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Albumin and IgG removal applications with gelatin based biosorbents

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Abstract:

Removal of the highest amount of albumin and IgG in the blood plasma for the determination of disease-specific proteins is of great importance in the field of diagnosis and treatment. In this study it is aimed to prepare the biosorbent to be used as a support material in affinity chromatography. Gelatin biosorbents were prepared by water in oil emulsion method and stabilized with glutaraldehyde as a biofunctional crosslinker to obtain spherical biosorbents. Prepared gelatin biosorbents were characterized by optical micrograph and SEM (scanning electron microcope). It was determined that the size/size distribution of the cellulose biosorbents prepared under optimal conditions was on average 3-10 µm. The results revealed that 85,7% of albumin and 79% of IgG in the aqua medium was successfully removed with gelatin biosorbents. It is thought that gelatin biosorbents will be very effective in removing albumin and IgG from high concentrations of proteins in the early diagnosis of low concentration proteins.

***Keywords**:* Gelatin, biosorbent, albumin, IgG, bioaffinity chromatography, proteomic.

Jelatin esaslı biyosorbentler ile albumin ve IgG uzaklaştırma uygulamaları

Öz

Hastalığa özgü proteinlerin belirlenmesinde kan plazmasındaki en yüksek derişimdeki albümin ve IgG'nin uzaklaştırılması, tanı ve tedavi bakımından büyük önem taşımaktadır. Bu çalışmada afinite kromatografisinde destek malzemesi olarak kullanılacak biyosorbentin hazırlanması amaçlanmıştır. Küresel biyosorbentler elde

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etmek için, jelatin biyosorbentler, su içinde yağ emülsiyon yöntemi ile hazırlanmış ve biyofonksiyonel çapraz bağlayıcı olarak glutaraldehit ile stabilize edilmiştir. Hazırlanan jelatin biyosorbentler, optik mikrograf ve SEM (taramalı elektron mikroskobu) ile karakterizasyon çalışmaları yapılmıştır. Optimal koşullar altında hazırlanan selüloz biyosorbentlerin boy/boy dağılımının ortalama 3-10 µm olduğu tespit edilmiştir. Sonuçlar, su ortamındaki albüminin % 85,7'si ve IgG'nin% 79'unun jelatin biyosorbentlerle başarıyla uzaklaştırıldığını ortaya koymuştur. Düşük konsantrasyonlu proteinlerin erken teşhisinde yüksek konsantrasyonlardaki proteinlerden albümin ve IgG'nin uzaklaştırılmasında jelatin biyosorbentlerin çok etkili olacağı düşünülmektedir.

Anahtar kelimeler: *Jelatin, biyosorbent, albümin, IgG, biyoafinite kromatografisi, proteomik.*

1. Introduction

The blood plasma is one of the basic material for the diagnosis of diseases. It contains high concentrations of protein (60-80 mg/mL protein). A very sensitive study and good observation should be performed to obtain small amount of proteins in the sample that could be a good marker. Identification of these proteins allow elucidation of mechanisms underlying the disease and normal biological processes with the development and evaluation of the data collected. The removal of high concentration proteins before proteomic analysis obtain advantages to identify marker proteins [1-4].
Proteomics studies require technologies for separation of proteins, quantification and identification of these proteins. Due to the complex nature of the proteome, very strong analytical techniques are needed. There are a wide variety of protein separation methods. Generally used techniques are one and two dimensional electrophoresis, mass spectrometry, capillary electrophoresis, chromatography (reverse phase, ion exchange, affinity etc.) and ultrafiltration techniques [5]. The addition of the affinity chromatography step before electrophoresis or mass spectrometry can be effective in reducing the complexity of proteome analysis [6,7].

Affinity chromatography is a method that used for the identification, purification and separation of macromolecules. It is based on specific and reversible binding ability of molecules in solution to complementary molecules, the ligands, usually immobilized on a solid chromatographic media like the support materials [8].

Gelatin is a biodegradable biopolymer, widely used in biomedical and biotechnological applications and can be prepared with desired properties to produce microparticles. However, due to the aqueous solubility and limited mechanical and thermal properties of gelatin microparticles, improvements, such as crosslinking reactions, are necessary in order to provide the use in long term applications [9-11].

In this study spherical gelatin biosorbents were prepared by the emulsion polymerization method and glutaraldehyde (GA) was used as crosslinker to improve mechanical and thermal properties of gelatin. The prepared biosorbents were then studied for IgG and albumin removal efficiency in aqueous solution in a batch system.

2. Materials and method

2.1 Chemicals

Gelatin (sigma), Olive oil(Komili), Bovine serum albumin (BSA) (Sigma), Immunoglobulin G (IgG) (Sigma), Acetone (Merck),Tween 20(Merck), All the other reagents used were of analytical grade and all solutions were prepared with distilled water.

2.2 Instruments

Scanning Electron Microscopy (SEM JEOL JSM-7600F FEG), UV Vis Spectrophotometer (Thermo Scientific Genesys 10S), Magnetic stirrer (IKA-WERKE RT 10), Refrigerated Centrifuge(MPW-65R).

2.3 Preparation of gelatin biosorbents

In the study stable gelatin biosorbents were obtained by water in oil emulsion method using the glutaraldehyde as crosslinker. Optimal conditions on the morphology, and size distribution of microspheres were studied in our previous study [12]. Briefly gelatin (0,1 g/ml) was dissolved in distilled water in a 37 °C water bath. After the water bath temperature was set at 50-60 ºC, the reactor vessel containing 12.5 ml olive oil (komili) was placed in the water bath. To ensure emulsification, tween 20 (4%)(v/v) was added on olive oil and mixed with a magnetic stirrer. The gelatin solution was added to the olive oil + tween 20 medium and started to be mixed at 750 rpm. Homogenization was provided for 5 minutes. After 10 minutes emulsification time, the reaction vessel was placed in a reaction medium of 4 °C, acetone and 25% GA were added to the medium at the same time. After the 30 min crosslinking time was completed, biosorbents were removed by centrifugation. Excess GA and oil were removed by washing several times with cold acetone. Optimum conditions were determined by this way.

2.4 Characterization of prepared gelatin biosorbents

Biosorbent preaparation with appropriate size and size distribution has a very important place in biosorbent applications. Therefore, in the optimization studies, some biosorbent was suspended in distilled water and dropped on the slide in order to determine the appropriate size and size distribution and stable form biosorbents. In order for the biosorbents to form a single layer, they were covered with coverslip and spread. Each of the synthesized biopolymeric biosorbents was imaged with the aid of an optical microscope (Leica microsystems, DFC 295) and photographed**.**

The size, shape and surface morphology of gelatin biosorbents were examined by scanning electron microscopy (SEM). For this purpose, gelatin biosorbents were attached to the SEM sample plate with a conductive adhesive. Then, the sample surfaces were coated with 200 Å thick metallic gold under vacuum to make the surface conductive. The prepared samples were placed in the SEM sample slot and photographed at various magnification rates (SEM JEOL JSM-7600 F FEG).

2.5 Albumin and IgG removal studies from aqueous solution

Prepared and characterized gelatin biosorbents were treated with buffer solution (pH: 7.4 Borate buffer) containing albumin and IgG, (pH: 7.4 Borate buffer) in the batch system respectively, and max adsorption amounts were determined.

For this purpose, albumin solution was prepared by dissolving 50 mg BSA in pH 7.4 Borate buffer and IgG solution was prepared by dissolving 17 mg IgG in pH 7.4 borate buffer separately. 2 ml of prepared albumin and IgG solutions were mixed with 50 mg gelatin biosorbents at room temperature at 150 rpm. At the end of 5 minutes, the magnetic stirrer was turned off and 5 minutes of resting was done. Then 50 µl of the sample was taken and protein was determined by Lowry method. The same processes were repeated at the end of 10, 30, 60, 120 minutes, and after determining the amount of albumin that was not adsorbed in the solution, the amount of adsorbed albumin and IgG were calculated by equation.

$$Q = \frac{(C_0 - C)V}{m}$$

where Q is adsorbed amount of albumin and IgG (mg/g); C_0 and C are the albumin and IgG concentrations in the initial (before mixing with biosorbent) and final solutions (after treating with biosorbent), respectively (mg/mL); V is the total aqueous volume of adsorption medium (ml); and m is the mass of the sample used for experiment (g). Adsorbed albumin and IgG values were calculated by taking the average of the three results.

3. Result and discussion

3.1 Preparation and characterization of gelatin biosorbents

Gelatin biosorbents were prepared in spherical microparticle form. Several methods can be used to prepare gelatin microparticles. The emulsification polymerization method was used to prepare gelatin biosorbents in microsphere form. The method requires only simple tools and can be easily applied in moderate conditions compared to other methods. Such advantages allow this method to be used frequently in the preparation of gelatin microspheres in pharmaceuticals and various medical applications.

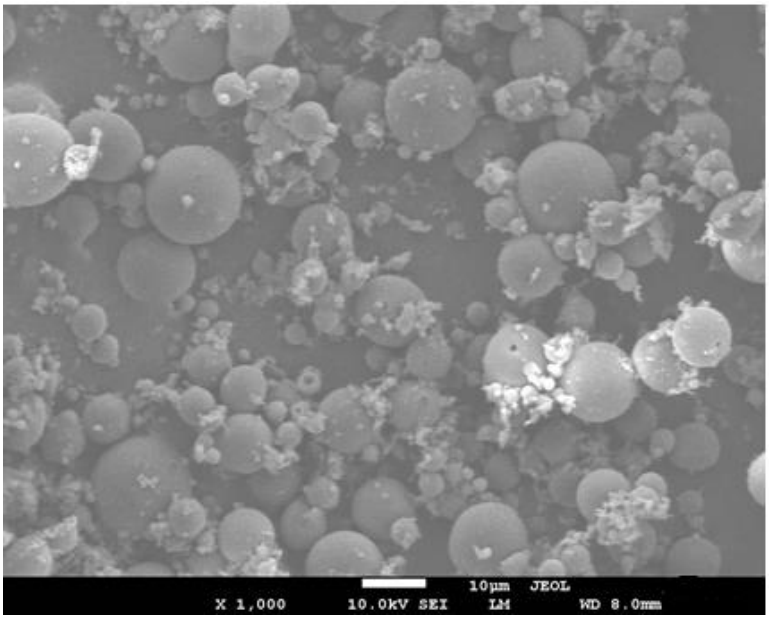

Figure 1. SEM images of the gelatin biosorbents.

Gelatin biosorbent are difficult to prepare due to various factors. It usually has large particle size and size distribution. Also, surface morphology is generally poor. In this study to minimize these features, biosorbents were first prepared in hot environment and then stabilized in cold environment. The effecting factors, such as gelatin concentration, emulsifier amount, emulsufication time, stirring rate on the morphology, and size distribution of microspheres were determined in our previous study. Gelatin biosorbents prepared at optimum conditions by using 0.1 mg/mL gelatin concentration, 4% emulsifier amount, 10 min emulsufication time, 750 rpm stirring rate. Figure 1 shows SEM photomicrographs of gelatin biosorbents. It was seem from the Figure 1, biosorbents are spherical, nonporous and with a smooth surface. Biosorbents obtained from natural polymers are not perfectly spherical because of the variations in molecular weight and other properties of the polymer [13]. Biosorbents having a microspherical form enhance the surface area. Affinity supports with high surface area to volume ratio, functional groups that can be easily modified for covalent bonding of ligands, minimum nonspecific binding properties, good flow properties and mechanical and chemical stability provides advantage in affinity purification methods due to its properties [8]. This is thought to offer high binding ability.

3.2 Albumin and IgG removal studies

There are many studies to remove albumin ve IgG serum proteins with high concentrations in order to improve the detection of low-abundance proteins. In the study, the biopolymeric biosorbents are prepared in the form of microspheres, making it possible to selectively separate the target molecule. Since the microsphere form provides a large surface area to the affinity support material, it increased the interaction between biosorbent and target proteins. It is reported that the support materials with particulate structure also provide better flow property with minimum diffusion limitation in column applications [14-16]. It is known that the use of particulate biosorbents provides the advantage of directly isolating the target molecules from the mixture, as well as significantly reducing processing time. In addition, the large surface area allowed less material to be used to separate the target molecule.

In our study, biosorbents were used in adsorption processes without any activation and ligand binding processes. It has provided an important advantage both in terms of economy and time.

The amount of albumin and IgG removed obtained from the graph plotted between mg albumin/g polymer and reaction time.

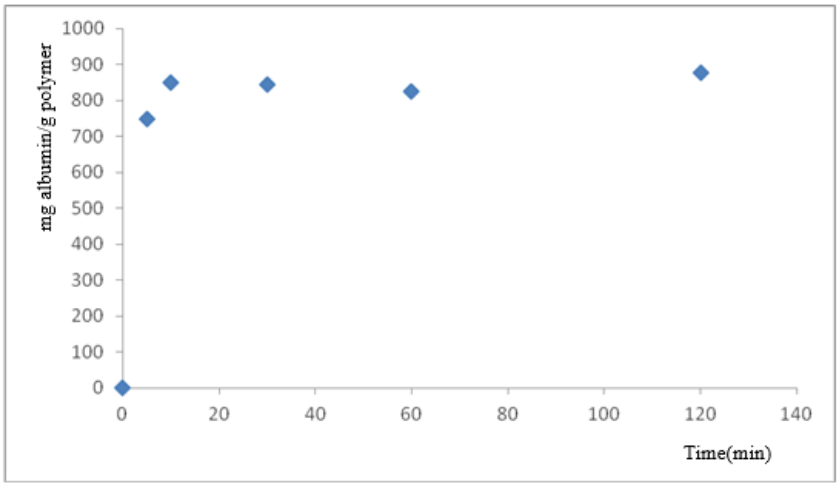

Figure 2. Albumin removal efficiency of gelatin biosorbents.

Figure 2 gives the removal efficiency data for the gelatin biosorbents. As shown in Figure 2 gelatin biosorbents started to immobilize albumin at the end of 5 min and the amount of albümin immobilized per g gelatin biosorbent at the end of 120 min was 877 mg albumin/g polymer. It has been found that 85.7% of the albumin was successfully removed with gelatin biosorbents.

In this study, it was determined that gelatin has capable albumin removal rate of 85.7% (877 mg albumin/g polymer) with biosorbents prepared under optimum conditions. This specific interaction was thought to result from the conformational and surface charge distribution state at albumin pH: 7.4, the interactions between regions with negative charge density on the gelatin biosorbent surface. As known, hydrophobic, electrostatic interactions and hydrogen bonds are the main forces responsible for specific adsorption in biological molecule adsorption [17]. When the results in our study are compared with the results in the literature, it is seen that a very good adsorption capacity has been reached. Unlike other studies, activation studies and ligand immobilization were not performed in our study. Thus, the target molecule could be separated from the liquid medium in which it is located in a single operation. This provides an important advantage in terms of economy and time.

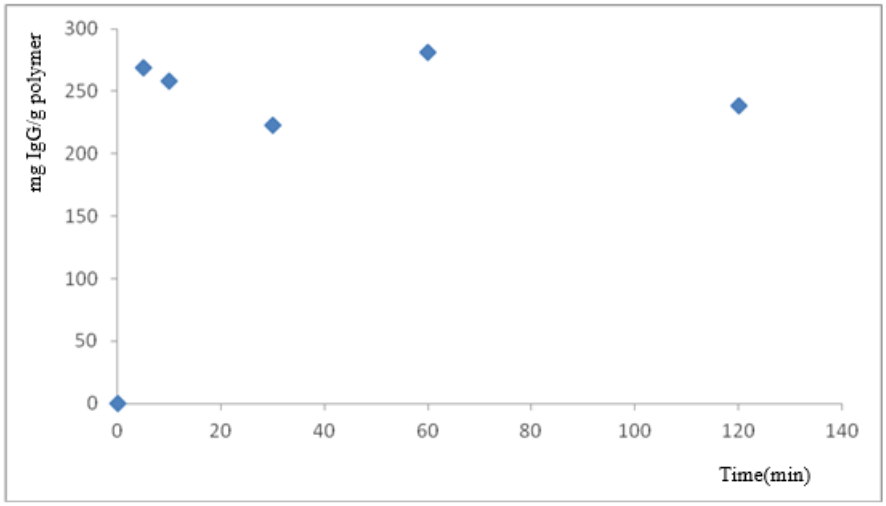

Figure 3. IgG removal efficiency of gelatin biosorbents.

As shown in the Figure 3, gelatin biosorbents started to immobilize IgG at the end of the first 5 min and immobilized 238.8 mg IgG per g gelatin biosorbent at the end of 120 min. Gelatin biosorbents prepared at optimum conditions were found to have an IgG removal capacity of 79% in aqueous solution. Gelatin biosorbents have been found to be not as efficient in IgG removal as albumin removal. The reason for this is thought to be the negative charge density on the gelatin biosorbent surface interaction with negative charge density of IgG on the Fc region.

There are many techniques in the literature to remove the wide dynamic range of proteins from plasma. However, IgG is often purified by affinity chromatography due to its high selectivity. The removal of IgG is commonly succeed by Protein A,G affinity adsorbents. However, these types of ligands are difficult to immobilize in the right direction required and also sensitive to degradation [18]. Polymeric particles are

attractive as an inexpensive and robust alternative to affinity reagents of biological origin, including antibodies [19].

Tamahkar et al. have found the maximum adsorption capacity of albumin as 600 mg/g with Cibacron Blue F3GA attached bacterial nanofibers [20]. Türkmen et al. have obtained the IgG adsorption as 800 mg/g with p(HEMA-MAPA) nanospheres [21]. Büyüktiryaki et al. have found albumin adsorption as 1100 mg/g and 825 mg/g IgG adsorption with twin affinity magnetic nanotraps [22]. The differences in IgG and albumin adsorption are thought to be due to the properties of each adsorbent such as structure, functional groups, ligand loading and surface area. In this study, unlike other studies, no activation or ligand immobilization was performed. So the target molecule can be easily separated from the batch systems quickly and efficiently with a single process.

High abundant proteins such as albumin and IgG are known to limit the total amount of protein that can be solved with 2-D IEF and inhibit the ability to visualize and detect low abundant proteins [23]. Therefore integrated removal of albumin and IgG from plasma enable an increase in the total protein sample load, resulting in enhanced detection of low abundant proteins.

4. Conclusion

The abundance of various proteins in the plasma are in a wide range of concentrations. This presents difficulties in elucidating potential biochemical markers for the detection and monitoring of various diseases. Detection of physiologically important but low-abundant proteins requires both analytically sensitive techniques such as 1D and 2D electrophoresis MS and techniques for enrichment of proteins of interest. We presented here a simple method for the efficient removal of albumin and IgG the most abundant proteins in plasma. By removing albumin and IgG allow the possibility of detecting and identifying low abundant proteins during analysis besides enables detection of an increased number of proteins.

As a result of removal studies, successful removal of albumin (85.7 %) and IgG (79%) with microspherical gelatin biosorbents is thought to be a great advantage for the identification of proteins at low concentrations in human plasma. The human organism has a total protein concentration of approximately 46 kg/m3. Among these proteins, albumin, immunoglobulins and IgG percentages are found in human plasma at 70%, 27.2% and 21.7%, respectively. A similar approach can be applied to identify complex molecules that are difficult to purify or lack separation methods by current proteomic technologies.

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