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The Effect of Polar Copolymers in Protein Adsorption on Contact Lenses Based on 2-Hydroxyethyl methacrylate (HEMA)

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ABSTRACT

For the purpose of increasing the permeability to oxygen and equilibrium water content in contact lenses, polar copolymers with nonionic monomers such as 2-hydroxyethyl methacrylate (HEMA) is used. By adding the polar monomers to (HEMA), the permeability to oxygen and equilibrium with water content is increased and also the adsorption of proteins on lens surface is changed. In this research, the acrylic acid (AA) was added to (HEMA) monomers in versatile percents and was applied as surface.

Three types of proteins incluging: Albumin, Lactoferrin and Lysozyme and UV-VIS spectroscopy is applied at 280 nm for the monitoring of adsorption on these surfaces. The amount of adsorption of these three proteins in different pH values (4.8, 6.8, and 8.8) is evaluated and the effect of pH on the protein adsorption is studied. By adding the polar monomer to the (HEMA) surface the adsorption of protein was changed and the amount of adsorption depends to pH.

Keywords: Protein adsorption, pH of protein solution, Contact lenses, Equilibrium water content

INTRODUCTION

Usage of contact lenses is increasing due to their several benefits. One of the problems in applying contact lenses is their opacity due to the adsorption of proteins.The study of protein adsorption in Contact lenses [1], Biosensors [2], Relaxation and controlling the drug [3], is done in this research.

It is well known that oxygen transport through contact lenses is of great importance for the proper functioning of comea. Oxygen permeability is a characteristic of the lens, which depends on temperature, pressure, water content, and thickness. When a contact lens is placed on the eye, the oxygen flux is altered and the normal corneal metabolism can no longer be maintained. Thus, oxygen permeability of soft contact lens has been a critical issue of performance of contact lens. It is shown that for better suitable hydrogel materials with improved oxygen permeability, silicone acrylate monomers and their analogs have been incorporated in hydrophilic polymers .In the studies by Yu-Chin, the resultant silicone-hydrogels showed higher oxygen permeability and increased tear strength [4].

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Hydrogel is coherent 3-dimensional polymeric networks that can absorb large quantities of water without dissolution of the polymer network. (Bruck SD, 1973), and currently is applied to be contact lens material. Kita et al (1990) showed an extensive study on Polyvinyl alcohol (PVA) hydrogel as soft contact lens material. PVA solution was mixed with solution containing water and water-miscible organic solvents to prepare PVA hydrogel. The experiment result showed that mechanical properties, such as tensile strength and elongation before failure, were stronger than other CL materials available. In addition, the oxygen permeability and water content were comparable to those of MMA/VA copolymer. Protein adsorption was significantly lower than that of other materials, and application of PVA hydrogel soft lens in rabbit eyes for 12 weeks resulted in no abnormal findings.

many researches try to improve physiological performance of lenses by many different ways, mainly by surface modifications and by introduction of new polymer hydrogel [5].Hydrogel is a type of polymer that can absorb water. The amount of water absorbed by hydrogel is called equilibrium water content (EWC) [6].

In this research, the polymers prepared from (HEMA) and (HEMA+AA) copolymers, are shown in the form of (HEMA) and (HEMA+AA), respectively. Also by addition of AA monomer to (HEMA), the considered surface is produced and the amount of protein adsorption at 280 nm is determined by UV-VIS spectroscopy.

EXPERIMENTAL

The materials used in the experiments were as follows:

The AA (molecular weight 72.06 g/mole), and HEMA (molecular weight 130.14 g/mole) .surfaces were prepared separately by the free radical solution polymerization in the presence of ammonium peroxo disulfate (APS), and sodium disulfite (SDS) for initiator; 0.01g ethylene glycol dimethacrylate (EGDMA) for the purpose of cross-link agent is used, for AA and 0.03g EGDMA (all from Merck company) for HEMA (from Sigma company) were used. Albumin, (cat#A-6418, with a purity>99.9%), Lactoferrin, (L-4860, with a purity>99.9%), from Sigma company, USA and lysozyme, (cat#107255, with purity>99.9%, roche molecular biochemical company, Germany) were purchased.

For preparing the (HEMA) and (HEMA+AA) surfaces, APS, SDS and EGDMA are used. Specific amount of (HEMA) monomer by weight percents of 0, 1, 2, 3, 4 and 5 is added to it. Then APS, SDS as a radical agent and EGDMA as a cross-link agent are added and the product is heated in thermostatic oven. After the formation of (HEMA +AA) surfaces, they are cut to the thickness of 12 mm similar to contact lenses [7].

The protein solution with concentration of 0.8 mg/ml in phosphate buffer solution with fresh deionized water was prepared and 5 ml of this , is inserted to glass vessels and the surfaceswere placed separately into protein solutions. The amount of adsorption at 280 nm in pH value of 6.8 and temperature of 22±0.1 °C after 5 days was measured by UV-VIS spectrophotometer (M350 Double Beam) .For this purpose, a precipitated lenses and another unprecipitated lenses was used as a reference. In studying the effect of pH on adsorption, solution with pH values of 4.8, 6.8 and 8.8 in phosphate buffer solution for proteins were prepared.

RESULTS AND DISCUSSION

In Figure 1, adsorption changes of albumin, lactoferrin and lysozyme on the (HEMA) surfaces with different amount of AA in pH=6.8 and temperature of 22±0.1 °C is measured .In these surfaces with increasing (AA), the amount of adsorption has been increased except for albumin which is due to mutual interactions between surface and protein molecules.

changes in albumin are less than the other proteins and is almost constant. The albumin molecule has negative charge in experimental pH value and by increasing the negative charge density on surface, the amount of adsorption decreases and then gets fixed. The reason this,is at first, tendency toward adsorption on nonionic surface (HEMA) is high. In terms of lysozyme and lactoferrin, the variations of adsorption are similar and with increasing the AA group, the amount of adsorption increases. But the higher adsorption of lysozyme is due the large isoelectric constant compared to lactoferrin [8]. In these two proteins, the amount of adsorption is increased by increasing the polar group of AA. Positive attraction between adsorbing surface and protein is the most important factor for adsorption. As is shown in this Figure, there is a direct relation between the increases in negative charge density of the surface and adsorption of the two proteins.

In Figure 2, the adsorption of albumin in mentioned pH values were shown. As it is obvious, the maximum adsorption occurs at pH=4.8 and the minimum adsorption occurs at pH=8.8 that is due to the negative surface charge and albumin protein. Also the amount of adsorption is constant by adding the polar group of AA. The amount of adsorption on (HEMA) surface is more than other surfaces. In Figures 3 and 4, the effect of pH on the lysozyme and lactoferrin adsorption is shovn. The

variations are similar in these two proteins and by increasing the polarity at (HEMA) surface, the amount of adsorption increases.

In Figures 3 and 4, the effect pH in albumin solutionand lactoferrin solution on the surface with AA copolymers, is shown. The concentration of proteins are constant (0.8 mg/ml) and temperature is 22±0.1 °C. In this case, the surfaces were added to the protein solutions with pH values of 4.8, 6.8 and 8.8.with increasing pH, the amount of adsorption decreases that is due to charge reduction of protein molecule. The reason for the higher adsorption of lysozyme than lactoferrin is the higher isoelectric constant of this protein.

Nowadays it is known that the lysozyme role in opacity of contact lenses is more than other proteins and the results of this research is the same as other experiments performed in this field.

CONCLUSION

It is observed that in pH=6.8, by adding (AA) polar group to (HEMA) surface, the lysozyme and lactoferrin adsorption is increased. But in the case of albumin, the adsorption first decreases and then gets fixed . This phenomenon is due to electrostatic repulsion between surfaces and albumin. By sttuding the pH effect, it is known that the variations are similar to the previous case and there aren't any definite charges about albumin. But in the case of lysozyme and lactoferrin, increasing pH will decrease the adsorption amount by reducing in protein charge. We can use the results of these experiments in manufacturing the lenses with higher facility.

CAPTION FOR FIGURES

Fig.1 The effect of acrylic acid percent on (HEMA) surfaces, on protein adsorption, pH=6.8, Temperature 22±0.1°C and concentration of proteins is 0.8 mg/ml.

Fig.2 The effect of pH albumin solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of albumin is 0.8 mg/ml.

Fig.3 The effect of pH lactoferrin solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of lactoferrin is 0.8 mg/ml.

Fig.4 The effect of pH lysozyme solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of lysozyme is 0.8 mg/ml.

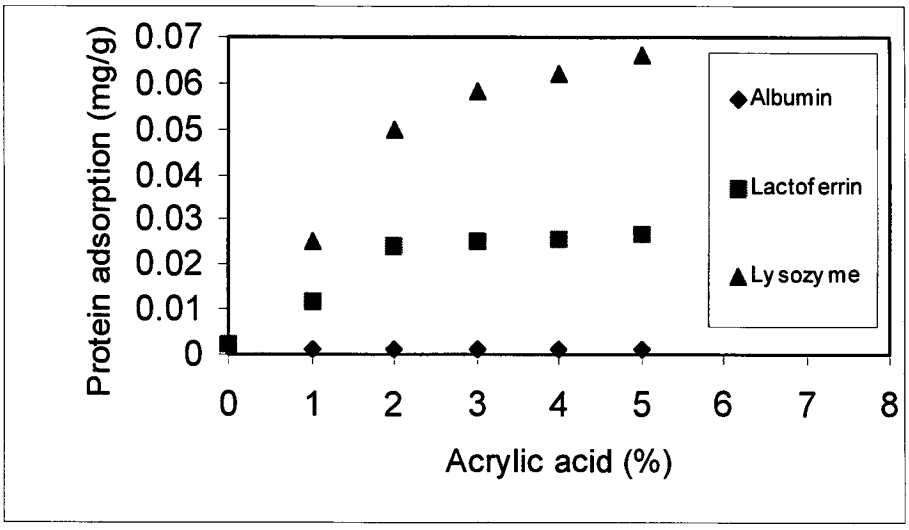

Fig.1. The effect of acrylic acid percent on (HEMA) surfaces, on protein adsorption, pH=6.8, Temperature 22±0.1°C and concentration of proteins is 0.8 mg/ml.

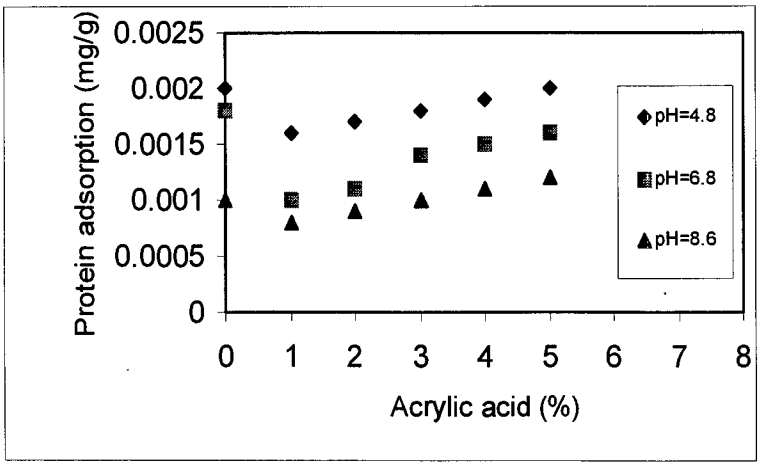

Fig.2. The effect of pH albumin solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of albumin is 0.8 mg/ml.

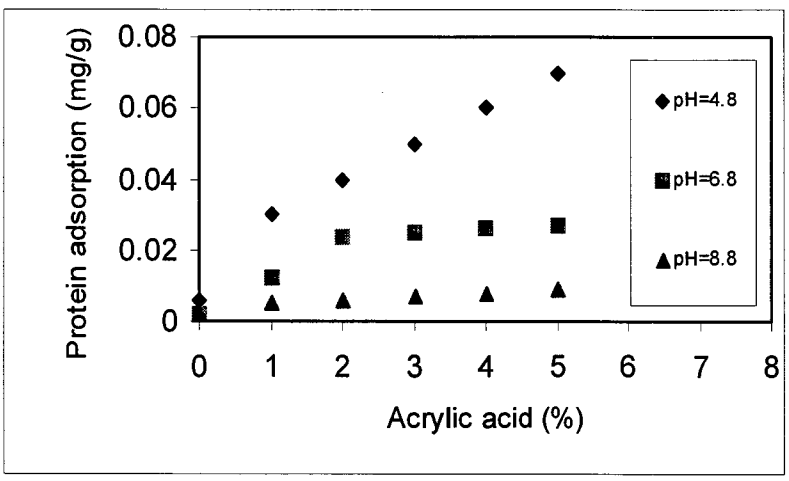

Fig.3. The effect of pH lactoferrin solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of lactoferrin is 0.8 mg/ml.

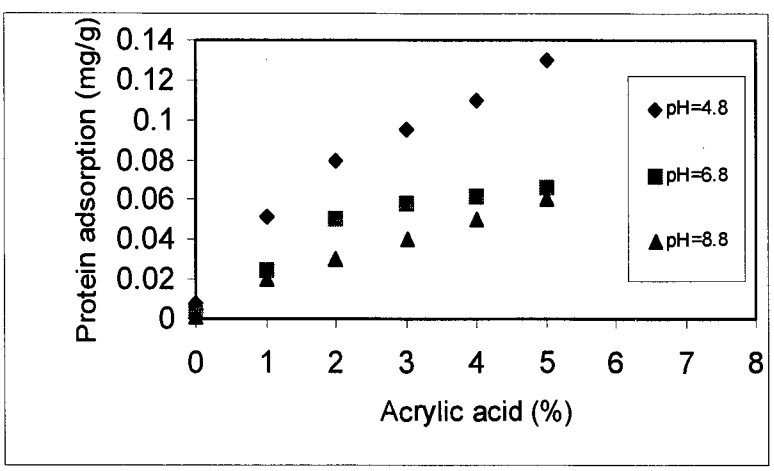

Fig.4. The effect of pH lysozyme solution onto acrylic acid percent on (HEMA) surfaces, pH=6.8, Temperature 22±0.1°C and concentration of lysozyme is 0.8 mg/ml.