EasyChair Preprint

№ 1877

Sorption of Human Serum Albumin on Surface IPN Acrylic Hydrogels Filled with Sodium Hyaluronate

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November 7, 2019

Sorption of human serum albumin on surface IPN acrylic hydrogels filled with sodium hyaluronate

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Abstract. In this work, IPN hydrogels based on a copolymer of acrylic acid and acrylamide filled with various amounts of sodium hyaluronate and bentonite were synthesized. For these hydrogels, the sorption capacity in relation to human serum albumin was studied, because protein sorption is one of the most important stages of cell adhesion. Shown, that due to an increase in the concentration of additional charged carboxylate groups, an increase in sorption capacity to 0.35 g of albumin per 1 g of hydrogel is observed with an increase in the percentage of sodium hyaluronate. With an increase in the proportion of benonite in the hydrogel composite, the sorption capacity for IPN gels filled with 0.05mas.% sodium hyaluronate also increases from 0.27 g to 0.38 g per 1 g of hydrogel.

1. Introduction

Hydrogels, due to their high hydrophilic properties, are biocompatible materials that are close in properties to the tissues of living organisms [1-5] Natural hydrogels have low mechanical properties and high biodegradation rates [6]. The formation of natural hydrogels occurs due to the physical crosslinking of molecules when exposed to temperature or radiation. Chemically crosslinked hydrogels have enhanced mechanical characteristics and hydrophilic properties that can be controlled during synthesis [7-12].

With the development of biotechnology and bioengineering, it became necessary to obtain various substrates for growing tissues and cells [13]. Many types of hydrogels used for these purposes have a neutral surface and are therefore quite inert to cell attachment. However, by producing polymers from monomers having charged functional groups, it is possible to impart a surface charge to the resulting polymers. For medical and bioengineering applications, various IPNs are often used [14]. IPN is a mixture of two or more polymers crosslinked by physical or chemical bonds. In addition, IPNs obtained on the basis of hydrogels with charged monomers are preferred in a number of medical and biological applications because of their specific unique biophysical properties, such as ease of manufacture of various geometric shapes, soft texture, minimal irritation of surrounding tissues, unusual resistance to biological environments [15]. IPN structures are widely used to control the overall hydrophilicity of hydrogel and drug release kinetics [16].

The presence of charged groups in the hydrogel structure will promote cell adhesion, one of the important stages of which is protein sorption by the material [17-19].

Thus, the study examined the sorption of human serum albumin (HSA) on the surface of IPN hydrogels based on acrylic acid (AA) and acrylamide (AAm) with sodium hyaluronate (SH) filler.

2. Materials and methods

2.1. Synthesis of hydrogels

The study prepared interpenetrating nets based on a copolymer of AA and AAm, 1-10% filled with bentonite and 0.01-1% SH. Composites were obtained by radical polymerization. To obtain semi-IPN polymers, acrylic acid was used, which was previously neutralized by 80%, then 3.5 ml of distilled water was added and mixed with AA and AAm in a ratio of 77:23. Methylene bisacrylamide (MBA) was used as a crosslinking agent, which was introduced in an amount of 0.08% by weight of monomers. Fillers of the composite SH and bentonite were introduced in various mass percent. As the redox system, 4 ml (2%) / 4 ml (2%) (NH4) 2S2O8 / TMED were taken.

2.2. Determination of protein sorption

To determine the protein sorption by the obtained hydrogels, a solution of HSA with a concentration of 10 g / L was used.

A portion of the hydrogel was placed in 20 ml of the solution and kept for 24 hours. The amount of albumin was recorded by UV-spectroscopy by help UV / VIS double beam spectrophotometr UNICO (USA). The protein sorption capacity of the hydrogel was determined by the formula:

$$q = \frac{\left(1-\frac{C_0}{D_0}D_t\right)\cdot V}{100\cdot m_0}, \quad (1)$$

where, C_0 is the initial concentration of the HSA solution, g / l,

D_0, D_t is the optical density of the initial solution and at time t, respectively, at a wavelength of 279 nm,

V is the volume of the solution,

m_0 is the mass of dry hydrogel.

2.3. FTIR – spectroscopy

The samples were identified using Fourier transform infrared spectroscopy with a Tensor 37 spectrometer (Bruker, Germany), employing an Attenuated Total Reflection (ATR) accessory, equipped with a diamond crystal cell, angle 45°. The spectra were acquired in the range 4000-600 cm^{-1} at a resolution of 2 cm^{-1} and the signal was averaged over 32 scans. Spectra were obtained by direct measurement.

3. Results and discussions

The object of the study in this work was the study of the adsorption of IPN protein by hydrogels based on acrylic acid and acrylamide with sodium hyaluronate filler. As a model protein, human serum albumin was used, which is a convenient and widely distributed model for studying the properties of globular proteins.

Figure 1 shows the time dependences of HSA sorption on the obtained various hydrogel composites.

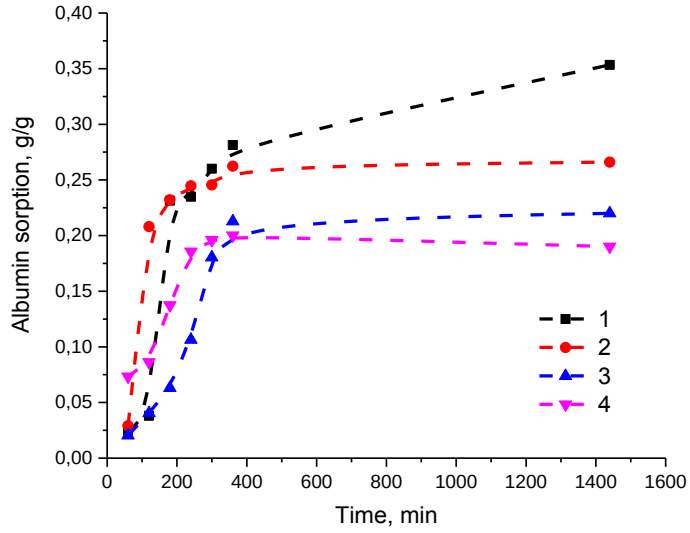

Figure 1. Sorption dependence of HSA (Halbumin / Hydrogel) for different hydrogels: 1 – AA-co-AAm IPN-hydrogel filled with 0,05mas.% sodium hyaluronat; 2 – AA-co-AAm hydrogel; 3 – AA-co-AAm hydrogel filled with 5mas.% bentonite and 0mas.% sodium hyaluronate; 4 – AA-co-AAm IPN-hydrogel filled with 5mas.% bentonite 0,05mas% sodium hyaluronate

Figure 1 shows that most of the albumin is adsorbed in the first 5-6 hours. It is known that sorption of albumin occurs due to the formation of ionic bonds between charged groups of the hydrogel matrix and terminal amino and carboxy grapples of amino acids that bind the domains of albumin. The slowest sorption is AA-co-AAm hydrogel filled with 5mas.% bentonite and 0mas.% sodium hyaluronate, because fewer charged hydrogel groups are involved in protein sorption, because part of the groups binds to the surface of bentonite particles. The slightly lower sorption rate of AA-co-AAm IPN-hydrogel filled with 0.05mas.% sodium hyaluronate compared to AA-co-AAm hydrogel can be explained by a decrease in the free space in the hydrogel cells, however, the sorption capacity of the hydrogel increases due to the introduction of additional charged carboxylate groups of sodium hyaluronate in the composition of the polymer matrix. Due to the presence of charged groups AA-co-AAm, IPN-hydrogel filled with 0.05mas.% Sodium hyaluronate shows the highest sorption capacity for albumin within 24 hours. Protein sorption reaches 0.35 g of albumin per 1 g of hydrogel.

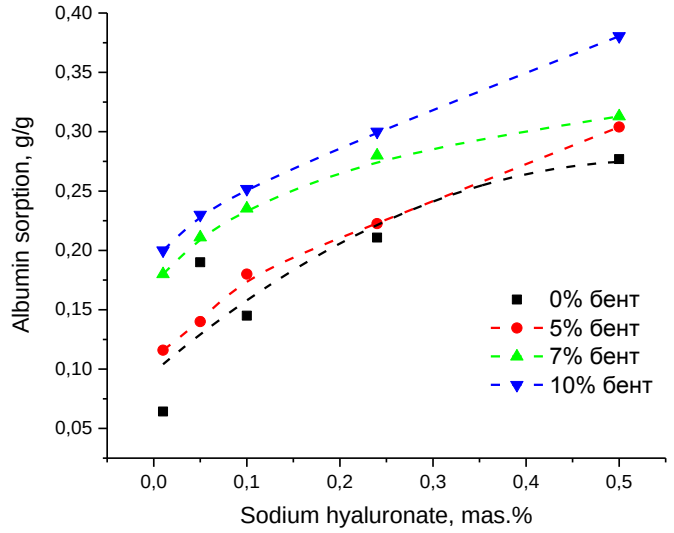

Figure2. Dependence of the absorption of HSA (g of albumin / g of hydrogel) on the concentration of sodium hyaluronate for different concentrations of bentonite

When varying the percentage of bentonite and sodium hyaluronate in IPN, the following dependences of albumin sorption are observed (Figure 2). As can be seen from the Figure 2, with an increase in the concentration of bentonite, the sorption capacity of IPN gels increases, which may be due to an increase in the free surface of bentonite particles, which can participate in protein sorption. With an increase in the concentration of sodium hyaluronate, the sorption capacity of the hydrogel composite also increases, because the number of charged groups of sodium hyaluronate in the composition of the polymer matrix increases.

For hydrogel composites with absorbed albumin, IR spectra of the surfaces of dried hydrogel samples were obtained. As shown in Figure 3, the IR spectrum of the hydrogel surface before protein sorption is significantly different from the spectrum after protein absorption. A significant increase in the optical density at the wave numbers corresponding to the peak of Amid 1 and Amid 2 and the appearance of an explicit peak of Amid A indicates the presence of a significant amount of absorbed protein on the surface of the hydrogel. Comparison of the spectra of dry HSA and dry AA-co-AAm hydrogel after sorption of albumin, shows almost complete coincidence of these spectra (Figure 4).

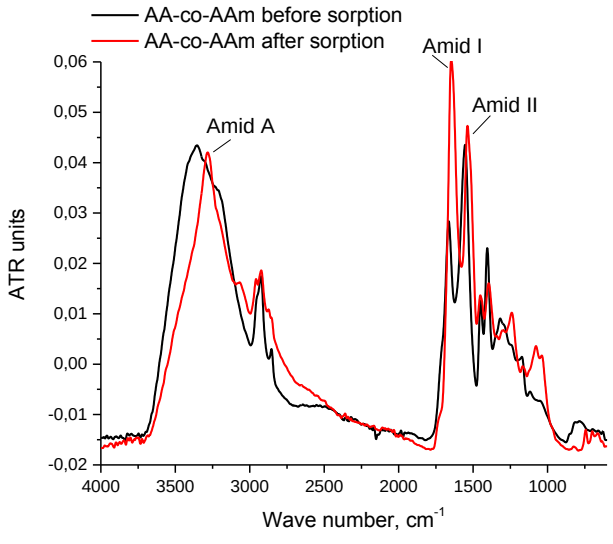

Figure 3. FTIR spectraAA-co-AAm hydrogel befor sorption and after sorption

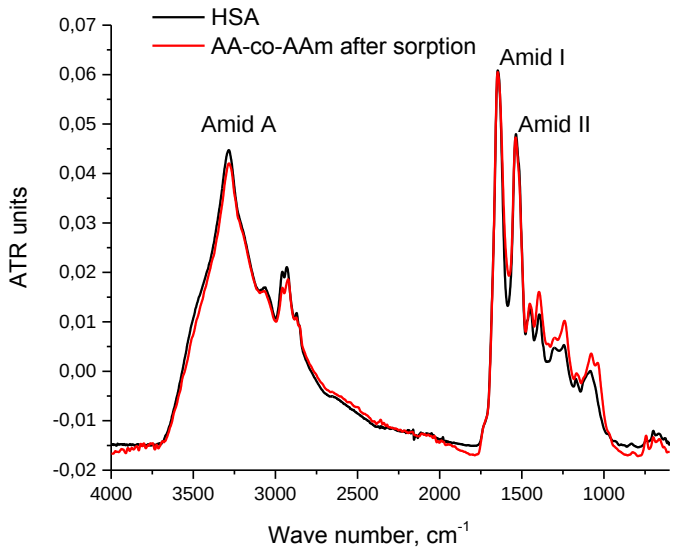

Figure 4. FTIR spectraAA-co-AAm hydrogel and HSA

4. Conclusions

In this work, we studied the effect of the composition of IPN hydrogel based on a copolymer of acrylic acid and acrylamide filled with sodium hyaluronate and bentonite on its sorption capacity relative to HSA. Shown, that with an increase in the percentage of hyaluronic acid, due to an increase in the concentration of additional charged carboxylate groups, an increase in sorption capacity to 0.35 g of albumin per 1 g of hydrogel is observed. With an increase in the proportion of bentonite in the hydrogel composite, the sorption capacity for IPN gels filled with 0.05 mas.% sodium hyaluronate also increases from 0.27 g to 0.38 g per 1 g of hydrogel.

5. References

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