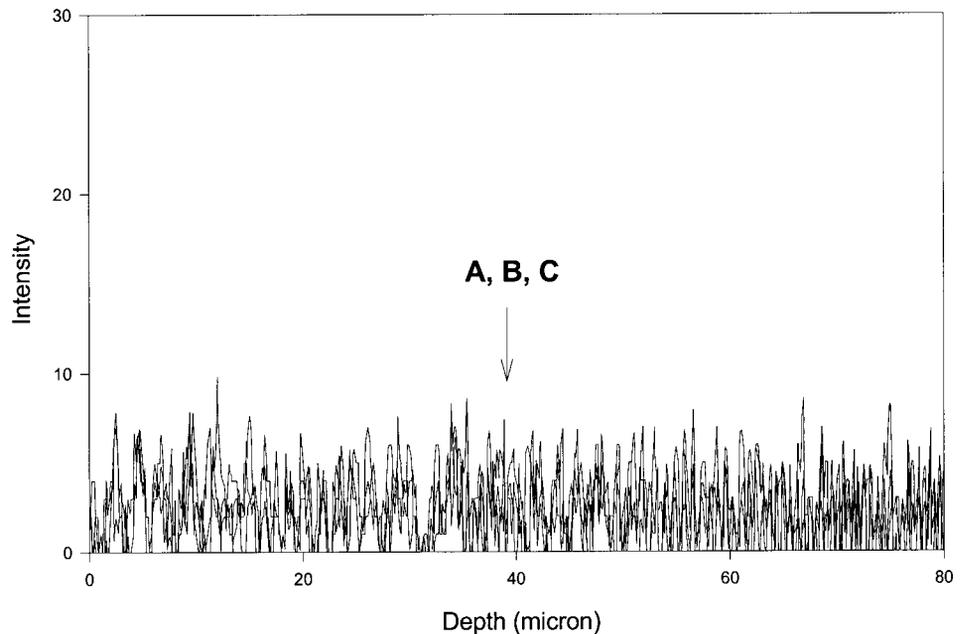


FIGURE 4. Profile of FITC-labeled HSA penetration into poly(HEMA-co-MAA) hydrogels having various percentages of MAA: A, 1%; B, 3%; and C, 5%.

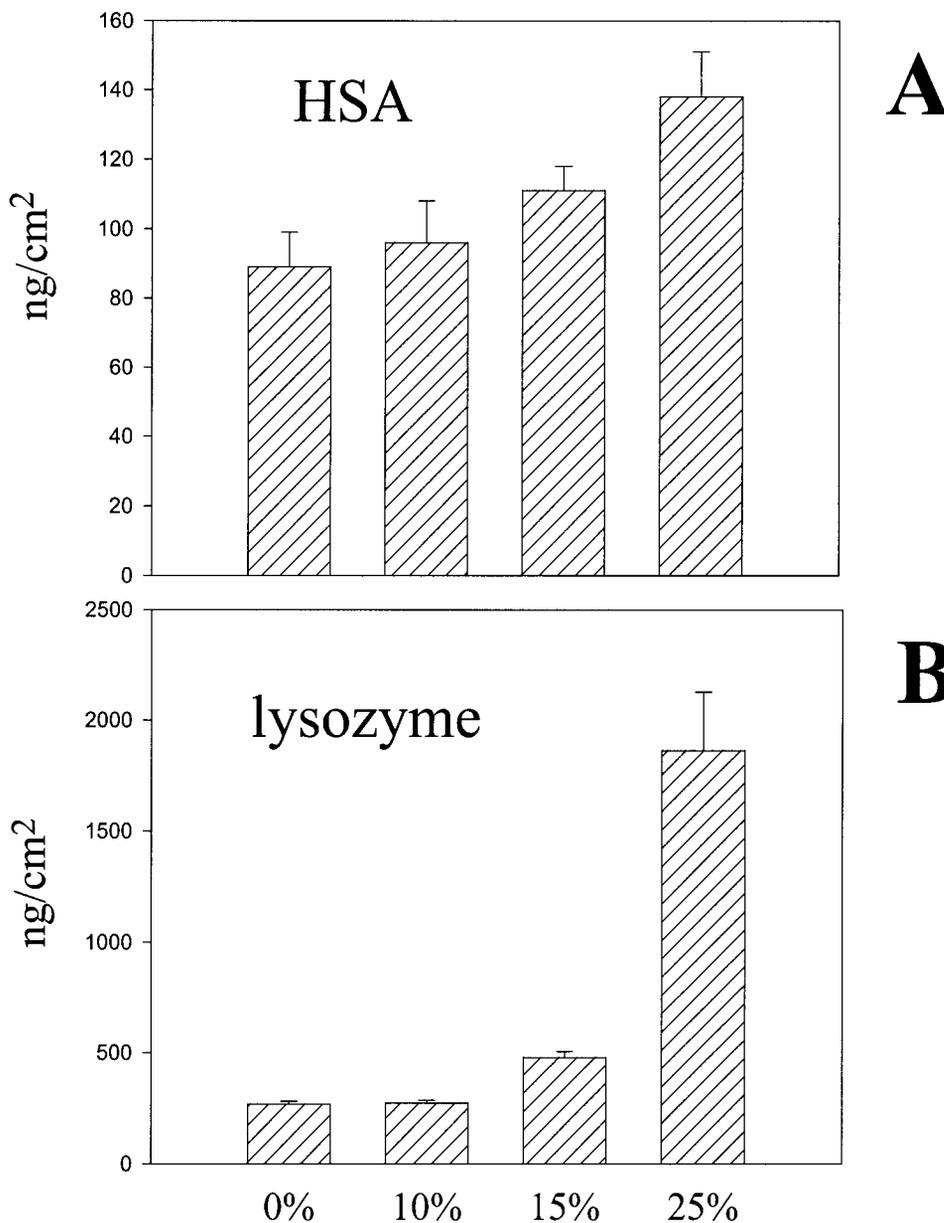


FIGURE 5. Apparent surface concentration (in nanograms per square centimeter) of protein HSA (**A**) and lysozyme (**B**) remaining on poly-(HEMA-co-NVP) hydrogels as a function of the percentage of NVP in hydrogels. Error bars: SD ($n = 3$).

bipolar property of the lactam moiety in the NVP component results in the poly(HEMA-co-NVP) hydrogels' being both partially negatively charged at the carbonyl group and partially positively charged at the nitrogen end closest to the relatively hydrophobic chain backbone. Thus, as a result of the electrostatic attraction between the negatively charged carbonyl group and the positively charged lysozyme, the adsorption of lysozyme increases as the NVP content increases. The same mechanism could be applied to the HSA adsorption, but based on the negatively charged HSA in association with the positive nitrogen present in NVP. However, this explanation is based on the assumption that the electrostatic interaction occurs between individual charge groups in NVP and protein molecules.

Alternatively, the increase in HSA or lysozyme adsorption with the increase in NVP content in hydrogels may be due to the attraction of protein to the surface lactam groups of NVP. In fact, a correlation between the presence of lactam groups

and adsorption of protein has been reported by several research groups,^{15,16,20} although the precise binding mechanism for this is unknown.

The plasticizing effect of water influences the polymer segments in the hydrogel and imparts some mobility that may result in fluctuating pore sizes. The theoretical models developed previously¹⁰ have been used to predict pore size from the water content of poly(HEMA-co-NVP) hydrogels (Table 3). The estimate of pore size indicates that the porosity is not quite large enough for the hydrogel containing even 25% NVP to allow penetration of lysozyme. However, the results from laser scanning confocal microscopy shown in Figures 6 and 7 indicate some penetration of lysozyme, but not of HSA, into the bulk hydrogel containing 25% NVP, albeit in rather small amounts in comparison with lysozyme penetration into poly-(HEMA-co-MAA) hydrogels (Fig. 3). This is also supported in that the lysozyme adsorption level for the hydrogel containing 25% NVP had approximately six to nine times the theoretical

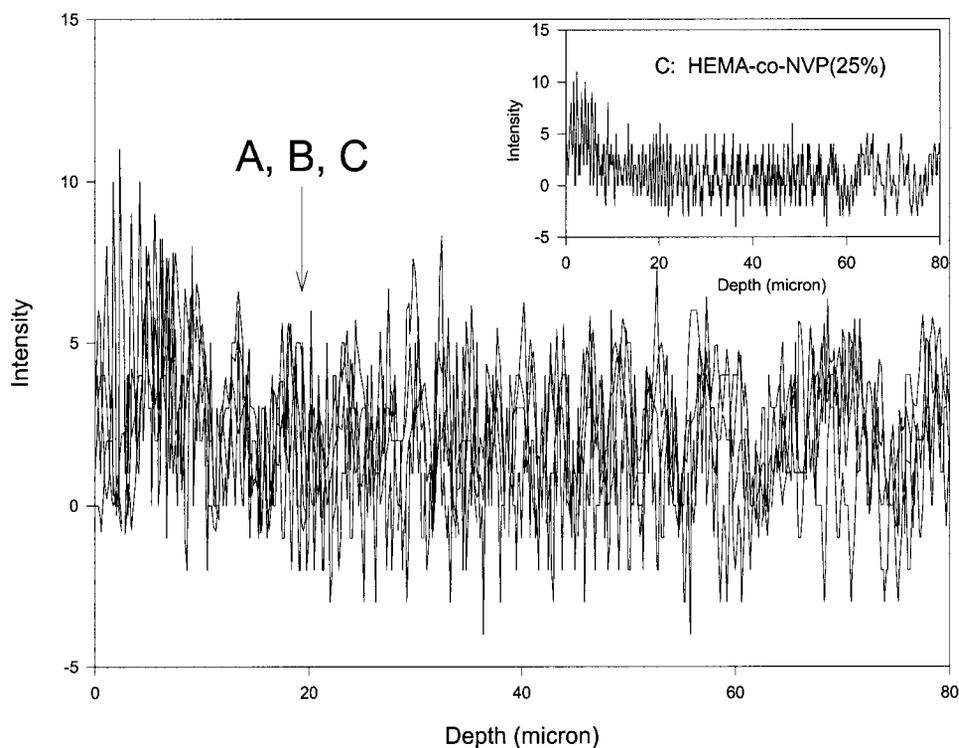


FIGURE 6. Profile of FITC-labeled lysozyme penetration into poly(HEMA-co-NVP) hydrogels having various percentages of NVP: A, 10%; B, 15%; and C, 25%. *Inset:* lysozyme penetration into a poly(HEMA-co-NVP) hydrogel with 25% NVP, C.

monolayer coverage (Fig. 5B). That there may be some variation in porosity and that there is a relative closeness of the pore size at 25% NVP content to the lysozyme dimensions may explain this penetration. It should be pointed out that the theoretical models of the hydrogel network assume that the hydrogel is a simple copolymer of HEMA and NVP. In reality, however, the reactivity ratios for HEMA and NVP are very different (relative reactivities of 32.7 and 0.00674 for HEMA and NVP, respectively²¹) and will probably result in long chains of PHEMA and PNVP. This will make prediction of

actual porosities from existing theoretical models more difficult.

Effect of Water Content or Comonomer Constituents

As discussed previously there are many factors that may control protein adsorption onto poly(HEMA)-based hydrogels. These factors include the effect of charges in hydrogels introduced by MAA or NVP, in particular MAA; the effect of lactam groups introduced by NVP; and the effect of water content.

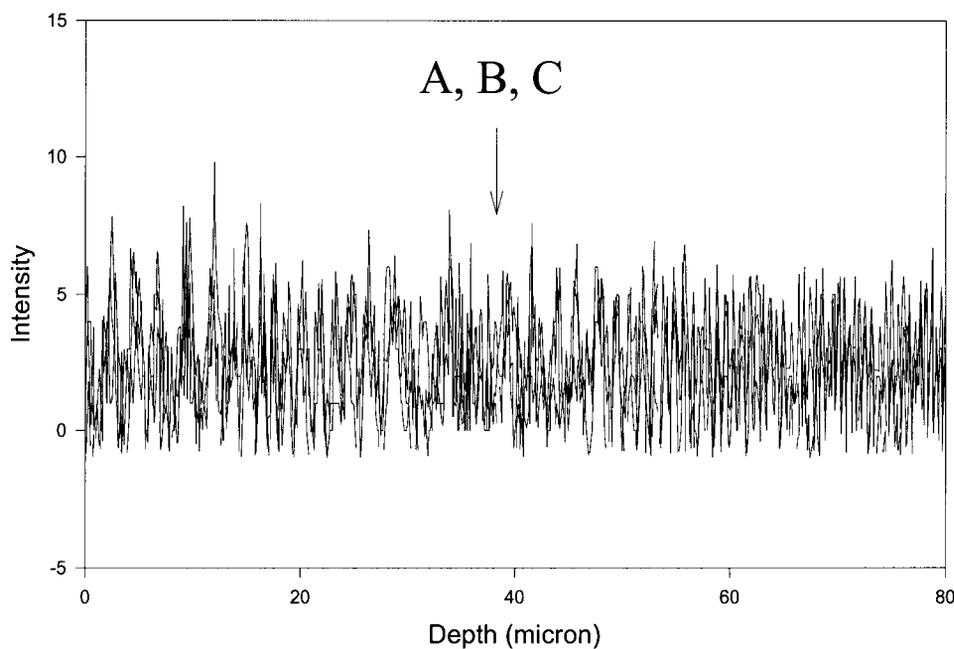


FIGURE 7. Profile of FITC-labeled HSA penetration into poly(HEMA-co-NVP) hydrogels having various percentages of NVP: A, 10%; B, 15%; and C, 25%.

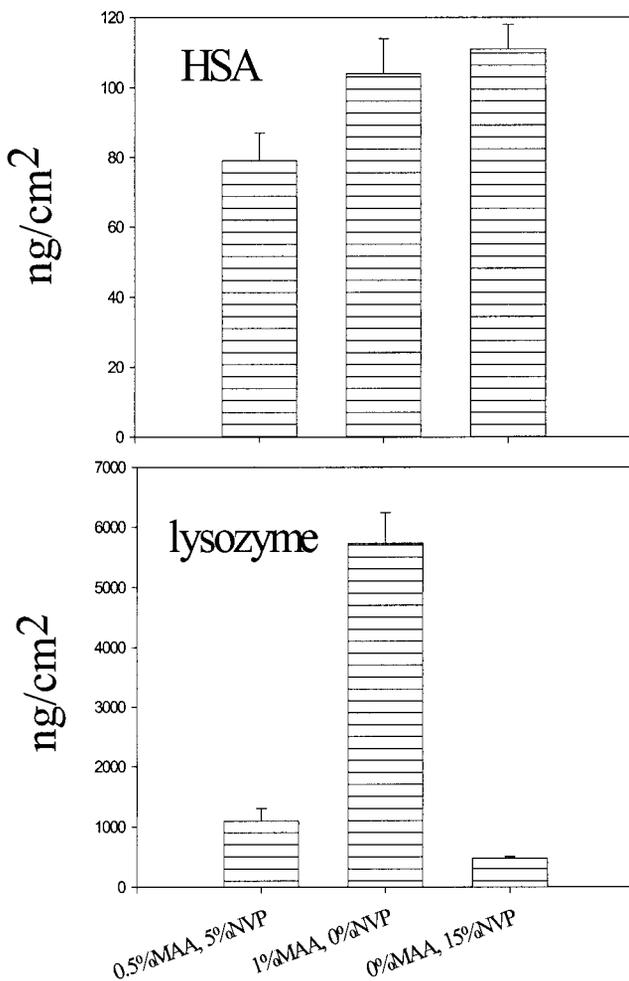


FIGURE 8. Apparent surface concentration (in nanograms per square centimeter) of protein (HSA, lysozyme) remaining on poly(HEMA)-based hydrogels with various percentage combinations of MAA and NVP: poly(HEMA, 0.5% MAA, 5% NVP), poly(HEMA, 1% MAA, 0% NVP), and poly(HEMA, 0% MAA, 15% NVP) but with a similar water content of 37.2%, 37.8%, and 38.3%, respectively.

However, because the water content is modulated by the charges as well as by the polar lactam groups, previous work was not able to separate the effects of the comonomer constituent and the water content in protein adsorption. Thus, to determine whether the effect of comonomers on protein adsorption is independent of the effect of water content, hydrogels with similar water contents but different comonomers or combinations of comonomers were studied.

A similar water content (37.8%, 38.3%, and 37.2%) had been produced, respectively, by copolymerization of HEMA with either 1% MAA or 15% NVP or terpolymerization of HEMA with 0.5% MAA and 5% NVP (Table 2). If a strong influence of water content on protein adsorption is assumed, then a similar level of protein adsorption to the hydrogels with a similar water content is expected. However, Figure 8 does not show such a phenomenon. Instead, there was a large difference in the amount of lysozyme adsorbed (Fig. 8B) but little variation in that of HSA (Fig. 8A) for these hydrogels. Furthermore, Figure 8B shows, for the adsorption of lysozyme, that the effect of MAA dominated that of NVP, with NVP acting as only a slight moderator. The sole driving force for the adsorption of ly-

sozyme, in this case, may have been electrostatic attractions between MAA and lysozyme. However, neither of the comonomers, MAA or NVP, seemed to have a dominant influence in the adsorption of HSA.

CONCLUSIONS

Variation in the water content of poly(HEMA)-based hydrogels was successfully obtained with the use of controlled amounts of MAA or NVP as comonomers. Inclusion of comonomers in hydrogels increased the water content, as well as having a strong impact on protein adsorption.

An increase in the amount of MAA in the poly(HEMA-co-MAA) hydrogels increased lysozyme adsorption and penetration but reduced HSA adsorption. Negatively charged carboxyl groups of the MAA may influence lysozyme sorption by electrostatic attraction and by increasing the possibility for small lysozyme molecules to penetrate into the hydrogels. However, the amount of protein adsorbed, for both HSA and lysozyme, increased with the amount of NVP in the poly(HEMA-co-NVP) hydrogels. Two possibilities may help to explain the attraction of proteins to NVP: electrostatic interactions and special interactions of the surface lactam groups of NVP with proteins.

In contrast to the marked effect of MAA on protein sorption—in particular, on lysozyme sorption—NVP had relatively little influence. Furthermore, there was a competitive effect between MAA and NVP on the sorption of protein. When hydrogel contained both MAA and NVP, MAA had the dominant effect on protein sorption—in particular, on lysozyme sorption.

Water content may not be a primary factor in determining protein adsorption. It appears that the monomer constituents, such as MAA or NVP, control the adsorption of certain types of protein (such as lysozyme), with changes in the water content a byproduct of the introduction of these monomers.

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