Human IgG Adsorption by Spherical Albumin Biosorbents in SBF Media

Küresel Albumin Biyosorbentlerle SBF ortamlarında İnsan IgG Adsorpsiyonu

Research Article

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ABSTRACT

The objective of this study was to investigate the removal efficiency of immunoglobulin G (IgG) by albumin biosorbents in batch wise in four different simulated body fluid (SBF) solutions at room temperature. Albumin biosorbents were prepared as microsphere form by emulsion polymerization method. 0.1 g/ml albumin concentration, 1000 rpm stirring rate, 1% glutaraldehyde (GA) concentration 30 minutes crosslinking time were determined as optimal conditions in our previous study. The prepared biosorbents were then studied for IgG removal efficiency in four simulated body fluid (SBF) solutions [N(Normal)-SBF, G(glucose)-SBF, H(hepes)-SBF, T(tris)-SBF] at room temperature. IgG capturing capacity was obtained as 320 mg IgG/g with albümin biosorbent (94,6%) in N-SBF. It was observed that there was no leakage at washing with water. It may be concluded that prepared albumin biosorbents are sufficient in terms of efficiency of IgG removal. It was seem that this method is easy to perform and is useful as a first step in the detection of diagnostic markers in body fluids by applying proteomics technologies. We believe that albumin biosorbents offered the promising approach with good removal specificity and efficiency of IgG.

Key Words
Biosorbent, Albumin, IgG, SBF

ÖZET

Bu **ç**alışmanın amacı albümin biyosorbentlerin, kesikli sistemde dört farklı yapay vücut sıvısı ortamında İnsan immunglobulin G (HIgG) uzaklaştırma kapasitesinin araştırılmasıdır. Albumin biyosorbentler, emülsiyon polimerizasyon yöntemi ile mikroküre formunda hazırlandı. Denysel parametreler 0,1 g/ml albumin derişimi, 1000 rpm karıştırma hızı, %1 glutaraldehid derişimi ve 30 dakika çapraz bağlanma süresi optimum şartlar olarak daha önceki çalışmamızda belirlenmiştir. Daha sonra, hazırlanan biyosorbentlerle, oda sıcaklığında dört faklı yapay vücut sıvısı (SBF) çözeltisi [N(Normal)-SBF, G(glukoz)-SBF, H(hepes)-SBF, T(tris)-SBF] ortamında, HIgG uzaklaştırma çalışmaları yapılmıştır. Albumin biyosorbentlerle N-SBF ortamında HIgG yakalama kapasitesinin 320 mg HIgG/g (%94,6) olduğu tespit edilmiştir. Saf su ile yıkama işlemlerinde kaçak olmadığı gözlemiştir. Hazırlanan albumin biyosorbentlerin HIgG uzaklaştırma verimliliği açısından yeterli olduğu sonucuna varılmıştır. Bu yöntemin, proteomik teknolojileri uygulayarak vücut sıvılarında teşhis belirteçlerinin saptanmasında, ilk adım olarak gerçekleştirilmesi, kolay ve yararlı olduğu görülmektedir. Albumin biyosorbentler, iyi bir uzaklaştırma seçiciliği ve etkinliği ile umut verici yaklaşım sunmaktadır.

Anahtar Kelimeler
Biosorbent, Albumin, IgG, SBF

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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) [1]. 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.

The cleavage product of Amyloid Precursor Protein (APP), A-42 (41 pg/ml) protein is exist at very low concentration in plasma. Its relationship with Alzheimer disease was proven. This protein is masked by the high concentration plasma proteins [2]. The removal of the large dynamic range of proteins will provide very important advantage at the diagnosis of Alzheimer disease [3].

Currently, there are several depletion technologies to remove the higher abundant proteins from serum. Among the depletion technologies affinity chromatography can be used to remove the large dynamic range of proteins in serum. The wide applicability of this method is based on the fact that any given biomolecule that one wishes to purify usually has an inherent recognition site through which it can be bound by a natural or artificial molecule. Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatography matrix. The technique is ideal for a capture or intermediate step in a purification protocol and can be used whenever a suitable ligand is available for the protein(s) of interest. With high selectivity, hence high resolution, and high capacity for the protein(s) of interest, purification levels in the order of several thousand-fold with high recovery of active material are achievable. In a single step, affinity purification can offer immense time-saving over less selective multistep procedures. It has played a central role in many "Omics" technologies, such as genomics, proteomics and metabolomics Target molecules can be purified from complex biological mixtures, native forms can be separated from denatured forms of the same substance and small amounts of biological material can be purified from high levels of contaminating substances. The technique can be used to separate active biomolecules from denatured or functionally different forms, to isolate pure substances present at low concentration in large volumes of crude sample and also to remove specific contaminants. In affinity chromatography applications, the choice of matrix and its preparation process are the important factors affecting the chromatografic performance [4].

To evaluate the in vitro studies the dissolution medium should have ion concentrations almost equal to those of the human plasma. A simulated body fluid (SBF) (which claim to mimic the acellular human blood) is a solution with an ion concentration close to that of human blood plasma, kept under mild conditions of pH and identical physiological temperature [5]. Simulated body fluid was developed initially to evaluate the surface structural changes of glass-ceramics used to manufacture artificial vertebrae, ileum, tooth roots [6], and bioactive material used to repair hard tissues such as artificial middle-ear bone and maxillofacial implants [7] Today as either supplemented with glucose or with amino acids and vitamins, and are commonly used in tissue engineering or cell culture studies. Solutions such as N-SBF, G-SBF, T-SBF, H-SBF do help to maintain intra- and extracellular osmotic balance, provide cells with water and certain bulk inorganic ions essential for normal cell metabolism, and provide a buffering system to maintain the medium within the physiological pH range (7.2–7.6) [8].

In the work reported here, we have investigated the efficacy of albumin biosorbents adsorption capacity for removing immunoglobulin G from different SBF solutions in batch wise. Albumin biosorbents were prepared by the emulsion polymerization technique The method is relatively rapid and easy and also reduces capital and separation costs. It was seem that this method is easy to perform and is useful as a first step in the detection of diagnostic markers in body fluids by applying proteomics technologies.

MATERIALS and METHODS

Chemicals

BSA (Bovine Serum Albumin) (Sigma), GA (J.T.Baker), diethylether (Merck), olive oil (Komili). BSA (Bovine Serum Albumin) (Sigma), GA (J.T.Baker), sodium hydroxide (Merck), sodium carbonate (Merck), sodium potassium tartrate tetrahydrate (Merck), copper(II) sulphate pentahydrate (Riedel de Haen), Folin & Ciocalteu's phenol reagent (Merck). All the other reagents used were of analytical grade and all solutions were prepared with distilled water.

Preparation of Albumin Biosorbents

Albumin biosorbents were prepared by using emulsion polymerization technique. Briefly, a weighed amount of BSA (25 mg) was dissolved in distilled water (0.25 mL). This solution was then added to 25 mL olive oil which was continuously stirred at various stirring rates (1000 rpm) at room temperature of 25°C. Stirring was continued for 5 min to obtain a water/oil (1:100) emulsion. 1% GA (25%, w/v) were added into the emulsion to crosslink the albumin present in the internal phase of the emulsion. Stabilization process was continued at various stabilization times (30 min). Biosorbents were formed then separated by centrifugation and washed with 30 mL of diethyl ether to remove excess GA and the olive oil.

Preparation of SBF solutions

SBF solution which claim to mimic the acellular human blood plasma is prepared in four different composition form to remove IgG. SBF solutions were prepared in 1 L of deionized water as mentioned amount (g) in Table 1. and stirred on a magnetic stirrer at room temperature.

Characterization of biosorbents

Surface Morphology of biosorbents

Biosorbents were charactherized firstly by inverted optical microscope (Leica microsystems, DFC 295). Scanning electron microscopy (SEM) of the albumin biosorbents was performed to examine the surface morphology. The biosorbentss were mounted on metal stubs and then coated with gold. Photomicrographs were taken using a Jeol scanning electron microscope (SEM JEOL JSM-5910 LV).

IgG Removal Studies

Affinity chromotography is a selective purifacition method. After the preparation of appropiate support material it is possible to remove proteins with high selectivity. In this part of the study adsorption of IgG on to the albumin biosorbents in SBF solutions was investigated batch-wise. Biopolimeric biosorbents that prepared under optimum conditions (0.05 g) were incubated with 2 mL of the SBF solutions (N-SBF, G-SBF, H-SBF, T-SBF) containing 17 mg IgG about 8h in flasks stirred at 150 rpm at room temperature. Samples were withdrawn at suitable time intervals and IgG concentration was determined by measuring the initial and final concentration of IgG within the adsorption medium using Lowry method as described by Lowry. The amount of adsorbed IgG was calculated using mass balance.

RESULTS and DISCUSSIONS

Charactherization Albumine Biosorbents

The effect of preparation parameters (Albumin concentration, stirring rate, crosslinker amount, crosslinking time) on the Albumin biosorbents size/size distribution and morphology were obtained in our previous study [11].

Albumin biosorbents prepared by using 0.1 $mg.mL^{-1}$ Albumin concentration, 1000 rpm stirring rate, 1% GA and with 30 min crosslinking time, were determined as optimum conditions. Figure 1 shows the morphological characteristics of Albumin 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 [12].

It was observed that prepared biosorbents are spherical with quite smooth surfaces. This result may be because of the low viscosity of olive oil, which was the external phase.

IgG removal studies

The human organism has a total protein concentration about 46 kg/m^3. Collectively,

Table 1. Preparation of 1 L SBF solutions with different chemical compasitions [9,10]

Chemicals	N(Normal)-SBF	G(glucose)-SBF	H(hepes)-SBF	T(tris)-SBF
NaCl	8.0	8.8	5.403	6.547
KCl	0.4	0.4	0.225	0.373
$CaCl_2$	0.14	0.14	0.293	--
$CaCl_2.2H_2O$	--	--	--	0.368
$NaHCO_3$	0.35	0.35	0.504	2.268
$MgSO_4.7H_2O$	0.2	0.2	--	--
$Na_2HPO_4.12H_2O$	0.12	--	--	--
$Na_2HPO_4.7H_2O$	--	0.06	--	--
$Na_2HPO_4.2H_2O$	--	--	--	0.178
KH_2PO_4	0.06	--	--	--
$KH_2PO_4H_2O$	--	0.1	--	--
K_2HPO_4	--	--	0.230	--
$K_2HPO_4.3H_2O$	--	--	--	--
Na_2CO_3	--	--	0.426	--
Na_2SO_4	--	--	0.072	0.071
$MgCl_2.6H_2O$	--	--	0.311	0.305
Tris	--	--	--	6.057
1M HCl	--	--	--	15 ml
$C_6H_{12}O_6$ (glukoz)	--	1.0	--	--
HEPES	--	--	17.892	--
0,2M NaOH	--	--	100 ml	--
1M NaOH	--	--	15 ml	--
1M HCl	--	--	--	*pH 7.4 te 37°C titrasyon*

albumin and immunoglobulin constitute over 80% of the human serum proteins [13].

Therefore, removal of IgG represents a fundamental improvement toward characterization of the serum ptoteins. In this study adsorption studies were performed by affinity chromotography method. IgG removal studies were aimed to determine AB42 protein that has an important role in diagnosis Alzheimer disease.

Adsorption studies were carried out in batch system in four different SBF solutions with albumin biosorbents prepared in optimum conditions. The amounts of adsorbed IgG were determined from the graph drawn between IgG amount (mg IgG/g polymer) and reaction time.

Figure 2 gives the adsorption data for the albumin biosorbents. As shown in Figure 2a about 130 min equilibrium is reached. IgG binding capacity of albumin biosorbents were 320 mg IgG/g biosorbents in N-SBF media at the end of the 300 min.

Adsorption studies revealed that the total IgG adsorption ratio was 94.6% with N-SBF solution. Adsorption capacity in another SBF solution was 270 mg IgG/g biosorbents at the end of the 420 min. The removal efficiency for IgG was 79% in G-SBF media. The best adsorption value was obtained with H-SBF media after N-SBF. IgG adsorption ratio was around 86% in H-SBF media. Adsorption capacity in H-SBF solution was 300 mg IgG/g biosorbents at the end of the 470 min. The lowest adsorption capasity was observed in T-SBF solution. The total protein adsorption was determined as 105 mg/g in T-SBF. The removal efficiency for IgG was only 30% in T-SBF media at the end of the 520 min.

According to these results N- SBF media is the best media compared to other SBF media for IgG

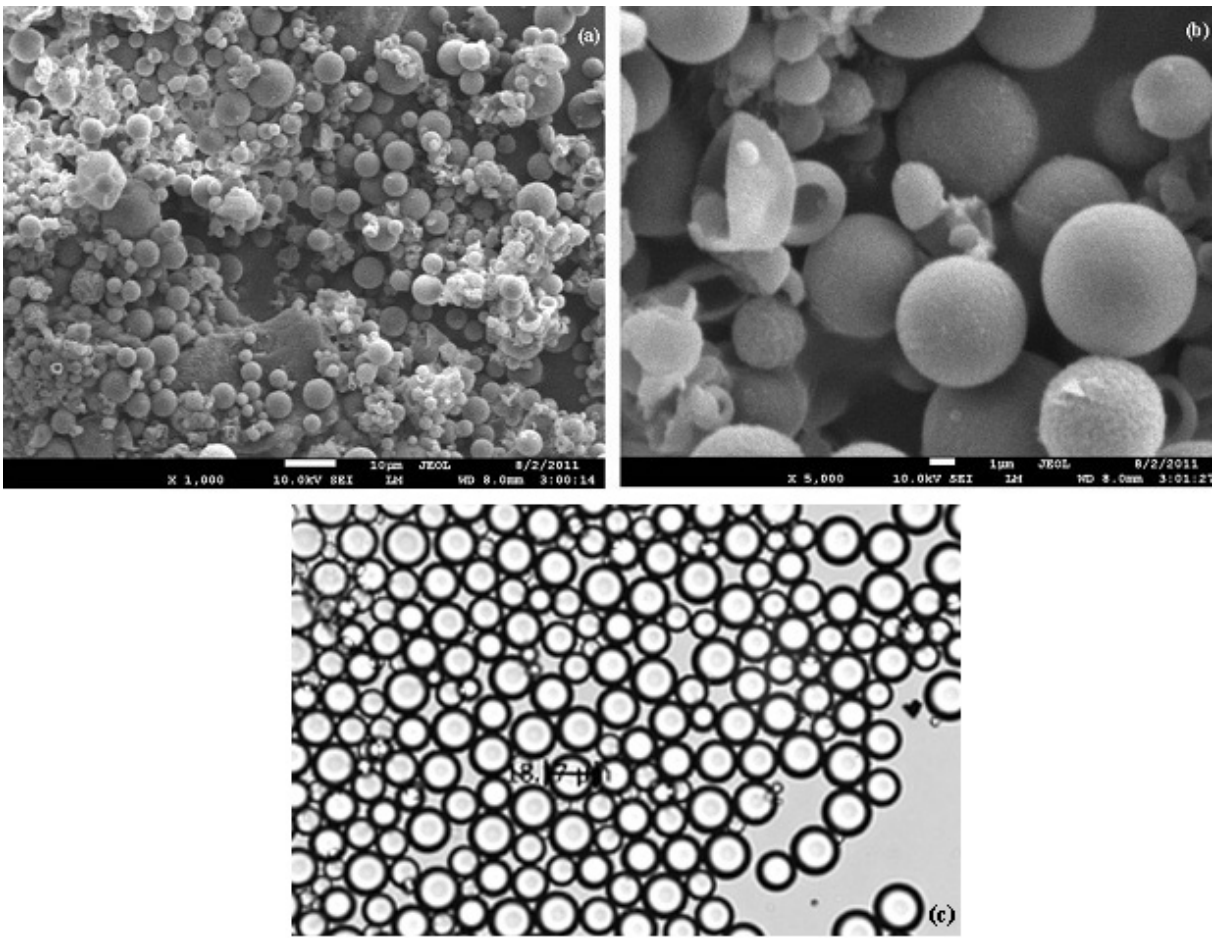

Figure 1. SEM and optic micrografs of albumin biosorbents. a) x1000; b) x5000; c) x100 magnifications.

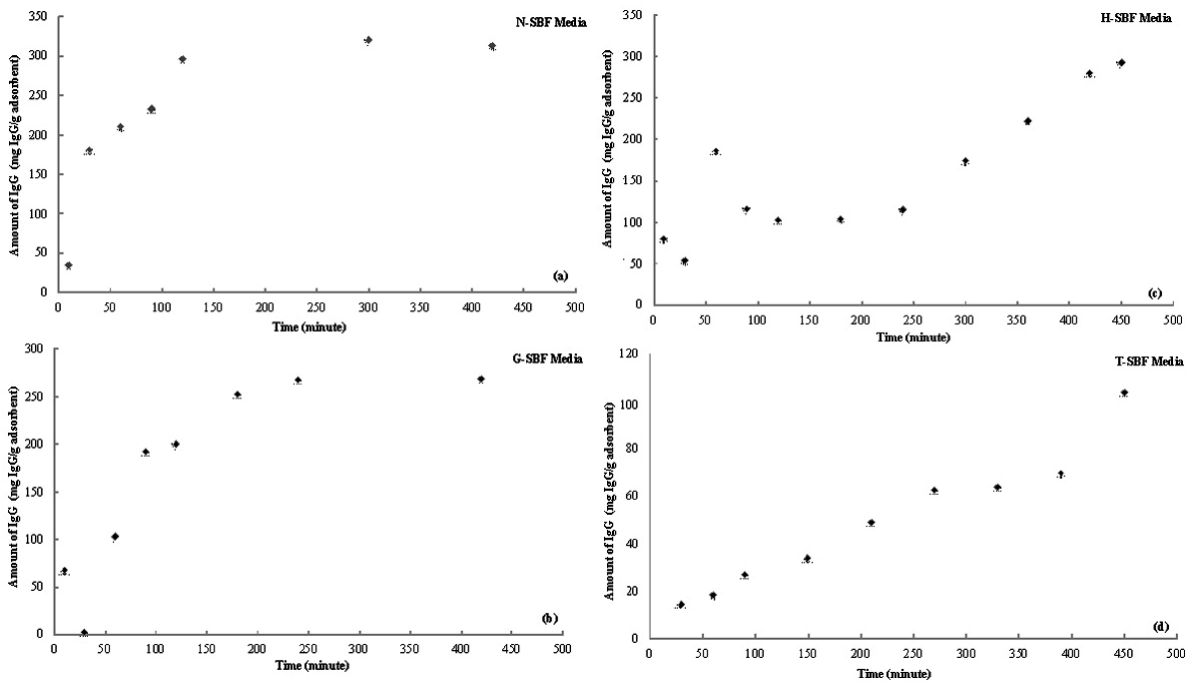

Figure 2. Adsorption capacities of albumin biosorbents. a) N-SBF; b) G-SBF; c) H-SBF; d) T-SBF.

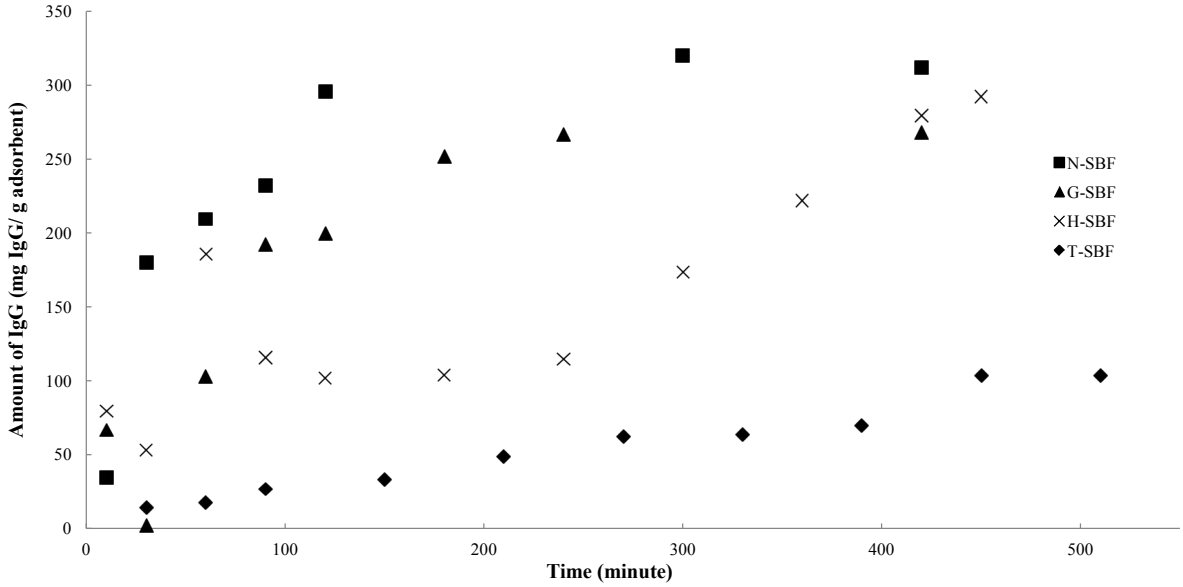

Figure 3. Comparison of Adsorption capacities of albumin biosorbents in different SBF media.

removal with albumin biosorbents.

In literature there are various techniques for removal of the large dynamic range of proteins in plasma. However, IgG are often purified by affinity chromatography because of its high selectivity. The depletion of IgG is commonly achieved by Protein A/G affinity adsorbents, which binds to the Fc region of the IgG. However, despite its high selectivity, the cost of these supports tends to be very high and these types of bioligands are difficult to immobilize in the proper orientation They are also susceptible to degradation.

The results compared in the literature with theresults obtained in this study, It was seen in N-SBF media good IgG adsorption capacity was achieved with albumin biosorbents. Unlike other studies Any ligand can be used in this study, in a single step by adsorption applications both time and is thought to be advantageous for economy. In this study adsorption stuides were performed without using any ligand in a single step unlike the other sudies. This difference is thought to be advantageous both in time and for economy.

In the literature different adsorption capacities were reported for IgG adsorption. Compared with these studies, our study shows that the results are quite good. Sitnikov et al. [14] applied Multiple Affinity Removal Column (Agilent Technologies, San Diego, CA, USA) for the depletion of blood plasma proteins under volatile conditions. The percentage of IgG depletion was greater than 99%. Altıntaş et al. [15] reached up to 98,2% (171.2 mg IgG/g polymer) IgG depletion with Cu^{2+} loaded poly (GMA)-IDA particules, Bereli et al [16] reported 93,6% (257 mg IgG/g polymer) adsorption capacity with composite cryogels. Seferovic et al., removed IgG from plasma by immunodepletion with anti-IgG and removed 94% of the IgG [17]. Karatas et al., reached up to 99.3% IgG depletion amount with Cu^{2+} loaded poly (EGDMA-MAH) beads [18]. Uzun et al [19] used nanoProA/pHEMA cryogels and they obtained 99.3% (293.7 mg IgG/g polymer) adsorption capacity. It may be concluded that prepared albumin biosorbents are sufficient in terms of efficiency of IgG removal. We demonstrated that albumin biosorbents exhibit quite good IgG binding capacities (320 mg/g) in N-SBF solution in batch system.

CONCLUSION

The analysis of proteins present in human serum is of great importance in the study of both health and disease. However, the diverse range of proteins present in the blood can lead to difficulties when looking for those present at low concentrations. In many techniques these are typically 'masked' by highly-abundant serum proteins such as albumin and IgG. There are several adsorbents for removing these proteins [20]. However, these adsorbents have somedrawbacks, such as high costs, required expensive instrumentation and

necessity of working with a small sample volume, which causes time consuming pre-analytical steps and generates too small of a sample for serial analyses, limiting repetitive measurements, intra- and intersample precision and reaching trustworthy data [21,22].

IgG Depletion with albumin biosorbents in N-SBF solution is fast and efficient prior to proteomics analysis, such as 1D and 2D electrophoresis and mass spectrometry. Depletion of IgG removes more than 60% of the total protein content in human plasma, whereby proteins normally obscured by IgG during analysis can be visualized. This also allows for a relatively higher load of less abundant proteins during analysis, enabling detection of an increased number of proteins. The results show that albumin biosorbents effectively adsorbes IgG in N-SBF media. The simple, quick and efficient removal of these proteins are thought to be allows increased resolution of lower-abundance proteins during 2-D electrophoresis.

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