The study of Lysozyme adsorption onto 2-hydroxyethylmethacrylates and Silicon Hydrogel Contact Lenses

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ABSTRACT

In order to increase the water content and the oxygen permeability of hydrogels used in the manufacture of contact lenses, the polar monomer Silicon Hydrogel Contact Lenses (SHCL), and 2-hydroxyethyl methacrylate (HEMA) were copolymerized with the hydrogels. Due to the presence of polar monomers in the conventional contact lenses, the major component of the human tear, lysozyme is extensively adsorbed onto their surfaces. The adsorption of lysozyme onto the contact lens' surface leads to limitations in its application. The present study concentration of lysozyme, adsorbed onto the surface of HEMA and SHCL hydrogels were measured by UV-Vis spectroscopy. The lysozyme employed was obtained from solutions with similar to concentration of artificial tear. The adsorption results were examined by the Langmuir adsorption isotherm and the constants of this isotherm were also evaluated.

Keywords: Protein adsorption; 2-hydroxyethyl methacrylate; Silicon Hydrogel; Contact Lenses; Langmuir isotherm; Hydrogels

INTRODUCTION

Since Wichterle and Lim proposed that their new polymer, poly hydroxyethylmethacrylate (pHEMA), could be used to make contact lenses half a century ago, the polymer hydrogel-based soft contact lenses have been extensively utilized for vision correction. Currently, in the United States, there are roughly 28 million soft contact lens wearers. A polymer hydrogel is a cross-linked hydrophilic polymer network solvated with water and it behaves like both solid and liquid. Like a solid, the hydrogel deforms under applied stress and recovers after the stress is released. Like a liquid, the hydrogel supports diffusion of the solutes when the size of solutes is smaller than the mesh size of the network. During blinking, the eyelid is sliding against the surface of the eye [1].

Silicone hydrogel contact lenses have been available in clinical use since 1999 in the United States and 2004 in Japan. These lenses appear to overcome many of the lens-induced hypoxic problems associated with contact lens wear, whereas several clinical complications have been reported to occur as a result of mechanical disturbance, infection, and deposition. In biomedical applications, 2- hydroxyethyl methacrylate HEMA, offers the greatest advantage over most other hydrophilic gels commonly encountered in biomedical applications with regard to stability to

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various parameters, for example, pH, and temperature. When the polymer is prepared in the absence of water, it is glassy and similar in many ways to poly methyl methacrylates. Thus, the permeability of the membranes, their mechanical properties, their surface properties and the resultant behavior at biological interfaces are all a direct consequence of the amount and nature of water held in this way [2].

The cross-linked 2- hydroxyethyl methacrylates HEMA are more commonly referred to as poly HEMAs. Hydrogels have been extensively studied in the biomedical and pharmaceutical fields for a variety of applications including soft contact lenses [3] and drug delivery devices [4]. Adsorption of proteins to hydrogel surfaces has been the subject of considerable investigation due to the fact that the presence of a protein film can in some cases modify the biocompatibility of the hydrogel surfaces. The amount of protein adsorbed increases with the anionic character of the hydrogels [5]. Despite the heterogeneity of the film, studies indicate that lysozyme is usually the most prevalent protein absorbed by ionic hydrogel contact lenses, due to its low molecular weight and the fact that it is positively charged at physiological pH, while ionic lenses are usually negatively charged. Furthermore, lysozyme is the most abundant protein in human tears constituting one third of the total protein content followed by lactoferrin and tears specific pre albumin [6]. Although many studies have been done on contact lens soiling, one central difficulty is the quantification of deposits as a response variable to different experimental methods. Some of the techniques used for the quantification of proteins include IR spectrometry [7], UV-VIS spectrometry [8], atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) [9,10].

SHCL are the latest kind of soft lenses commercially available. This type of lens provides excellent oxygen transmissibility to the cornea on account of silicone's high oxygen transmissibility when compared to the conventional hydrogel SHCL [7, 11]. Silicone is a hydrophobic polymer, and for this reason most of the silicone-based SHCL possesses surface treatment, which decreases the surface hydrophobicity. The reduction in hydrophobicity gives a greater comfort to the wearer and additionally prevents the formation of deposits such as lipids and proteins, as well as microbial colonization [12]. The reduction in the lens surface hydrophobicity can be obtained through two methods. The first one consists in performing a treatment on the lens surface, which can be achieved in a gas plasma reactive chamber by creating an ultra-thin permanent coating in the cases of Lotrafilcon A and Lotrafilcon B (Ciba Vision), or by plasma oxidation, transforming the silicone into silicate compounds, in the case of Balafilcon A (Bausch & Lomb, Inc.) (Table 1). The second method consists in the incorporation of a wetting agent such as polyvinyl pyrrolidone (PVP), which is the case of Galyfilcon A (Table 1) (Johnson & Johnson Vision Care). Silicone hydrogel CL, despite the advantages they offer due to their high oxygen transmissibility, also present some pitfalls, which are related to the migration of the silicone hydrophobic moieties to the lens surface [13]. As mentioned earlier, less hydrophobic surfaces are advantageous, since they prevent protein adsorption and microbial colonization.

In the present study, UV spectroscopy was used to determine the amount of protein adsorbed onto the surface of the two types of contact lenses, HEMA and SHCL. The results were examined by Langmuir adsorption isotherm and constants of this isotherm were evaluated.

Table 1. Contact lenses properties.

Commercial name	Manufacturer	Material	FDA group	Water content(%)
Acuvue	Johnson & Johnson Vision Care	Etafilcon A	IV	58
Acuvue and AdvanceTM	Johnson & Johnson Vision Care	Galyfilcon	I	47
Purevision	Bausch & Lomb, Inc.	Balafilcon A	III	36
Focus1 Night & Day	CIBA Vision	Lotrafilcon A	I	24
O2Optix	CIBA Vision	Lotrafilcon B	I	33

THEORY

Langmuir isotherm

Many different isotherm models have been proposed for the adsorption of solutes from a liquid solution onto a solid surface. Most of those models are essentially empirical although theoretical derivations have been accomplished in some cases. Among all models, the Langmuir model is probably the most popular due to its simplicity and its agreement with experimental data. The Langmuir model is expressed by [11]:

$$q = \frac{q_m K_d C}{1 + C K_d} \qquad (1)$$

where q (mg/g) and c (mg/ml) are the equilibrium concentration of protein in solute in solid adsorbent and liquid bulk phases, respectively. Constant q_m (mg protein adsorbed/g adsorbent) and K_d (mg protein adsorbed/ml volume of protein solution) are Langmuir parameters. The constant q_m represents the maximum binding capacity and K_d is the dissociation coefficient of the solute-adsorbent complex, which represents the affinity between the solute and the adsorbent.

The Langmuir isotherm has been widely accepted as a practical method for integrating experimental data of protein adsorption onto hydrogels surfaces [12, 13]. It is therefore more favourable to obtain the dissociation coefficient and maximum protein binding capacity by rearranging Equation (1). This gives a linear equation as follows

$$\frac{C}{q} = \frac{K_d}{q_m} + \frac{1}{q_m} C \qquad (2)$$

Graph of C/q versus C gives a line of an intercept of K_d/q_m and a slope of $1/q_m$.

Measurement of adsorption isotherms is an important first step in the characterization of the interaction between protein and adsorbent. The values of q_m give an indication of the maximum possible capacity of the adsorbents although it must be remembered that these maximum values will not be achieved under most operating conditions. The values of K_d give some indication as to what concentrations of adsorbate are needed to achieve capacities approaching the maximum values, q_m. If the adsorption stage is carried out with protein concentration of the same order as the value of K_d or smaller, only a fraction of the maximum capacity of the adsorbent will be utilized. Only if the concentration of protein is far greater than the value of K_d, will the adsorbent show a capacity approaching q_m and even this capacity will occur only in circumstances where the protein and adsorbent have been contacted for a sufficient period for equilibrium to be reached [13].

Excess Gibbs Surface

The extent of adsorption related to the adsorption free energy ΔG_{ads} by [14]:

$$C_s = C_b \int_0^\infty (e^{-\Delta G_{ads}/kT} - 1)dz \qquad (3)$$

where C_s and C_b are the solute concentration on the surface and in the bulk, respectively, and Z is the solute-surface distance. The amount of ΔG_{ads}

depends on the solute configuration on the surface. The primary contribution to ΔG_{ads} is, of course, from solute -surface interactions, but the effect of interactions among adsorbed solute particles is an essential feature that must also be included.

The lateral interactions of primary interest are the electrostatic interactions. These interactions are computed within the framework of the linearized Poisson-Boltzmann equation [14]. In the present approach, the interactions are treated as pair wise additive, and this assumption allows ΔG_{ads} to be decomposed into solute-surface (ΔG_{I}) and solute- solute (ΔG_{II}) contributions, namely:

$$\Delta G_{ads} = \Delta G_{\mathrm{I}} + \Delta G_{\mathrm{II}} \qquad (4)$$

Further simplification results from additional approximations that arise from the fact that, ΔG_{ads} is typically several kT in magnitude for solute close to the surface and decays with increasing Z consequently, the integral in Equation (3) is dominated by those solute particles closest to the surface, specifically, a monolayer of particles differing little in their positions relative to the surface, Z. Thus (ΔG_{II}) can be assumed to be independent of Z, and a function only of the configuration of particles in the plane of the adsorbent surface. Thus Equation (3) may be approximated as:

$$C_s = C_b \int_0^z (e^{-\Delta G_{\mathrm{I}}/kT})dz = C_b K \quad (5)$$

where

$$K = e^{-\Delta G_{\mathrm{I}}/kT} \qquad (6)$$

The Henry's law constant K has the units of length.

The concentration of solute adsorbed on the adsorbed surface (mg/cm^2) can be expressed as:

$$C_s = \frac{N_s(M_M/N_0)}{A} \qquad (7)$$

where N_s are the number of solute particle adsorbed on surface area (A), M_w and N_0 are respectively, the molecular weight and Avogadro number. The area per solute particle adsorbed on surface is expressed as: $\sigma = \dfrac{A}{N_s}$. Then the fractional of coverage of surface is $\theta = \dfrac{\sigma_0}{\sigma}$, where σ_0 the actual coverage area by a solute particle is. For a spherical of particle of diameter d, $\sigma_0 = \pi d^2$, then Equation (7) can be written as:

$$C_s = \frac{\theta M_w}{\pi d^2 N_0} \qquad (8)$$

The molar mass of lysozyme is 14600 g/mole and diameter d for the globular protein, lysozyme at pH=7.2 is $d = 1.2$ nm [15].

MATERIALS AND METHODS
The materials used in the experiment are as follows;

HEMA and SHCL prepared from Bausch & Lomb, Inc. Company, monobasic sodium phosphate and dibasic sodium phosphate- for buffer solutions- Merck Company. Hen white egg lysozyme (cat#107255) with purity >99% and Roche Molecular preparation from Biochemicals Company. The concentration of the adsorbed lysozyme was measured through UV spectroscopy from a calibration curve made for lysozyme solution of known concentrations at wavelength 280 nm [16]. All buffers were freshly prepared for the experiment. HEMA and SHCL were used as contact lenses as adsorbents [17-19]. The solutions were prepared with similar constituents as the human tear [20]. The

lysozyme aqueous solution with the following concentrations of 0.8, 1, 1.2, and 1.4 mg/ml, were prepared using double distilled and deionized (Milli-Q treated) water. The concentration of NaCl was in the range of 0.05 - 0.2 M [21]. The contact lenses were placed in phosphate buffer solution, pH=7.2 [22] similar to that of the tears [23]. The lenses were placed in the solution and kept for five days at temperature 22 ±0.1ºC, to allow the protein adsorption onto the lenses to be completed and to reach the equilibrium state. Samples of solution were taken and the absorbance was measured at 280 nm- M350 Double Beam UV spectroscopy. The concentrations of the adsorbed lysozyme onto the contact lenses were determined through a calibration curve for the known lysozyme concentrations in the solution [24].

RESULTS AND DISCUSSION

Various factors such as protein concentration, pH, ionic strength and temperature affect the quantity and quality of protein adsorption. The amounts of lysozyme adsorbed onto SHCL and HEMA contact lenses were also measured and are shown in Fig.1.
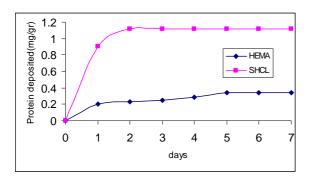

Fig. 1. The effect of protein concentration on adsorption on SHCL and HEMA surfaces, protein concentration is 1.2 mg/ml, pH 7.2, 22ºC.

The temperature was set at 22±0·1ºC, lysozyme concentration is 1.2 mg/ml and the pH of the experiments at 7.2, similar to that of human tears. Lysozyme was chosen as protein in the study. This protein has isoelectric point pH 11, thus this protein has positive charge at pH experiment [25].

In order to obtain Langmuir parameters, we need a different concentration of protein. We made lysozyme with concentration of 0.8, 1, 1.2 and 1.4 mg/ml in phosphate buffer at pH 7.2 and 22±0·1ºC, than the amount of lysozyme adsorbed is measured. Than with plot C/q versus C, we can be obtain Langmuir parameters. In Figure 1, the adsorption is rapid initially and then gradually levels off with longer contact times until it reaches equilibrium state. Approximately 85% of adsorbed mass are reached within 1 day of time for both surfaces. As indicated in Figure 1, the concentration of lysozyme on SHCL and HEMA surfaces is at its highest on the fifth day. The results also indicate that the lysozyme is adsorbed to a greater degree onto the SHCL surfaces. In a previous paper, [26] also obtained strong adsorption of positively charge lysozyme on a positively charged surface. Using a semiconducting tin oxide layer as substrate and varying surface charge by varying the applied interfacial potential, it was found that at pH 9.9, that is, below the isoelectric point of lysozyme, more protein adsorbed as the surface was rendered more positively charged. The results on lysozyme adsorption obtained by [26] are consequently in accordance with the results of this paper. A positively charged protein, even a hard one, can adsorb in high amounts onto negatively charged SHCL surface, as shown in our work by lysozyme at pH 7.2. Between days 0 and 1, the increases adsorbed lysozyme on SHCL rapidly than HEMA surface. Then between days 1 and 5, the amount of lysozyme increase is adsorbed on HEMA slowly, but for lysozyme adsorbed on SHCL, the amount of protein reaches equilibrium and

increase of time the amount of adsorbed is constant. Between 5 to 7 days, the amount of lysozyme adsorbed on both surfaces is constant. The results on adsorption are obtained in accordance with the results of this paper [27]. This anomalous adsorption behavior has been observed before [28]. The reason for this can be explained in terms of the negative surface charge of SHCL, due to the presence of a carboxylate group, and positive lysozyme charge at pH 7.2 [29]. The HEMA surface, on the other hand, has no charge, thereby less affinity for protein adsorption [30], also observed this behavior [31]. They adsorbed cytochrome C from a solution onto $Si(Ti)O_2$ surfaces.

Fig. 2 demonstrates the effect of the ionic strength of the solutions on lysozyme adsorption. In these experiments, NaCl - the major electrolyte in the human tears, 0.05-0.2 M was added to the protein solutions (lysozyme concentration 1.2 mg/ml).
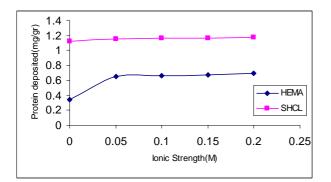

Fig. 2. The effect ionic strength of solutions on SHCL and HEMA surfaces, at pH 7.2 and 22ºC concentration of protein is 1.2 mg/ml.

Fig. 2 shows the effect of the solution's ionic strength on the amount of adsorption of protein onto SHCL and HEMA surfaces. The influence of ionic strength on the adsorption has been studied by determining protein adsorbed on SHCL and HEMA surfaces at four concentrations of NaCl, 0.05, 0.1, 0.15 and 0.2 M. The amount of lysozyme adsorbed, increasing

concentration NaCl, would lead to a lower affinity between the lysozyme and both surfaces at pH 7.2. This can best be tested at low surface coverage, where the shape of the figure is essentially determined by the lysozyme-surface interaction. The electrolyte concentration primarily exerts its influence on protein adsorption in a different way, for example, by affecting the conformational stability of the protein and or being adsorbed simultaneously [29]. We are not able to draw conclusions about the influence of concentration of NaCl, on the interaction between the lysozyme and SHCL surface [32]. As can be seen upon an increase in the concentration of the protein, the adsorption increases, a phenomenon attributed to the favorable orientation of protein molecules in the presence of sodium ion which causes more protein attraction per surface area of both SHCL and HEMA [15, 33]. However, the electrolyte concentration does not have a significant effect on the amount of protein adsorption; this is probably due to the competitive adsorption of lysozyme and NaCl on the hydrogel surfaces.

Fig. 3 illustrates the pH effect on the adsorption of lysozyme on surfaces of AA and HEMA. In Figs. 4 and 5, the fractional coverage of SHCL and HEMA surfaces, based on Equation (8), are shown versus protein concentration at pH 7.2 and 22±0·1ºC.
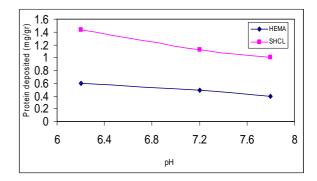

Fig. 3. The effect of pH on HEMA and SHCL surfaces, at pH 7.2 and 22ºC, concentration of protein is 1.2 mg/ml.

Fig.3 shows the effect of pH on lysozyme adsorption onto SHCL and HEMA surfaces. The effect of solution pH on protein adsorption appears to be clear and depends on the physicochemical properties of a protein, that is, size, dimensions and electrostatic charge [34]. The maximum lysozyme adsorption occurred at pH 6.2 and the lysozyme adsorbed at pH 7.8 was minimally onto both surfaces. Lysozyme has positive charge at pH 6.2 and is an isoelectric point of pH 11.1 [25]. Hence the charge of lysozyme is positive throughout the pH range used in this investigation. In comparison with SHCL and HEMA surfaces, SHCL is readily expectable that the strongest electrostatic interaction may occur around pH 6.2. But at this pH (6.2), the surface charge of HEMA is neutral and the amount of lysozyme adsorbed in this surface is less than SHCL surface. With increase in pH protein solution (to 7.8), lysozyme has positive charge, but the magnitude of positive charge, much less pH 6.2. At this pH (7.8), the amount of lysozyme adsorbed is reduced for both surfaces. An increase in pH causes a decrease in the adsorption rate. Also, as expected the amount of adsorption is observed to decrease with increasing pH protein solution. The reason for which can be explained in terms of the lysozyme reduced surface charge as the pH of the protein solution increases. Figure 3 also illustrates the greater adsorption onto SHCL surfaces as opposed to the HEMA surfaces. This behavior was also observed for lysozyme onto Octacalcium phosphate crystal film [5].

Figs. 4 and 5 are showing the fractional coverage of surface versus concentration of protein on the surfaces. These figures show that as the protein concentration increases so does the surface coverage (note the different scales at the axis). According to the random sequential adsorption (RSA) model [35], there is a maximum surface coverage beyond which further adsorption becomes impossible (54.7% coverage for spherical particles). RSA has been successfully used to explain and understand lots of the experimental result [36]. In concordance with the previously published results [37]. At low coverage of both surfaces, the shape of the isotherm is essentially determined by the protein-surface interaction. At high coverage, lateral interactions between adsorbed protein molecules may also play a role in the adsorption process [37].

Fig. 6 shows the effect of temperature on protein adsorption onto SHCL and HEMA surfaces for a 0.8 mg/ml lysozyme concentration.

Table 2 reports the values for Langmuir parameters for SHCL and HEMA surfaces.

Fig. 6 shows the effect of temperature on the lysozyme adsorption. An increase in temperature, to some extent (37°C), increases lysozyme adsorption rate on both surfaces, but at higher temperatures where denaturation of the protein occurs and the amount of protein adsorption is reduced. However, the highest adsorption is at 37±0·1°C, which is the human body's physiologic temperature. This behavior was also observed by Roscoe et al. [37].

The values of Langmuir parameters (K_d and q_m) shown in Table 2, for SHCL surfaces are higher than those of HEMA surfaces. This indicates the higher dissociation coefficient of the protein-surface and the maximum protein binding capacity of the SHCL surface. The higher value of q_m on SHCL surfaces as compared to HEMA is indicative of the higher amount of adsorbed protein on SHCL surfaces. Also the higher K_d value for SHCL surfaces as compared to HEMA leads us to believe that there exists strong binding between lysozyme and the surface of the SHCL contact lenses. The main reason for which can to attributed to the

presence of positive charges on lysozyme protein (under experimental pH) and negative charge of the SHCL contact lens surface. In concordance with the

previously published results [38-43] similar findings were noted, however, in the case of non-ionic HEMA and lysozyme.
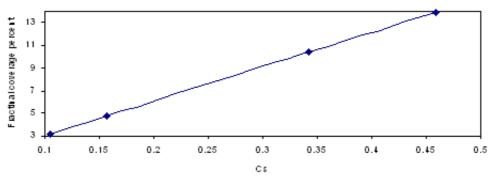

Fig. 4. The fractional coverage surface HEMA versus concentration of protein on the surface at pH 7.2 and 22ºC.
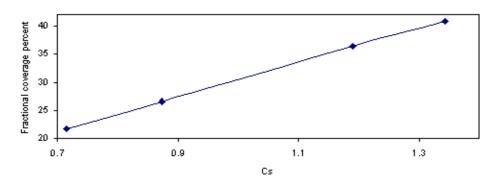

Fig. 5. The fractional coverage surface SHCL versus concentration of protein on the surface at pH 7.2 and 22ºC.

Table 2. The Langmuir parameters for HEMA and SHCL surfaces at 22 ºC, pH 7.2.

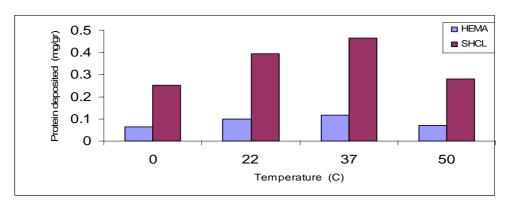| Samples	$q_m(mg/g)$	$K_d (mg/ml$
HEMA	*0.1289*	*1.7944*
SHCL	7.6162	9.3427

Fig. 6. The effect of temperature (ºC) on protein adsorption on SHCL and HEMA surfaces, concentration of protein is 0.8 mg/ml at pH 7.2.

CONCLUSION

UV-Vis spectroscopy was used to determine the amount of lysozyme adsorbed onto SHCL and HEMA hydrogel surfaces. The effect of temperature, pH, ionic strength and concentration of protein on the rate of adsorption were examined. Lysozyme adsorbed higher on SHCL surface than HEMA surface at all pH experimental. Lysozyme on the other hand, has its isoelectric point at pH 11.1 and is thus strongly positively charged at pH 7.2. Also, the AA surface carries a negative charge at this pH. Thus, positively charge lysozyme is adsorbed in higher amounts on SHCL surface. This anomalous adsorption behavior has been observed before. But, lysozyme adsorption less on HEMA surface, because this surface is non ionic compound. The based on the study's findings, through an increase in the concentration of the lysozyme protein, the amount of adsorption onto HEMA and SHCL surfaces increased at constant temperature and pH. As for the effect of pH, it can be said that as a result of an increasing in pH the amounts of protein adsorption will decreased by the HEMA and SHCL surfaces. This finding leads researchers to believe that through the preparation of contact lens washing solutions with a low pH, less than that of tears, one can decrease the amount of protein adsorption. As for the effect of ionic strength, with an increase in the ionic strength of protein solutions, the amount of protein adsorption will be increased. However, the amount of lysozyme adsorption is not influenced as a result of an increase in the solution's electrolyte concentration. The Langmuir adsorption isotherm was applied and the constants of this isotherm were evaluated. The K_d and q_m values for lysozyme protein and SHCL surfaces were higher than those for the HEMA surfaces, due to the presence of a positive charge on the lysozyme protein and a negative charge on the SHCL surface. The results of adsorption were discussed in terms of the protein and hydrogel surface properties.

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