Specificity and Biological Activity of the Protein Deposited on the Hydrogel Surface

Relationship of Polymer Structure to Biofilm Formation

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The in-situ lens-bound protein layer (LBPL) was characterized on hydrogels of varying water content and ionic-binding capacity. The LBPL proved to be critically dependent on the ionic binding capacity of a given hydrogel. On nonionic polymers the LBPL invariably was thin and largely insoluble. Histochemical staining allowed the detection of all major types of tear proteins. Amino acid analysis revealed a variable composition. Extractable protein proved devoid of active lysozyme. Electrophoresis of pooled samples revealed a variable mixture of acidic, neutral, and basic bands. To what extent variability is dependent on tear film composition and lens structure awaits use of more sensitive analytic procedures. On anionic hydroxyethylmethacrylate copolymer lenses, the LBPL proved radically different. Here the LBPL invariably was much thicker and composed primarily of loosely bound protein. Electrophoresis and enzymatic analysis revealed a homogenous layer consisting primarily of lysozyme much of which retains enzymatic activity. The amino acid analysis of the insoluble protein suggests a similar composition. Specificity of deposition can be attributed to ionic affinity. Conformational integrity can be attributed partly to the unique stability of lysozyme. Electrophoresis of a pooled anionic lens extract revealed an unknown, highly mobile, basic protein. This presumably represents the selective accumulation of a highly basic trace or transient constituent of the tear film. The specificity and biological activity of the LBPL on the anionic lens may modify hydrogel biocompatibility affecting risk of spoilage, microbial colonization, and propensity to trigger an inflammatory and immune response. Invest Ophthalmol Vis Sci 28:842–849, 1987.

Increased oxygen permeability and ease of fit have made the hydrogel, or soft contact lens, the lens of choice for the vast majority of contact lens wearers.1 Related polymers are being considered for a variety of other biomedical applications in which they will be in direct contact with body fluids. While these polymers, in general, are bioinert, like all foreign objects once in the body they are rapidly coated with a biopolymeric layer, which may modify the hydrogel biocompatibility.2 How the hydrogel structure and its environment affects the nature of the biofilm is not well understood. The ease of access, extent of use, and the degree to which the tear film composition has been characterized makes the contact lens surface an ideal vehicle for such study. On the contact lens surface, this ever-thickening layer impairs visual acuity, limits the useful life span of the lens and necessitates the use of expensive and less than optimal cleaning agents.3 As a possible mechanical, toxic, and antigenic irritant, the biofilm is suspected of contributing to the risk of allergic and inflammatory reactions.4,5 As a source of microbial contamination,6 the biofilm is also likely to serve as a risk factor for ocular infection, especially with extended wear (EW) use.7 Thus, the origins, composition, and effect of the biofilm on contact lens biocompatibility is of considerable clinical and industrial concern.

Numerous substances of both ocular and foreign origin have been reported within the biofilm matrix. The most common components, undoubtedly derived from the tear film constituents, are cations, lipids, and proteins. The tear film lipids (with or without calcium) tend to form discrete deposits termed “jelly bumps.” These are particularly endemic to the high-water content extended wear contact lens (EWCL).8 The tear proteins, in contrast, have long been associated with film-like deposits that are ubiquitous to all contact lenses. The lens-bound protein layer (LBPL) is widely perceived as a highly denatured entity. Partial to complete denaturation is believed to occur on binding as a direct result of the high interfacial tension inherent...
in the lens-tear film interphase. This is exacerbated by routine exposure to thermal or chemical disinfection cycles theoretically rendering any remaining protein little more than an ever-thickening insoluble film.\textsuperscript{4,5,10} The composition of this film is believed to be highly specific. More than a decade ago, Karageozian\textsuperscript{11} used amino acid analysis to identify virtually pure lysozyme on the nonionic Softlens\textsuperscript{8} (polymacon) hydrogel. Because lysozyme represents approximately 30% of the tear proteins,\textsuperscript{12} deposition must be a highly selective process. This conclusion has since been supported in subsequent studies often without citing experimental documentation.\textsuperscript{10,13,14} Recent work indicates that the above model of the LBPL may be in need of revision. Spectrophotometric observation of lysozyme bound to a hydrogel (of unspecified composition) suggests that conformational integrity is retained to a considerable degree.\textsuperscript{15} Such a conclusion is consistent with the results of an enzymatic assay of extracts obtained from poly-2-hydroxyethyl-methacrylate (PHEMA) copolymer hydrogels as reported from this laboratory.\textsuperscript{13} To what extent, composition, and conformational integrity is dependent on hydrogel structure is unknown. Furthermore, the composition of the LBPL may not be nearly as homogeneous as widely believed. Welder\textsuperscript{16} solubilized and separated protein deposited on pooled polymacon lenses and found six discrete bands. Whether these bands represented minor deposit constituents, polymerized lysozyme, or a heterogenous deposition was not determined. Laboratory-simulated protein deposition studies support the contention that the LBPL may be variable and highly dependent on polymer structure in a manner inconsistent with Karageozian’s findings.\textsuperscript{17-19} Whether laboratory conditions accurately mimic the in-situ environment is uncertain. The ocular environment is complex, and the tear film is known to contain at least one surfactant that inhibits the binding of protein to hydrogel-like surfaces.\textsuperscript{20} In order to resolve these questions, the authors have analyzed the extent, composition, and conformational integrity of the LBPL indigenous to hydrogels of varying water content and ionic-binding characteristics, and yet be representative of the broad spectrum of hydrogels in common use in the New York City area. For purposes of discussion, all PHEMA copolymer lenses are referred to herein as being of anionic or acidic composition. Lenses of other structures will be referred to as being of nonionic composition. The rationale and significance of this classification will be elaborated on later. EW use is thought to result in a more extensive build-up of deposits.\textsuperscript{21} To avoid complications resulting from this variable, lenses used on an EW and a daily wear (DW) basis were analyzed separately. Heavily deposited, torn, or otherwise unusable lenses at the end of their normal useful life cycle were randomly selected from those donated by patients to clinicians. Complete records were obtained for these lenses with respect to length and type of use (DW or EW), past or present history of adverse reactions to lens wear, and mode of cleaning and disinfection. Careful questioning of patients and clinicians quickly revealed that the actual wearing, cleaning, and disinfecting habits of many individuals were quite variable. These aspects of our recorded lens history were viewed as being of questionable reliability. Lenses were mailed to this laboratory in a moist state within a few days of collection and stored at \(-20^\circ\text{C}\) until needed. In some instances, perfectly good lenses were recovered directly from the patient’s eye, dipped in 2 ml of distilled water to remove residual tear film, and then used for analysis. (Replacement lenses were provided courtesy of various manufacturers.) Before chemical analysis, most lenses were examined under a phase-contrast microscope and characterized as to the severity and morphology of the deposit. This process was often repeated after extraction. At times, segments of individual lenses were removed with a scalpel and put aside for comparative histochemical staining or protein analysis.

### Materials and Methods

#### Reagents

Rabbit antiserum to human lysozyme and lactoferrin were purchased from Accurate Chemical and Scientific Corp. (Westbury, Long Island, NY). Immunoelectrophoresis and electrophoresis supplies were purchased from Beckman Instruments (Fullerton, CA) or Gelman Sciences (Ann Arbor, MI). Other antisera, chemicals, and reagents, unless stated otherwise, were obtained from Sigma Chemical Co. (St. Louis, MO.).

#### Lenses

Analysis was carried out on over 200 PHEMA copolymer (buficon A and perificon), PHEMA (polymacon), lidofilcon A and B (Sauflon 70, Genesis 4, and B&L 70, 79), and crofilcon A (CSI) lenses. These hydrogels were selected to provide a sample population with variable water content and ionic-binding characteristics, and yet be representative of the broad spectrum of hydrogels in common use in the New York City area. For purposes of discussion, all PHEMA copolymer lenses are referred to herein as being of anionic or acidic composition. Lenses of other structures will be referred to as being of nonionic composition. The rationale and significance of this classification will be elaborated on later. EW use is thought to result in a more extensive build-up of deposits.\textsuperscript{21} To avoid complications resulting from this variable, lenses used on an EW and a daily wear (DW) basis were analyzed separately. Heavily deposited, torn, or otherwise unusable lenses at the end of their normal useful life cycle were randomly selected from those donated by patients to clinicians. Complete records were obtained for these lenses with respect to length and type of use (DW or EW), past or present history of adverse reactions to lens wear, and mode of cleaning and disinfection. Careful questioning of patients and clinicians quickly revealed that the actual wearing, cleaning, and disinfecting habits of many individuals were quite variable. These aspects of our recorded lens history were viewed as being of questionable reliability. Lenses were mailed to this laboratory in a moist state within a few days of collection and stored at \(-20^\circ\text{C}\) until needed. In some instances, perfectly good lenses were recovered directly from the patient’s eye, dipped in 2 ml of distilled water to remove residual tear film, and then used for analysis. (Replacement lenses were provided courtesy of various manufacturers.) Before chemical analysis, most lenses were examined under a phase-contrast microscope and characterized as to the severity and morphology of the deposit. This process was often repeated after extraction. At times, segments of individual lenses were removed with a scalpel and put aside for comparative histochemical staining or protein analysis.

#### Analysis of Insoluble Portion of the LBPL

The insoluble portion of the LBPL was characterized by histochemical and amino-acid analysis.

**Histochemical analysis:** Enzyme-linked histochem-
ical and immunochemical staining techniques were adapted to visualize acidic (albumin), relatively neutral (immunoglobulin A [IgA] and lactoferrin), basic (lysozyme) proteins, and glycoproteins (presumably mucin) on the lens surface. By carrying out the analysis (immunoglobulin A [IgA] and lactoferrin), basic (lysozyme) adapted to visualize acidic (albumin), relatively neutral probe visualized by standard colorimetric assay for intensity of a yellowish-green deposit. Readings were by washing five times with 0.5 ml of buffer. The lens extraction of each of these substances. Commercially available horseradish peroxidase (HRP)-linked concanavalin A was used to detect glycoprotein. In order to detect lysozyme, albumin, IgA, and lactoferrin, comparable HRP-linked antibody probes were synthesized from commercially available antibodies (rabbit antibodies to lysozyme and IgA and goat antibodies for albumin) and HRP (Z = 3.0). Synthesis was carried out by a two-step sodium periodate, sodium borohydrate procedure as detailed by Wilson and Nakane. The resulting enzyme-linked antibodies were stored at 0°C in phosphate buffer and diluted 1-1000 or 1-500 V/V before use. In order to detect a given protein, the lens was presoaked in goat serum and then incubated with the appropriate enzyme-linked probe. The unbound probe was removed by washing and the bound probe visualized by standard colorimetric assay for HRP activity. For example, in order to detect glycoprotein the lens or fragment was preincubated for 10 min with 0.5 ml of 5% goat (or rabbit) serum at 37°C in a “cell well compartment” (Corning Glass Works; Corning, NY). The serum was removed, and the lens then soaked for 1 hr at 37°C in 0.5 ml of diluted HRP-linked concanavalin A (prepared by reconstituting Sigma product number L-4010 1:500 v/v in 0.01 M phosphate buffered saline; pH, 7.4). Residual unbound HRP-linked concanavalin A was removed by washing five times with 0.5 ml of buffer. The lens was transferred to a new reaction well where the five-step washing procedure was repeated. The lens was subsequently incubated for 1 hr at room temperature with 0.5 ml of 0.4% phenylenediamine hydrogen chloride and 0.012% hydrogen peroxide in 0.1 M phosphate-citrate buffer (pH, 5.0) and the reaction was terminated by rinsing in buffer. The lens was removed from the reaction well, and the presence of glycoprotein was then graded on a 0-6 basis by the increasing intensity of a yellowish-green deposit. Readings were measured against controls consisting of unused lenses of similar composition that had been treated in an identical manner. (Unused lenses were provided courtesy of various manufacturers.) The specificity and threshold of sensitivity for each reaction was determined by staining lenses impregnated with known amounts protein standards. In order to prevent false-positive reactions it proved essential to adhere to all of the previously mentioned preincubation and lens washing procedures carefully.

Analysis of Amino Acids

Representative exhaustively extracted lenses were sent to a commercial laboratory (Genetic Design; Watertown, MA) for acid hydrolysis and analysis of amino acids. The resulting amino-acid composition was contrasted to that of human lysozyme.

Extraction and Analysis of the Soluble Portion of the LBPL

Saline extraction: Individual lenses were extracted four times by stirring overnight at room temperature in 2-ml aliquots of 0.9% NaCl. Extracted lenses were recovered and sectioned to be stained histochemically or subjected to Lowry assay. The resulting extracts were used directly for lysozyme or protein assay or concentrated to 40 μl by centrifugal ultrafiltration in a Centricon (Amicon Corp.; Danvers, MA) microconcentrator (molecular weight cut-off, 10,000 daltons). Samples requiring further concentration were evaporated with nitrogen.

Extracted proteins were separated and characterized by electrophoretic and immunoelectrophoretic (IE) assays on cellulose acetate or mylar-backed nitrocellulose acetate membranes in veronal buffer (pH, 8.8) at 230 V according to modified procedures outlined in the appropriate Beckman microzonal electrophoresis or Gelman manuals. Protein was quantitatively visualized by Ponceau’s staining followed by counterstaining with the more sensitive Coomassie blue stain. (Attempts to increase sensitivity by use of a modified biotin/avidin-HRP linked protein detection system (BioRad, Richmond, CA) proved unsatisfactory because of the lack of reactivity on the part of highly charged tear proteins.) Appropriate standards were employed for identification of specific proteins. These included human serum, milk (a gift), albumin, lysozyme, lactoferrin, mucin, tear, and denatured tear samples. Tear samples were collected from the lower fornix using a microcapillary tube. Samples were either transferred directly to an electrophoretic applicator for analysis or first denatured by heating within a sealed microcapillary tube at 90°C for 30 min. Identification of lysozyme, lactoferrin, IgA, and immunoglobulin G (IgG) was carried out by IE or immunodiffusion assay using antisera specific to each of the above proteins.

Lysozyme Assay

Extracts were assayed for lysozymal activity by the spectrophotometric measurement of the lysis of a fresh suspension of Micrococcus lysodeikticus (Sigma). The assay was carried out as described elsewhere except for the temperature of the assay, which was 21°C in this study. The specific activity of the extract was then
normalized against pure human lysozyme (Sigma), which had been assayed under identical conditions.

Protein Assay

Insoluble and soluble protein was assayed by a modified micromethod of Lowry. In order to analyze the insoluble protein, this material had to be first eluted off the lens. This was accomplished by stirring the lens or fragment for 1 hr in 0.5 ml of 0.1 N NaOH. The pH of the resulting elutant was adjusted to 9.0 and Lowry assay was performed. Readings were carried out against a blank consisting of an unused lens of identical composition treated in a similar manner. Calibration was implemented with a bovine serum albumin standard.

Results

Histochemical Analysis

The threshold of histochemical sensitivity for all protein, except lysozyme, approached 2 ng. In the case of lysozyme, the probe was approximately fivefold less sensitive. In interpreting histochemical data it is important to realize that many factors, such as solubility of deposited protein and chromogen, antigenic conformational integrity, degree of penetration of enzyme-linked probe, availability of antigenic-binding sites, and interference from other deposit constituents are all capable of interfering with a quantitative reaction. Thus, the intensity of reaction should be viewed as reflective of the surface availability rather than the actual concentration of a given substance. Depending on thickness of deposition, surface availability can differ dramatically from the actual concentration of a substance within the deposit. Approximately 150 heavily deposited or prematurely discarded (damaged or poorly tolerated) PHEMA copolymer, PHEMA and non-PHEMA DWCL, and EWCL were used for histochemical study. Many of these lenses were covered with hazy-white or yellowish film-like deposits. A few lenses contained a small number of jelly bumps, and others exhibited a pink-to-brown discoloration around their edges. Regardless of past history, all lenses were found coated with a continuous layer of mucin and protein. This layer must form rapidly because it was observed on lenses removed from the eye after as little as a few hours use. The pattern of staining often proved irregular, with local foci apparent on the surface of many lenses. Lens structure had a pronounced effect on the anionic as opposed to nonionic lens (Table 1). With the exception of albumin, staining was more intense for all substances on the anionic as opposed to nonionic lens (Table 1). Although the intensity of staining sometimes correlated with the overall deposit morphology for a significant fraction of lenses, this relationship did not hold. Some lenses that were heavily discolored (pink to brown) or covered with a film-like deposit stained no more intensely than lenses of comparable composition with little or no visual evidence of spoilage.

Biochemical Analysis

The extent of protein deposition proved highly dependent on the ionic-binding capacity of a given hydrogel (Table 2). Not only did the insoluble film prove

<table>
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<tr>
<th>Table 1. Intensity of reaction to various enzyme-linked specific probes contrasted with basic (lysozyme), relatively neutral (lactoferrin and immunoglobulins), and acidic proteins (albumin) on acid and nonionic hydrogels*</th>
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<tbody>
<tr>
<td><strong>Acidic-matrix</strong></td>
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<tr>
<td>(PHEMA copolymer)</td>
</tr>
<tr>
<td><strong>Nonionic matrix</strong></td>
</tr>
<tr>
<td>(polymacon)</td>
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<tr>
<td><strong>DW</strong></td>
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<td><strong>EW</strong></td>
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<td><strong>DWi EW</strong></td>
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<tr>
<td>Lysozyme</td>
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<tr>
<td>Lactoferrin</td>
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<tr>
<td>Immunoglobulins</td>
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<tr>
<td>Albumin</td>
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<td>Glycoprotein</td>
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* Intensity of reactions is expressed on a 0-6 scale based on increased intensity of chromogenic reaction. Note that the extended wear acidic matrix lenses were not assayed for albumin.

† DW = daily wear.

‡ EW = extended wear.

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<tr>
<th>Table 2. Hydrogel structure contrasted with the extent of deposition of soluble and insoluble protein and the presence of active lysozyme in the lens extracts*</th>
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<tr>
<td><strong>Acidic-matrix</strong></td>
</tr>
<tr>
<td><strong>% Water content</strong></td>
</tr>
<tr>
<td><strong>Soluble protein (μg)</strong></td>
</tr>
<tr>
<td><strong>Insoluble protein (μg)</strong></td>
</tr>
<tr>
<td><strong>% Protein active lysozyme</strong></td>
</tr>
<tr>
<td>Perifcon A</td>
</tr>
<tr>
<td>Bufilcon A &amp; B</td>
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<tr>
<td>Nonionic</td>
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<tr>
<td>Polymacon</td>
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<tr>
<td>Crofilcon A</td>
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<tr>
<td>Lidofilcon A &amp; B</td>
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* Note that the extent of deposition appears to be dependent hydrogel ionic-binding capacity rather than water content.
thicker on the acidic matrix lens, but, in addition, these lenses were coated with an extensive saline-soluble layer. A comparable layer was almost nonexistent on the nonionic lens (Table 2).

Amino-acid analysis failed to confirm the findings of Karageozian.11 The hydrolyzate that most closely approximated the composition of lysozyme was that found on the acidic matrix lens. Even here, the contribution of minor nonlysozymal components must be considerable (Table 3).

Electrophoretic analysis of the extracts obtained from 16 individual PHEMA copolymer lenses revealed a highly homogeneous population consisting of samples with a single prominent band identified by IE assays as lysozyme (Figs. 1, 2). In only one extract was there even a faint hint of an additional band. This had a mobility consistent with either lactoferrin or IgA. Lysozyme assay further revealed that much of the extracted protein retained its biologic activity (Table 2).

Minor extract constituents could be evidenced by carrying out analysis on pooled PHEMA copolymer lens extracts. Identifiable proteins consisted of IgA, lactoferrin (not shown) and an unknown, highly mobile basic protein. The latter protein or polypeptide (sample being concentrated directly with nitrogen) detected in the extract obtained from one of two groups of 25 pooled EW acidic matrix lenses (Fig. 3) proved nonreactive to antibodies to human lysozyme, lactoferrin, or serum proteins. A comparable band was not evidenced in either individual or pooled tear samples (Fig. 2).

The sensitivity constraints imposed by our electrophoretic procedures precluded the analysis of individual nonionic contact lens extracts. Analysis was limited to pooled extracts obtained from groups of six to eight crofilcon, lidofticon, or polymacon lenses. These revealed a heterogeneous composition containing faint acidic, neutral, and basic bands, the exact location and composition of which varied in a manner similar to that encountered with denatured tear samples (Fig. 1). At no time was there any evidence for a selective deposition of lysozyme. In fact, in some samples there seemed to be a limited preferential deposition of acidic tear proteins (Fig. 1). Lysozyme assay consistently revealed the absence of appreciable activity (Table 2).

**Discussion**

As a source of spoilage, antigenicity, and possible biological activity, the LBPL have been the object of interest and study. The prevalent view is that the LBPL is highly homogeneous consisting almost entirely of denatured lysozyme.10,11,13 These results do not support such a conclusion. Instead, they suggest that the LBPL is variable in its extent, composition, and conformational integrity in a manner highly dependent on hydrogel structure. Several contradictory theories have been advanced as to whether and how hydrogel water content and/or surface chemistry affects the nature of the LBPL.4,9,10,13,17,18,19 Part of this confusion may have resulted from past failure to delineate adequately or consider the unique nature of the hydrogels under study and/or a lack of appreciation of the interrelationship that often exists between hydrogel water content and ionic-binding capacity. As recognized by Parker24 and elaborated on by Stone et al15 hydrogels can be classified into three distinct groups of polymers based on their water content and matrix chemistry. These consist of low-water content, nonionic hydrogels (ie, polymacon and crofilcon), high-water content, nonionic hydrogels (ie, lidofticon), and high-water content, anionic hydrogels (ie, PHEMA copolymers). Comparison of the deposition on these three classes of hydrogels unequivocally shows that the ionic-binding capacity, rather than the water content, plays the dominant role in determining the nature of the resulting LBPL (Tables 1–3, Fig. 1). These results are compatible with and extend the findings of laboratory deposition studies of others.17–19 On the anionic PHEMA copolymer lens surface, the LBPL consists primarily of a thick, loosely bound layer of lysozyme, much of which retains its conformational integrity. This layer is the precursor of the eventual insoluble film as evidenced by its amino-acid composition (Table 3). On the nonionic hydrogel surface, the LBPL is radically different. Here, the LBPL is much thinner and likely to consist of a mixture of

<table>
<thead>
<tr>
<th>Amino acid leucine ratio</th>
<th>Ly‡</th>
<th>Polymacon</th>
<th>PHEMA copolymer</th>
<th>Crofilcon</th>
</tr>
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<tbody>
<tr>
<td>Lysine</td>
<td>0.63</td>
<td>1.3</td>
<td>1.6</td>
<td>1.7</td>
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<tr>
<td>Arginine</td>
<td>1.8</td>
<td>0.3</td>
<td>1.8</td>
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<tr>
<td>Aspartic</td>
<td>1.0</td>
<td>0.80</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.63</td>
<td>0.71</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Serine</td>
<td>0.75</td>
<td>0.64</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.1</td>
<td>0.71</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Proline</td>
<td>0.25</td>
<td>0.14</td>
<td>0.75</td>
<td>0.0</td>
</tr>
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* PHEMA = poly-2-hydroxyethyl-methacrylate.
† Amino-acid composition of human lysozyme as listed in Dayhoff.24
‡ Data is expressed as a molar ratio to that of leucine.
§ Lysozyme assay further revealed that much of the extracted protein retained its biologic activity (Table 2).

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933366/)
Fig. 1. A typical electrophoretic pattern obtained from acidic matrix lens (lane A). Pooled extract from six polymacon lenses (lane B). Tear sample; specific identified proteins lysozyme (L), lactoferrin (I), IgA (I), prealbumins (P) (lane C). P usually appears as two separate bands. Serum sample (lane D).

Fig. 2. Immunoelectrophoretic pattern obtained on analysis of the extract from a single acidic matrix lens. Troughs above and below sample, respectively, contained antiserum to lysozyme and a mixture of antiserum to lactoferrin, lysozyme, and human serum proteins.

Fig. 3. Electrophoretic pattern obtained with pooled poly-2-hydroxyethyl-methacrylate copolymer lens extract. Individual acidic matrix lens extract (lane A). Pooled acidic matrix lens extract (lane B). (p) is an artifact at the point of application; (ly) is lysozyme; (u) is an unknown, highly mobile, basic protein. Serum sample (lane C). Note that pooled lens extract was dried directly under nitrogen without prior ultrafiltration.
denatured tear proteins (Table 3). This is not meant to imply that the actual composition of the LBPL on different nonionic hydrogels is necessarily the same or that no selectivity is involved in this process. Comparison of the amino-acid composition of residual protein on croficon and polymacon lenses suggests otherwise (Table 3). Neither do we intend to suggest that the deposit on individual nonionic lenses is always the same or unaffected by the known variability of tear film chemistry. Quite the opposite would be expected. Study of these questions awaits use of procedures with an analytic sensitivity beyond the scope of those employed herein. Although in theory the biotin–avidin HRP detection system that the authors employed had the needed sensitivity to allow such analysis, its direct use on cellulose acetate membranes was hampered by a highly variable response of different tear proteins. Resolving this problem or adapting a silver stain coupled with gel electrophoresis, a system under consideration, may allow such an analysis.

Although lysozyme is widely cited as being selectively deposited on all hydrogel surfaces, the only data, to the authors' knowledge, that documents such a deposition on the nonionic lens is the widely quoted study of Karageozian et al.11 These findings are at variance with those of the authors, those of laboratory-simulated deposition studies,4,17–19 and electrophoretic results as reported by Welder.16 Re-examination of Karageozian's analysis reveals that it is in error because the amino-acid composition that was assumed for lysozyme11 is somewhat incorrect. Using the currently accepted amino-acid composition of lysozyme,27 the identity of deposition is less certain. Furthermore, the authors are at a loss to provide a rational explanation for a selective deposition of lysozyme on the polymacon lens. Selective deposition on other hydrogels possibly can be attributed to ionic affinity or to a molecular sieving effect. Neither of these factors should be relevant when considering the polymacon lens.

It has been suggested that lysozymal deposition increases with an increase in hydrogel water content.13 Our data shows that this is not necessarily the case (Table 3). Deposition, instead, appears directly related to the anionic-binding capacity of a given hydrogel. The rationale for this relationship is readily understood. The presence of methyl acrylic acid anions within the PHEMA copolymer matrix allows this group of hydrogels to function much as a cation exchange, with resins selectively adsorbing the more basic of the tear constituents. The tear film proteins consist of a diverse mixture of ionic species at physiologic pH, of which only lysozyme is of sufficient positive charge (pK\text{a} = 10.528) to have a marked affinity for the anionic lens. Being highly compact, lysozyme reportedly can penetrate the normally restricted PHEMA copolymer matrix.29 Thus, selective deposition has, in part, been attributed to a selective sieving effect.19 This, however, appears to play a limited role in determining the nature of deposition. Lidofilcon hydrogels are of high water content and presumably also have a large pore size. Yet here deposition of lysozyme is minimal (Table 2). To what degree lysozyme enters into the lens matrix is unknown. If most of it remains on the surface, then the very extent of deposition necessitates formation of a multilayered complex. Other ionic species in the tear film probably participate in complex formation. What these factors are and why only some users of acidic matrix lenses appear to be rapid and heavy protein depositors is unknown.

Most striking is the extraordinary degree to which conformational integrity is retained on the acidic matrix lens. Highly active lysozyme can be extracted from moist lenses that have been kept in storage at room temperature for 4 or more years. This fact may be more reflective of the properties of lysozyme than of its benign environment. In this study, lysozyme is remarkably stable. It survives heating for a few minutes at 90°C and exposure to 3% hydrogen peroxide: conditions reflective of the extremes to which the lens is exposed during disinfection.

The LBPL on the acidic matrix lens must be considered from a new perspective a highly selective, biologically active layer. This layer may transform the lens' physical characteristics (ie, modify its wetting angle, tear break-up time, permeability, and so forth) and impart to its surface a distinctive physiologic activity. For example, a viable mucin, lysozyme, and IgA coat could affect the capacity of microorganisms to adhere to and colonize the hydrogel surface, much as it does on external tissue surfaces.30 Thus, by studying the effect of the LBPL on microbial deposition, it may prove possible to identify a hydrogel surface less conducive to microbial contamination. This, in turn, could reduce the risk of spoilage and ocular infection.

Several highly basic trace or transient proteins are known or suspected of being present in the tear film.31,32 These substances would be expected to accumulate on the acidic matrix lens especially with EW use (one such protein, the cosinophilic major basic protein, has been reported in elevated concentration in the tear film of atopic individuals.31 The EMBP with a pK, near 12 (Personal communication: G. Gleich), would be expected to adhere tightly to the PHEMA copolymer surface. This might exacerbate or prolong an inflammatory response. Whether an unknown, highly basic protein (Fig. 3) represents an artifact or a tear constituent is under study.

On the PHEMA hydrogel, the dynamics of adsorption are radically different. Pore size is sufficiently small, preventing the penetration of even the smallest of proteins.29 Deposition must be treated entirely as a surface adsorption phenomenon. The scarcity of cat-
ionic and anionic binding sites most likely limits both the extent and specificity of deposition. Any protein that manages to bind to the lens surface presumably is held, in part, by hydrophobic and nonionic hydrophilic interactions. These forces would favor exposure of nonionic amino acids normally buried within the protein interior. This explains the presence of a thin, heterogeneous mixture of denatured protein on the nonionic hydrogel.

Whether lens structure significantly influences the risk of spoilage is of obvious clinical and economic concern. Our results suggest that the acidic matrix lenses would be particularly prone to cationic species induced spoilage. Because the degree of incorporation of methyl acrylic acid varies with specific patented PHEMA copolymers, some hydrogels would be more susceptible than others. The permalens would seem to be at particular risk in this regard (Table 2). Decreasing the risk of spoilage or contamination presumably calls for careful control of charged contaminants such as insoluble cations and amines in ophthalmic solutions, and complete and exhaustive soaking of lenses in saline before disinfection.

Key words: contact lens, contact lens biocompatibility, contact lens deposits, hydrogel, lysozyme, tear proteins

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