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Albumin and IgG Removal by Cellulose Biosorbents

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Original Research Article

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Abstract: Proteomic analysis is a great and effective biomarker discovery tool that is being used to discover and identify various diseases biomarkers. The complexity of the plasma content exceeds the existing methods to determine lower abundance proteins that may prove to be informative biomarkers. The aim of this study was to investigate the removal efficiency of albumin and IgG (immunoglobulin G) by cellulose biosorbents in batch wise at room temperature. Polysaccharide based cellulose biosorbents were prepared and stabilized by solvent remove/solvent evaporation method in chitosan media. 2% chitosan amount, 1400 rpm stirring rate, 40^{o}C solvent evaporation temperature were determined as optimal conditions in our previous study for polysaccharide based cellulose biosorbents. The prepared spherical biosorbents were then studied for albumin and IgG removal by cellulose biosorbents. 976,6 mg albumin/g polymer and 209 mg IgG/g capturing capacity was obtained with cellulose biosorbents.
Keywords: Cellulose, Biosorbent, Albumin, IgG, Bioaffinity, Proteomic.

INTRODUCTION

The blood plasma is one of the basic materials for the diagnosis of diseases [1]. It contains high concentrations of protein (60-80 mg/ml protein). The analysis of proteins present in human plasma is of great importance in the studies for the diagnosis of diseases. The complexity of the plasma content exceeds the existing methods to determine lower abundance proteins that may prove to be informative biomarkers [2-4].

The most abundant proteins in the plasma are albumin and immunoglobulins. If these proteins are not removed before two-dimensional gel electrophoresis, it is almost impossible to determine disease specific proteins. Bioaffinity chromatography is often preferred choice for the purification, determination or removal of many biologically active substances due to its excellent specificity, ease of operation, yield and throughput. There are many studies on IgG and albumin proteins. Büyüktiryaki *et al*. investigated the adsorption of albumin and IgG on the surface of superparamagnetic nanoparticles conjugated with Cibacron Blue F3GA and Protein a (SPNs) for the specific depletion of the proteins in human serum [5]. A purified polyclonal rabbit anticamel antibody that detecting all camel IgG subclasses as well as their derived nanobodies were characterized by Haddad *et al*. Bormotova *et al*. prepared a composite sorbent enabling the depletion of HSA and IgG from serum by single step affinity chromatography [6]. More detailed revisit of the conformation of IgG purified by protein an affinity chromatography was investigated by Gagnon *et al*. [7].

Cellulose is the most abundant natural, biodegradable, and biocompatible biopolymer with high biocompatibility and good hydrophilic properties [8], has long been used as a bioaffinity carrier material [9]. Cellulose has wide ranging applications, e.g. as separation medium, carrier system and as adsorbent in extracorporeal blood purification [10-16]. Ethyl cellulose (EC) is a derivative of cellulose in which some of the hydroxyl groups on the repeating anhydrous glucose units are modified into ethyl ether groups, largely called as non-ionic ethyl ether of cellulose [17]. There are many applications and preparation methods for cellulose micro particles in the literature. Amin *et al*. prepared spray-dried bacterial cellulose micro particles to provide new insight on the potential applications as a pharmaceutical excipient [18], Li *et al*. developed mannan-decorated mucoadhesive thiolated hydroxypropylmethyl cellulose phthalate (HPMCP) microspheres (Man-THM) that contain ApxIIA subunit vaccine[19], Bodmeiers investigated the effect of solvent type on the solidification rate of ethyl cellulose (EC) micro

particles and particle size/distribution of emulsion droplets/hardened micro particles[20].

In the present study it was planned to prepare spherical biosorbents that will be used as support material in the affinity chromatography. For this reason, various processing and formulation parameters such as stirring speed, volume of processing medium and evaporation temperature were optimized to have narrow size distribution. The optical micrographs and SEM images of biosorbents were taken for the characterization studies after preparation. Then immobilization of albumin and IgG on to the cellulose biosorbents at pH: 7.4 in borate buffer solution were investigated batch-wise separately.

MATERIALS AND METHODS

Chemicals

Ethyl cellulose (sigma) Chitosan (Merck) Bovine serum albumin (BSA) (Sigma), Immunoglobulin G (IgG) (Sigma), Acetone (Merck), Methanol (Merck), Dichloromethane (Merck), All the other reagents used were of analytical grade and all solutions were prepared with distilled water.

Preparation of cellulose microspheres

Ethyl cellulose biosorbents were prepared by using solvent remove/solvent evaporation method in chitosan media [21]. Briefly, ethyl cellulose (0.5 g) was dissolved in organic solution mixture of 10 ml dichloroethane, 20 ml methanol and 10ml acetone. Chitosan medium (2%, w/v) was prepared by dissolving chitosan in diluted acetic acid (3% v/v). Then the cellulose dissolved in methanol, acetone and dichloromethane organic solvent mixture was added drop wise to the chitosan media at 1400 rpm stirring rate. At the end of the 30 min. homogenization period, the reaction vessel was allowed to stand in a 40°C incubator for 3 hours to remove the organic solvent. Cellulose biosorbents were removed by centrifugation after evaporation of the organic solvent mixture. The biosorbents washed with distilled water and optimization studies were carried out.

Characterization of microspheres

Microspheres Surface Morphology was characterized firstly by inverted optical microscope (Leica microsystems, DFC 295). Scanning electron microscopy (SEM) of the cellulose biosorbents was performed to examine the surface morphology. The microspheres were mounted on metal stubs and then coated with gold. Photomicrographs were taken using a Jeol Scanning Electron Microscope (SEM JEOL JSM-5910 LV).

Removal of Albumin

Affinity chromatography is a selective purification method. After the preparation of appropriate support material, it is possible to remove proteins with high selectivity. In this part of the study removal of albumin by cellulose biosorbents at pH: 7.4 in borate buffer were investigated batch-wise. Biopolimeric biosorbents that prepared under optimum conditions (0.05 g) were incubated with 2 mL of the borate buffer containing 50 mg albumin about 8h in flasks stirred at 150 rpm at room temperature. Samples were withdrawn at suitable time intervals and albumin concentration was determined by measuring the initial and final concentration of albumin within the removal medium using Lowry method as described by Lowry. The amount of immobilized albumin was calculated using mass balance.

Removal of IgG

IgG removal studies were carried out as same as albumin removal studies in batch wise at pH: 7.4 borate buffer solutions with cellulose biosorbents prepared in optimum conditions. Cellulose biosorbents that prepared under optimum conditions (0.05 g) were incubated with 2 mL of the borate buffer containing 17 mg IgG and studies were performed as mentioned at 4.

RESULTS AND DISCUSSIONS

Characterization of Cellulose Biosorbents

The effect of preparation parameters (stirring speed, volume of processing medium and evaporation temperature) on the cellulose biosorbents size/size distribution and morphology were obtained in our previous study [22].

Cellulose biosorbents prepared by using 1400 rpm stirring rate, 2% chitosan media and 40ºC evaporation temperature were obtained as optimum conditions. Figure 1 shows the morphological characteristics of cellulose biosorbents. The SEM photomicrographs of the biosorbents reveal that they are spherical, nonporous and uniform with a smooth surface. It was reported that biosorbents obtained from natural polymers are not perfectly spherical because of the variations in molecular weight and other properties of the polymer [23]. It was observed that prepared biosorbents are spherical with quite smooth surfaces.

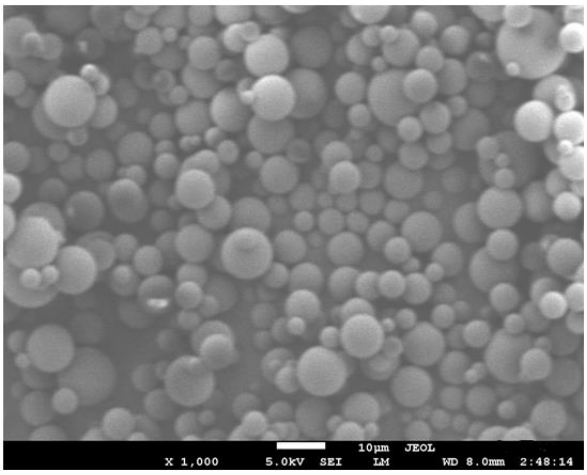

Fig-1: SEM images of the cellulose microspheres

Albumin removal studies

Human plasma contains tens of thousands of proteins making it difficult to study specific proteins and their roles in health and disease. Albumin removal studies were carried out in batch wise at pH: 7.4 borate buffer solutions with cellulose biosorbents prepared in optimum conditions. The amounts of removed albumin were determined from the graph drawn between mg albumin/g polymer and reaction time.

Figure 2 gives the removal efficiency data for the cellulose biosorbents. As shown in Figure 2 cellulose biosorbents started to immobilize albumin at the end of 30 min and the amount of albumin immobilized per g cellulose biosorbent at the end of 120 min was 976,6 mg. It has been found that 97.6% of the albumin was successfully removed with cellulose biosorbents.

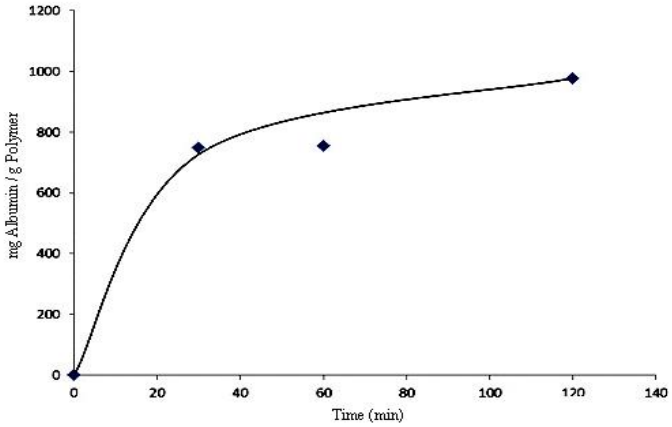

Fig-*2*: *Albumin* removal efficiency of cellulose microspheres

In literature there are various techniques for removal of the large dynamic range of proteins in plasma [24-27]. As a result of albumin removal studies with cellulose biosorbents very successful data have been obtained with the use of cellulosic biosorbents. The reason why cellulose biosorbents perform so well is thought to be the interactions between the regions with negative charge density on the cellulose biosorbent surface, with albumin pH: 7.4 conformational and surface charge distributions. Hydrophobic, electrostatic interactions and hydrogen bonds in the biological molecule immobilization, an interface event, are the main forces responsible for specific bounding [28]. Comparing the results in the literature with the results obtained in our study, it is seen that a very high immobilization capacity is reached.

IgG removal studies

Removal studies were aimed to determine a non-abundant protein that has an important role in diagnosis many diseases. Albumin and immunoglobulin have become focused targets for protein removal because together they represent over 75% of all of the proteins present in plasma [29].

IgG removal studies were carried out as same as albumin removal studies in batch wise at pH: 7.4 borate buffer solutions with cellulose biosorbents prepared in optimum conditions. The amounts of

removed IgG were determined from the graph drawn between IgG amount (mg IgG/g polymer) and reaction time.

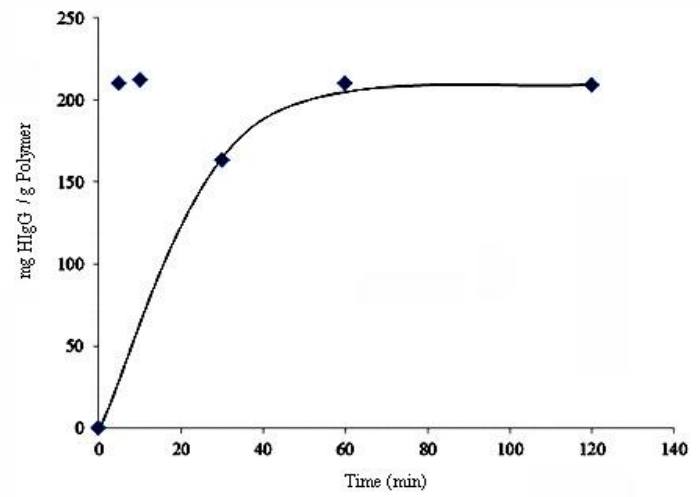

Fig-3: IgG removal efficiency of cellulose microspheres

As shown in the Figure 3, cellulose biosorbents started to immobilize IgG at the end of the first 5 min and immobilized 209 mg IgG per g cellulose biosorbent at the end of 120 min. Cellulose biosorbents were found to have an IgG removal capacity of 61.47% in the aqueous solution. IgG removal studies in aqueous solution did not yield good results with cellulose biosorbents prepared at optimum conditions. The reason for this is thought to be the negative charge density on the cellulose biosorbent surface interaction with negative charge density of IgG on the Fc region.

In the literature, studies on albumin and IgG removal show that we have very good results when we compare the albumin and IgG studies that we have made with the biopolymeric biosorbents in batch wise. In Sitnikov et al.'s study, 99% of the IgG was removed with the Multiple Affinity Removal Column [30]. Altıntaş et al removed 98.2% IgG (171.2 mg IgG / g polymer) with Cu^{2+} loaded poly (GMA) -IDA particles [31], in a study by Soskic *et al*. 41.9% albumin was removed by hexadecanodioic acid immobilized Sepharose 4B [32], Bereli *et al*. removed 89.4% albumin (342 mg albumin / g polymer) and 93.6% IgG (257 mg / g polymer) with the composite cryogels [33]. There are many studies on the proteomic analysis of plasma after removal of high concentration of proteins in plasma [34-37]. As a result of removal of high concentration of proteins, 325 different proteins could be identified with 2DE [38]. In the literature, it has been reported that the removal of albumin and IgG in the plasma provided 4-5 times higher sample loading in 2DE analyzes [39].

CONCLUSION

As a result of removal studies successful removal of albumin (97.6%) and IgG (61.47%) with micro cellulose biosorbents is thought to be a great advantage for the identification of proteins at low concentrations in human plasma.

Human blood plasma contains information that directly represents information for a variety of diseases. But the levels of high abundance proteins such as albumin and IgG should be removed in the first step before many analytical applications. So, micro particles synthesized from various biomolecules showed great potential for this purpose. The removal of albumin and IgG from plasma proteins with different micro particles will play an important role in the identification of low concentration biomarkers in many diseases.

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