

The Effects of Concentration, pH, and Ionic Strength on Lysozyme Adsorption onto AA and HEMA Contact Lenses

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ABSTRACT

Due to the presence of polar monomers acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) in the conventional hydrogel contact lenses, the major protein component of the human tear, lysozyme is extensively adsorbed onto their surfaces. The adsorption of lysozyme onto the surface of contact lenses leads to limitations in their applications. The presence of electrolytes in the human tear, in particular NaCl, affects the adsorption of lysozyme. The present study measures the concentration of lysozyme adsorbed from solutions with similar concentrations of artificial tear onto the surfaces of AA and HEMA hydrogels by UV spectroscopy. The adsorption results are treated by the Langmuir adsorption isotherm and the constants of this isotherm are evaluated. The effect of various factors such as protein concentration, ionic strength, pH and temperature on the adsorption of lysozyme are examined and discussed in the light of the obtained results.

Key Words:

protein adsorption;
acrylic acid;
2-hydroxyethyl methacrylate;
Langmuir isotherm;
contact lenses;
hydrogels.

INTRODUCTION

Although the principles involved in the formation of hydrogel polymers have not come to light in the recent years, it has been since the late 1970s that their potentials have begun to be realized. There is no precise and limiting definition of the term hydrogel

and problems always arise when attempts are made to apply such definitions to the range of materials that are encompassed by the term. Possibly the most entailing description that could be formulated is that, hydrogels are water-swollen poly-

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mer networks of either natural or synthetic origin. It is the cross-linked, covalently bonded, synthetic hydrogels whose biomedical application has grown most dramatically in recent years.

In biomedical applications, acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA), offer the greatest advantage over most other hydrophilic gels commonly encountered with regard to stability to various parameters, e.g. pH, and temperature. The amount of water absorbed by these polymers is expressed as the equilibrium water content (EWC) [1]. The EWC is the most significant single property of the gel since it is the water held within the polymer substrate that gives hydrogels their unique properties. Thus, the permeability of hydrogel, their mechanical properties, their surface properties and the resultant behaviour at biological interfaces are all a direct consequence of the amount and nature of water held in this way [2]. In order to increase the water content and the oxygen permeability of contact lenses the polar monomer acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) are polymerized into hydrogels. Hydrogels AA and HEMA 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 in some cases can 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 hydrogel contact lenses, due to its low molecular weight and the fact that it is positively charged at physiological pH, while hydrogel 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].

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, acrylic acid AA and 2-hydroxyethyl methacrylate HEMA. The results were examined by Langmuir adsorption isotherm and constants of this isotherm were evaluated.

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 [10]:

$$q = \frac{q_m \times C}{C + K} \quad (1)$$

Where, q (mg/g) and C (mg/mL) are the equilibrium concentration of protein in solid adsorbent and liquid bulk phases, respectively. Constant q_m (mg protein adsorbed/g adsorbent) and K (mg protein adsorbed/mL volume of protein solution) are Langmuir parameters. The constant q_m represents the maximum binding capacity and K is the coefficient for the solute-adsorbent complex formation, which represents the affinity between the solute and the adsorbent.

The Langmuir isotherm for the adsorption of solute from liquid solution was first expanded directly from the corresponding isotherm of gas-solid adsorption and was later derived thermodynamically, kinetically, and stoichiometrically. All these derivations are based on a few common assumptions, namely: (i) all binding sites are equivalent, distinguishable and independent, (ii) each binding site combines with only one solute molecule, (iii) a molecule adsorbed onto one binding site does not influence the adsorption of another molecule on a neighbouring site [11].

The Langmuir isotherm has been widely accepted as a practical method for integrating experimental data of protein adsorption onto hydrogels surfaces [11]. It is, therefore, more favourable to obtain the coefficient (K) and maximum protein binding capacity (q_m) by rear-

ranging eqn (1). This gives a linear equation as follows:

$$\frac{C}{q} = \frac{K}{q_m} + \frac{1}{q_m} C \quad (2)$$

Graph of C/q versus C gives a line of an intercept of K/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.

EXPERIMENTAL

Materials and Methods

The materials used in the experiment were as follows; HEMA, Sigma Company USA; AA, ethylene glycol dimethacrylate (EGDMA), ammonium peroxydisulphate (APS), sodium disulphite (SDS), monobasic sodium phosphate and dibasic sodium phosphate for buffer solutions were obtained from Merck Company, Germany, and Hen white egg lysozyme (cat#107255, purity >99%) was obtained from Roche Molecular Biochemicals Company Germany.

The concentration of the adsorbed lysozyme was measured by UV spectroscopy (M350 double beam UV spectrometer) from a calibration curve made for lysozyme solution of known concentrations at wavelength 280 nm [8]. All buffers were freshly prepared for the experiments. HEMA and AA were used as monomers, APS and SDS as initiators and EGDMA as a cross-linking agent. The hydrogels were prepared by the free radical solution polymerization in the presence of APS, SDS and EGDMA [12-14]. The hydrogels were made in the size of commercial lenses with a diameter of 12 mm. The solutions were prepared with similar constituents as the human tear [15]. The lysozyme aqueous solution with the concentrations of, 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 [16]. The contact lenses were placed in phosphate buffer solution, pH 7.2 [17] similar to that of the tears [15] and kept for five days at temperature 22 ± 0.1 °C, 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. The concentrations of the adsorbed lysozyme

onto the contact lenses were determined through a calibration curve for the known lysozyme concentrations in the solution [18].

RESULTS AND DISCUSSION

Table 1 reports the values of Langmuir parameters (K and q_m) for lysozyme adsorption on AA and HEMA surfaces at 22 ± 0.1 °C and pH 7.2. These parameters are evaluated from a plot of C/q versus C according eqn (2) for lysozyme adsorbed on AA and HEMA. From these results it is evident that lysozyme is adsorbed to a greater extent on the AA surface. This is indicated by the higher coefficient (K) of lysozyme on the AA surface. The higher value of q_m on AA surface as compared to HEMA proves that higher amount of lysozyme is adsorbed on AA surface. The reason for this can be explained by the presence of positive charge on lysozyme (at pH 7.2) and negative charge of the AA surface. This observation is in concordance with the previously published results [19, 20] and it is confirmed by the results presented in Figure 1 where it shows the positively charged lysozyme is strongly adsorbed on the negatively charged AA surface. Figure 1 also shows that the first stage of lysozyme adsorption (day 1) on both AA and HEMA surface is a very rapid process and then it is slower in the second stage, days 1 to 3, and then approaches a maximum and remains constant between days 5 to 7.

The values of q_m as presented in Table 1, 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 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 or

Table 1. The Langmuir parameters for AA and HEMA surfaces at pH 7.2 and temperature 22 ± 0.1 °C.

Monomer surface	q_m (mg/g)	K (mg/mL)
HEMA	0.1289	1.7944
AA	7.6162	9.3427

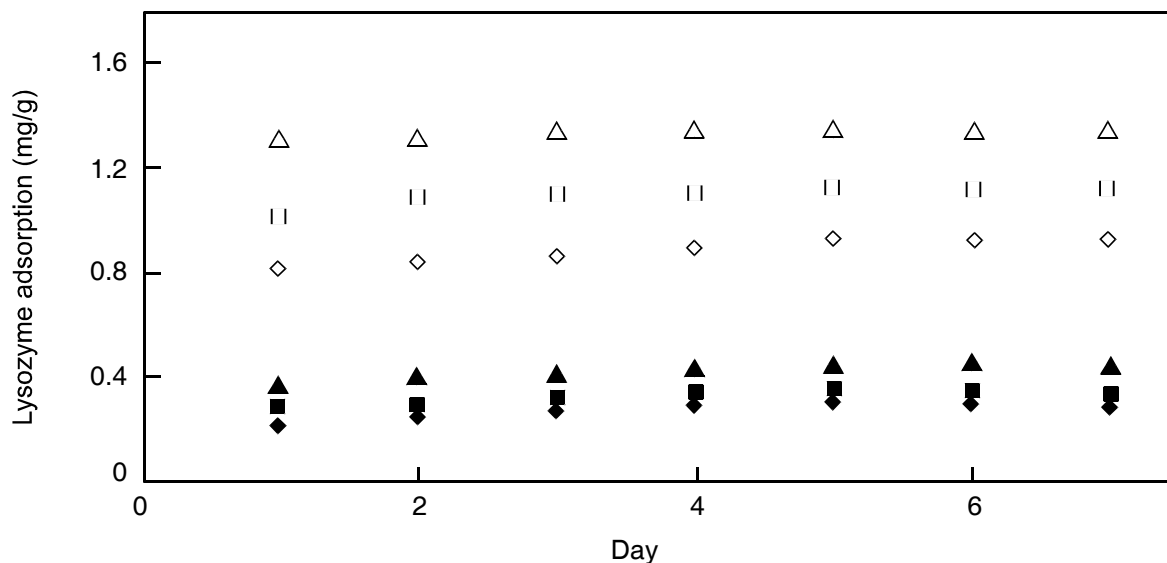


Figure 1. The effect of lysozyme concentration on adsorption onto AA and HEMA surfaces, (lysozyme concentration 1, 1.2, 1.4 mg/mL, pH 7.2 and temperature $22 \pm 0.1^\circ\text{C}$).

(◆) 1.0 mg/mL AA; (■) 1.2 mg/mL AA; (▲) 1.4 mg/mL AA; (◇) 1.0 mg/mL AA; (□) 1.2 mg/mL AA; (△) 1.4 mg/mL AA).

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 , 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.

Figure 2 shows the effect of ionic strength on lysozyme adsorption to the AA and HEMA surfaces measured on the day 5 after attainment of equilibrium. As it is seen from this figure by increasing the concen-

tration NaCl in lysozyme solution (1.2 mg/mL) the ionic strength of the protein solution increases and this leads to slightly higher equilibrium adsorption of lysozyme on AA surface, whereas increasing the ionic strength of lysozyme solution (1.2 mg/mL) has a considerable effect on the equilibrium adsorptions of lysozyme on HEMA surface. It has been reported that the electrolyte exerts its influence on protein adsorption by affecting the conformational stability of the protein as being adsorbed [21]. This phenomenon is attributed to the favorable orientation of lysozyme molecules in

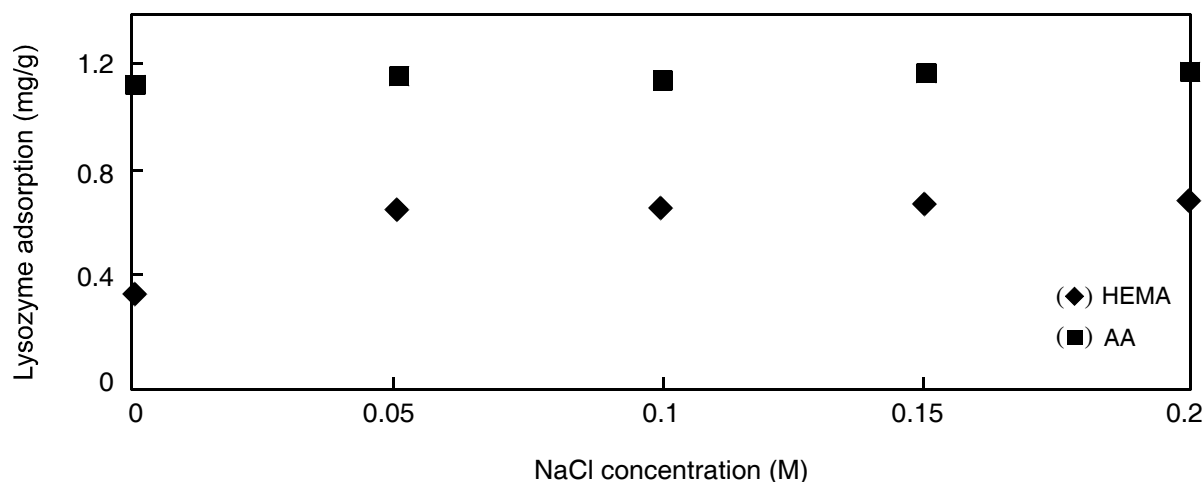


Figure 2. The effect of ionic strength of lysozyme solutions on adsorption onto AA and HEMA surfaces, at pH 7.2 and temperature $22 \pm 0.1^\circ\text{C}$ (lysozyme concentration 1.2 mg/mL).

the presence of sodium ion, which causes more lysozyme attraction per surface area of both AA and HEMA [22, 23]. The results presented in Figure 2 are in agreement with these observations. Since lysozyme has been adsorbed on the AA surface strongly, and the surface is saturated with this protein, therefore, increasing the ionic strength of solution has a small effect on adsorption of lysozyme on AA surface. While on HEMA surface, lysozyme is not strongly adsorbed and at equilibrium only a small fraction of surface is covered by the protein. Then an increase on electrolyte concentration has a significant effect on the amount of protein adsorption on the HEMA surface. But at higher concentration of electrolyte, probably due to the competitive adsorption of lysozyme and NaCl on the HEMA surface, the equilibrium adsorption does not change significantly.

Figure 3 shows the effect of pH on lysozyme adsorption onto AA and HEMA surfaces. The effect of solution pH on lysozyme adsorption appears to be evident and depends on the physicochemical properties of the protein, such as its electrostatic charge [24]. In the pH range used in this work, the maximum lysozyme adsorption occurred at pH 6.2 and the minimum lysozyme adsorption was at pH 7.8 onto both surfaces. This can be explained by the fact that lysozyme has an isoelectric point at pH 11.1 [25]. Hence lysozyme has positive charge in the pH range used in this investigation. In comparison, it is readily expected that the strongest electrostatic interaction may occur around pH 6.2 for AA surface. However, as HEMA has no surface charge the amount of lysozyme adsorbed on this sur-

face is less than AA. By increasing the pH to 7.8, the positive charge of lysozyme decreases in magnitude and then it will be adsorbed less than that of pH 6.2. In over-all, Figure 3 illustrates the greater adsorption of lysozyme onto AA surface compared with the HEMA surface which is in agreement with similar results obtained by Lee et al. [26] for the adsorption of lysozyme onto octacalcium phosphate crystal films.

Figure 4 shows the fractional coverage of AA and HEMA surface versus the amount of the adsorbed lysozyme q (mg/g). The fractional coverage is defined as [27]:

$$\theta = \frac{A_0}{A} = \frac{N_s \sigma_0}{A} \quad (3)$$

Where, A is the surface area of the absorbent, A_0 is the area covered by the adsorbed molecules, N_s is the number of adsorbed molecules on the surface and σ_0 is the actual surface area of a molecule. For a spherical protein molecule of diameter d , the actual surface area of a molecule can be approximated as $\sigma_0 \approx d^2$ and N_s can be expressed in terms of concentration of adsorbed protein q (mg/g) and its molecular weight \bar{M}_w (g/mol) in the following form:

$$N_s = \left(\frac{W_q}{W_w} \times 10^{-3} \right) N_0 \quad (4)$$

Where, N_0 is the Avogadro's constant ($N_0 = 6.02 \times 10^{23} \text{ mol}^{-1}$) and W is the weight of adsorbent (g). On the other hand from Eqns (1), (3) and (4) the fractional

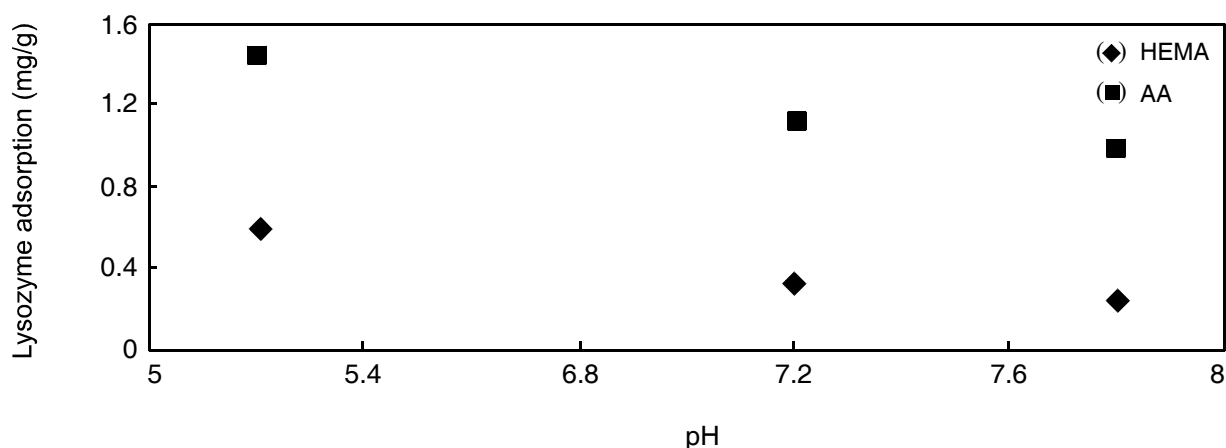


Figure 3. The effect of pH on equilibrium lysozyme adsorption on HEMA and AA surfaces, temperature $22 \pm 0.1^\circ\text{C}$ (lysozyme concentration 1.2 mg/mL).

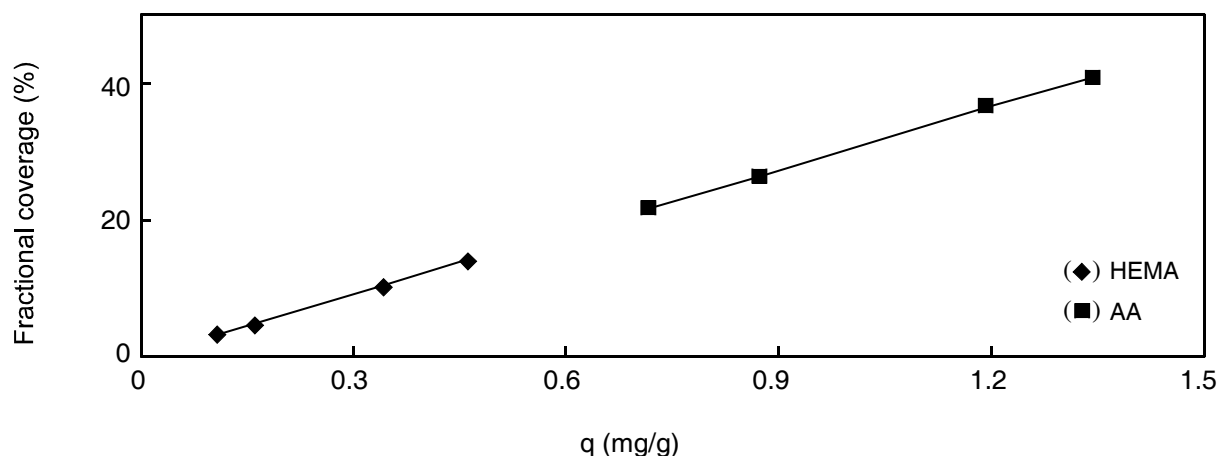


Figure 4. The fractional surface coverage percent of AA and HEMA versus concentration of adsorbed lysozyme, at pH 7.2 and temperature $22 \pm 0.1^\circ\text{C}$.

coverage can be expressed as [27]:

$$\theta = \frac{q}{q_m} = \frac{10^{-3} N_0 d^2 W q}{A \bar{M}_w} = \left(\frac{10^{-3} N_0 d^2}{\sigma \bar{M}_w} \right) q \quad (5)$$

Where, q_m is the maximum protein concentration on the surface given by Langmuir isotherm (eqn 1) and $\sigma_s (\text{cm}^2/\text{g}) = A/W$ is the specific surface area. σ_s is an important characteristic of an adsorbent, and can be evaluated via eqn (5) and by utilizing the results obtained in this work. The \bar{M}_w of lysozyme is 14600 g/mol and the diameter of the globular protein lysozyme at pH 7.2 is $d=1.2$ nm [25]. Therefore, the values of σ_s for AA and HEMA surfaces are evaluated, respectively, as 8.8130 and 3.4866 (cm^2/g). These values indicate that AA hydrogel has a higher available

surface area for protein adsorption. However, the random sequential adsorption (RSA) model [28], predicts a maximum surface coverage for an adsorbent beyond which further adsorption becomes impossible. This maximum surface coverage is about 54.7% for spherical particles. RSA has been successfully used to explain many of the experimental results [29]. The maximum surface coverage for AA is 40.87% and for HEMA is 13.94% which is in agreement with RSA model and also it is in concordance with the previously published results [30]. It is worth noting that at low surface coverage, the adsorption is essentially determined by the protein-surface interaction, but at high surface coverage, the lateral interactions between the adsorbed protein molecules may play a determining role in the adsorption process [30].

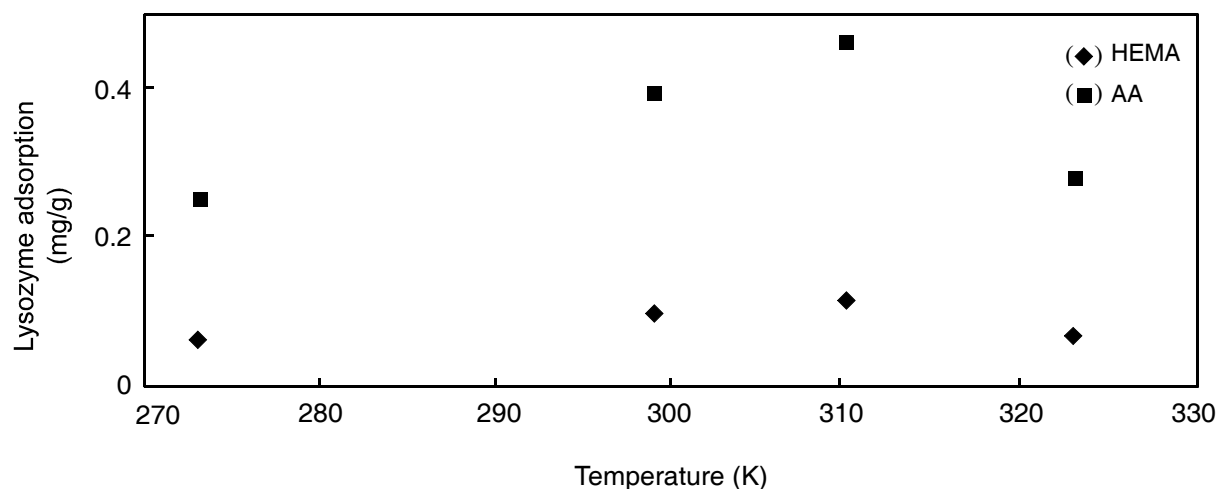


Figure 5. The effect of temperature (K) on lysozyme adsorption on AA and HEMA surfaces, (lysozyme concentration 0.8 mg/mL) at pH 7.2.

Figure 5 shows the effect of temperature on the lysozyme adsorption. An increase in temperature, to some extent (310 K), increases lysozyme adsorption on both surfaces. But at higher temperatures where denaturation of the protein occurs, the amount of protein adsorption is reduced. However, the highest adsorption is at 310–0.1K, which is the human body physiological temperature. Similar results were obtained by Roscoe et al. [31].

CONCLUSION

UV Spectroscopy was used to determine the amount of lysozyme adsorbed onto AA and HEMA hydrogel surfaces. The effect of temperature, pH, ionic strength and concentration of protein on the adsorption were examined. Lysozyme was adsorbed higher on AA surface at the studied pHs. This was explained by the fact that the lysozyme isoelectric point is at pH 11.1 and it is thus strongly positively charged at pH 7.2. Also the AA surface carries a negative charge at this pH. Thus, positively charge lysozyme was adsorbed on AA surface in higher amounts. The results indicated that lysozyme concentration increased the amount of adsorption onto both HEMA and AA surfaces at 22–0.1°C and pH 7.2. As for the effect of pH the amounts of protein adsorption decreased at higher pHs. Therefore, through the preparation of contact lens washing those with a low pH solution, less than that of tears, can decrease the amount of protein adsorption. The ionic strength of protein solution increased the amounts of protein adsorption. However, the amount of lysozyme adsorption is not influenced by the electrolyte concentration significantly. The Langmuir adsorption isotherm was applied to treat the adsorption results and the constants of this isotherm were evaluated. The coefficients K and q_m values for lysozyme adsorption on AA surface were higher than those for the adsorption on to the HEMA surface.

The results of adsorption were discussed in terms of the protein and hydrogel surface properties.

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