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Albumin Adsorption From Aqueous Solutions And Human Plasma With Cibacron Blue F3GA Attached Microporous PTFE Membranous Capillary

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ABSTRACT

Affinity dye-ligand cibacron blue F3GA (CB F3GA), was covalently coupled with poly(vinyl alcohol) grafted on the inner surface of microporous poly(tetrafluoroethylene) (MPTFE) membranous capillary via nucleophilic reaction between the chloride of its triazine ring and the hydroxyl of the PVA under alkaline conditions. The grafting degree of PVA and the amount of CB F3GA immobilized onto membranous capillary are 23.5mg/g and 89.6μmol/g, respectively. These dyed membranous capillaries were chemically and mechanically stable, could be reproducibly prepared. Human serum albumin (HSA) was selected as model proteins. Their adsorption on and desorption from the dye attached membranous capillary was investigated. About 85.3 mg HSA/g dye attached membranous capillary was adsorbed at saturation by the membranous capillary from a 0.01 M Tris-HCl/0.01M NaCl, at pH 5 solution. The capacity of nonspecific adsorption for HSA is less than 0.3 mg/g. High human serum albumin adsorption (125.8 mg HSA/g) was observed from human plasma. High desorption rations (over 95% of the adsorbed albumin) were achieved by using 0.5 M NaSCN (pH 8.0).

KEYWORDS

Albumin adsorption;
Cibacron blue F3GA;
PVA;
PTFE;
Human plasma.

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INTRODUCTION

Immobilized dye-ligand affinity chromatography has become recognized as an important technique for the separation, purification and recovery of a wide range of proteins and enzymes, because inexpensive, stable and group specific dyes are available, which can be used for the separation and evaluating the proteins and drug-proteins. Various dyes were coupled to different bead-based matrices, such as dextran[1], agarose[2], polyacrylamide[3], cellulose[4], poly(vinyl alcohol)[5] and silica gel[6]. However, their use is limited by the compressibility of the soft gels at high flow rates, the high pressure drops for small size beads and the mass-transfer limitation due to the internal diffusion. In recent years, the adsorptive membrane has emerged as an alternative to the traditional column chromatography, since it brings the solute into close proximity to bound ligands through convective transport, thus reducing the mass-transfer resistance and enabling lower pressure drops and higher flow rates[7-9]. But the capacity of membranes is lower than that of column. In addition, membrane fouling is other problem.

CB F3GA has a structure similar to bilirubin (one of two kinds of binding sites in albumins), and was widely employed for plasma fractionation and purification. CB F3GA immobilized on microporous and macroporous chitosan membranes for albumin adsorption have been reported[10-11]. The latter membranes had a much higher affinity and adsorption toward some proteins. In addition, the transport mechanism in microfiltration membranes is generally controlled by steric factors, specifically the solute:pore size ratio. It is, however, well-known that transport in microporous membranes can be affected by factors such as pH[12], ionic strength[13], and solution composition[14].

In the present, the preparation and application of a new form of adsorbent is an interesting work. The coating method is the simplest way to modify the membrane surface by introducing hydrophilic and functional polymers. In our previous work, the membranous capillaries were modified by HSA using a radiation-induced graft polymerization technique, and by CB F3GA using chemical method for bilirubin removal from human plasma[15,16]. In this paper, this new sorbents is composed of CB F3GA as dye affinity ligand, and microporous membranous poly (tetrafluoroethylene) (MPTFE) capillary as the carrier matrix. In order to prevent non-specific interactions between the hydrophobic MPTFE surface and protein molecules, and also to attach the ligand (i.e., CB F3GA) to the matrix, these capillaries are coated with a hydrophilic layer of poly(vinyl alcohol) (PVA) after activation. The results of albumin adsorption-desorption studies from aqueous media and human plasma are presented in this paper. To our knowledge, the similar method which used MPTFE capillaries for HSA adsorption has not been reported before.

EXPERIMENTAL

Chemicals

Microporous membranous PTFE capillaries provided by Prof.K.Watanabe, were used for the present study. These capillaries have an internal diameter of 2 mm, a wall thickness of approximately 0.5 mm and an average surface aperture of 1 μm. Scanning electron microscopy of MPTFE capillary inner walls has been presented elsewhere[17]. PVA (average M_r 14000, 100% hydrolysed) was purchased from the chemical reagent company of Shanghai, China. CB F3GA and HSA (lyophilized, Fraction V) were purchased from Sigma. The blood samples were obtained from a healthy human donor. Reagents such as terephthaldehyde, sodium chloride, sodium carbonate, hydrochloride acid, etc. were analytical reagent grade (purchased from chemical reagent company of Shanghai, China). The fresh piranha solution was formed concentrated sulfuric acid and hydrogen peroxide (1:1, v/v).

Apparatus

A flow injection system (Model FI-2100, Beijing Haiguang Instrument Co., China) was used for the feeding of solution. The concentration of protein in the samples was determined by 7060 automated analyzer (HITACHI, Japan) (Total protein reagent Kit: Cat. No. TP7143 and Albumin reagent Kit: Cat. No. AB7162, Randox Laboratories Ltd., UK). Fibrinogen kit was obtained from Stago (Cat. Ref. No.:

00608 and 00625, Diagnostica Stago, Asnieres-sur-Seine, France).

Cibacron blue F3GA-attached MPTFE

1. Activation of the unmodified MPTFE capillaries

MPTFE capillaries were firstly activated by fresh piranha solution[18]. The capillaries were incubated in a solution of 20 ml fresh piranha solution at room temperature for 40 min. After that, the capillaries were washed several times with distilled water.

2. Coating of MPTFE capillaries with PVA

MPTFE capillaries were coated with PVA by a two-step procedure[16]. In the first step, PVA was deposited on the surface of capillary by a simple adsorption process carried out in an aqueous medium. The initial PVA concentration was 40 mg/ml. The solution (MPTFE capillaries added) was stirred for 2 h with a magnetic stirrer at 200 rpm at room temperature.

In the second step, PVA molecules adsorbed on the MPTFE capillaries were chemically cross- linked to give a stable PVA coating on the surface. After adsorption of PVA from solution, the final acid concentration of the medium was adjusted to 0.1 M by adding HCl. A 10-mg amount of terephthaldehyde was dissolved in 10 ml of water and this solution was added to the previous medium. Then stopping stirring the medium, the temperature increased to 80 °C and cross-linking was completed in 2h. The capillaries were washed several times with hot distilled water. The PVA-coated capillary was stored under distilled water.

The PVA adsorbed onto the capillaries was determined by measuring the initial and final concentrations of PVA within the adsorption medium, according to the KI-I_2 method, spectrophotometrically at 690nm[19].

3. Immobilization of cibacron blue F3GA

CB F3GA was immobilized onto the capillaries by the methods of Xia and Zhang[33]. Briefly, a 0.6 g amount of CB F3GA was dissolved in 20 ml of water, then the PVA-coated capillaries were immersed for 60 min at 60 °C and 1 g of NaCl was added. After this, 0.5 g of Na_2CO_3 were added after 30 min to adjust the pH value of the (to about pH 10) solution. The reaction then took place in the following 4h. The capillaries were washed with distilled water and methanol several times until all the unbound dye was removed. The dye immobilized capillaries was stored in phosphate solutions (pH 7.0) containing 0.02 wt. % sodium azide at 4°C to prevent microbial contamination.

The dye content of the capillaries was determined spectrophotometrically by first hydrolyzing the capillaries in 12 M hydrochloric acid aqueous solution, at 80 °C, for 30 min. The solution was then diluted to 6 M with distilled water, and neutralized with 6 M NaOH aqueous solution. Then the concentration was determined spectrophoto metrically at its maximum absorbance wavelength (λ_{max}=610 nm)[20].

The leakage of the CB F3GA from the capillaries in the aqueous solution and human plasma were followed by treating the capillaries with aqueous solution or fresh human plasma samples for 24 h at room temperature. CB F3GA released after this treatment was measured in the liquid phase spectrophotometrically at 630 nm[21].

Character of on-line adsorption and analytical system

In order to study the dynamic adsorption of albumin, we adopted a kind of FIA system with double channels. The sample solution of containing albumin different concentration (i.e, HSA solution in 0.01 M Tris/0.01 M NaCl, at pH 5 or plasma) was transferred to MPTFE and deposited on it when six valves was fixed at the position of sampling. Then stop pump, and change six valves to the position of eluting, started pump and transferred the eluent (0.01 M Tris/ 0.5 M NaSCN, at pH 8) and developer (arsenazo K) at same time to elute the adsorbed albumins. The eluted solution was send to flow cell to on-line determine adsorbed albumins at 611nm after it was reacted adequately by a K-R (a kind of knitted PTFE capillary). To choose arsenazo K as a developer is because it can react with albumin rapidly, and has same sensitivity of determination for protein.

It is very convenient to determine the adsorption capacity of albumin by this kind of system. Moreover, the system is fit for dynamic adsorption studying of albumins and clinic analysis.

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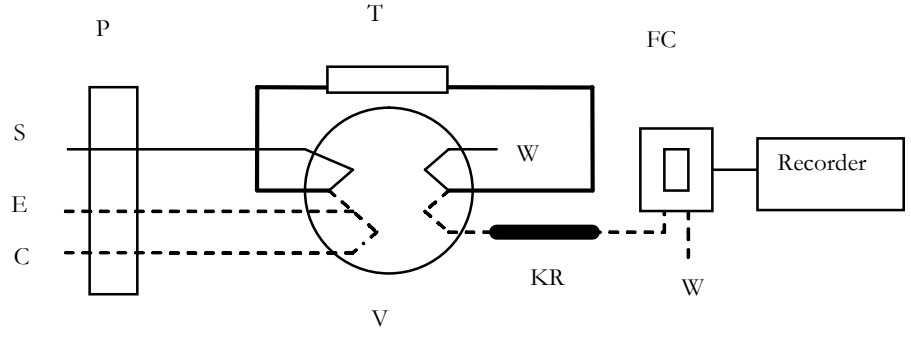

Figure 1: The system of on-line adsorption/ determination analysis.

S : sample, E : eluent, C : developer, P : peristaltic pump , W : waste, V : sixway valve, KR : knotted tube, T : affinity membranous capillary, FC : flow cell, Thin real line : loading flow path, Broken line : eluent, coloration and detection flow path, Thick real line : sameness flow path

Human serum albumin adsorption from aqueous solution

The adsorption/desorption studies were carried out in a flow injection system. The affinity MPTFE capillaries (5 cm long MPTFE capillary was used) connected to the flow injection system directly. Because the modified capillaries were still soft, we added a bushing (a around 5 cm common PTFE capillary) for protection of the capillary. And the bushing has also played a role of rivet for immobilization of the capillary. Recirculation of solution was achieved by changing rotation direction of pump. The affinity capillary was equipped with a water bath apparatus for controlling temperature. This system was controlled by computer, so it could achieve automatic operation.

The amounts of adsorbed HSA (or adsorption capacity) from the solution were described by the following equation:

$$T = (C_0\text{-}C)V/m \quad (1)$$

where T is the amount of HSA adsorbed onto unit mass of the capillary (mg/g polymer); C_0 and C are the concentrations of the HSA in the initial (before adsorption) and in the final solution (after adsorption) (mg/ml), respectively; V is the volume of the human plasma; and m is the mass of the capillary (g). The concentration of albumin in solution (aqueous solution or plasma) was determined by using HITACHI 7060 automated analyzer. The amount of HSA adsorbed onto unit mass of the capillary can be also determined by using a tailored FIA on-line system (see Figure 1).

In a typical flow injection system (see Figure 1), 50 ml of the aqueous solution of HSA was recirculated through the modified capillary for 2 h. The temperature was kept constant at 25°C. The HSA adsorption capacity was determined by measuring the initial and final concentrations of HSA within HITACHI 7060 automated analyzer.

In the first group of experiments, the flow rate of the aqueous protein solution (HSA initial concentration 5.0 mg/ml) was varied between 0.5 and 2.5 ml/min. The initial pH of the solution was 5.0. Ionic strength of the medium was 0.01 (adjusted by NaCl).

The second group of experiments, HSA adsorption from aqueous solution containing 5.0 mg/ml was studied at different pH values and ionic strengths. The pH of the adsorption medium was changed between 4.0 and 7.5 by using different buffer systems (CH_3COONa-CH_3COOH for 4.0-6.0, K_2HPO_4-KH_2PO_4 for pH 7.0-7.5 and NH_3-NH_4Cl for pH 8.0). To investigate the effect of ionic strength on the equilibrium binding of HSA to the affinity capillaries, the adsorption of HSA was carried out at different NaCl concentrations (varied between 0 and 1.0 M, in buffer solution, pH 5 and 8).

In the last group, adsorption rates were obtained both in the continuous recirculation flow injection system. Aqueous solution containing different amounts of HSA was used in these experiments. Changes of the HSA concentration (0.5-5.0 mg/ml) with time were followed to obtain the adsorption rate curves. The flow rate was 1 ml/min. These studies were performed at a constant temperature of 25°C.

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The HSA desorption experiments were performed in a buffer solution containing 1.0 M NaSCN at pH 8.0. Briefly, 25 ml of the NaSCN solution were recirculated through the containing HSA adsorbed affinity capillary for 30 min. Desorption ratio was calculated by using the following expression:

$$DR (\%) = D \times 100 / A \quad (2)$$

where DR is the desorption ratio (%); D and A are the amount of albumin desorbed and adsorbed on the affinity capillaries, respectively.

Human serum albumin adsorption from human plasma

Human serum albumin adsorption studies were carried out in the same flow-injection system. Blood samples were centrifuged at 600 × g for 20 min at room temperature to separate the plasma. The initial human plasma of the donor contained 42.3 mg/ml of HSA as determined by using albumin reagent kit within 7060 automated analyzer. In a typical continuous flow-injection system, 25 ml of the plasma freshly separated from the human blood were recirculated through the CB F3GA affinity capillaries for 2 h. The amount of human serum albumin adsorbed was obtained by measuring the decrease in the HSA concentration in the plasma by the same assay. Phosphate-buffered saline (PBS; pH:7.4, NaCl: 0.9 %) was used for dilution of human plasma. In this experiment, the flow-rate of the human plasma was 0.5 ml/min. The temperature was constant at 25 °C. The same desorption procedure was applied for desorption of adsorbed as mentioned above. In order to observe the performance of affinity capillaries in this group of experiment, the purity of HSA was also studied. Total protein concentration was measured by using total protein reagent kit and HSA concentration was determined by using Albumin reagent kit within 7060 automated analyzer. Chronometric determination of fibrinogen according to the Von-Clauss method on plasma was performed by using fibrinogen kit[22]. λ-globulin concentration was determined from the difference. The initial human plasma contains albumin (42.3 mg/ml), fibrinogen (2.9 mg/ml) and λ-globulin (18.7 mg/ml).

Purity of HSA was calculated by using the following expression:

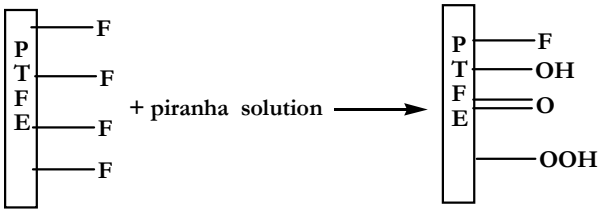
$$P (\%) = D_H \times 100 / D_P \quad (3)$$

where P is the purity of HSA (%); D_H and D_p are the amount of human serum albumin desorbed and total protein desorbed on the affinity capillaries, respectively.

RESULTS AND DISCUSSION

Surface modification of PTFE capillary membranous capillary

The reactive equation of activating the PTFE with piranha solution is as following:



The adsorption capacity of albumin is only 0.408±0.028 albumin molecules/mm^2 when the activated PTFE was coupled indirectly with dye[17]. However, the grafting PVA onto the activated PTFE membranous capillary has some difficulties in technique. But it will be easy job if the process of graft can be manipulated by certain way. The experimental results show that the effect of coating is best at 5 cm of the capillary length and 40 mg/ml of PVA solution. Here, the maximum adsorption capacity of HSA is 85.3 mg /g polymer. The relation between the concentration of PVA and the amount of PVA immobilized onto the PTFE membranous capillary

TABLE 1: Aspect and amount of PVA immobilized on the PTFE membranous capillary

Concentration of PVA (mg/ml)	Immobilized mass of PVA (mg/g)	Aspect
5	Undetected	
10	0.6	Uneven
20	5.9	Homogenized partly
40	23.5	Homogenous
50	*	Uneven

***: The membranous capillary is easily plug up because of flocculation of PVA.**

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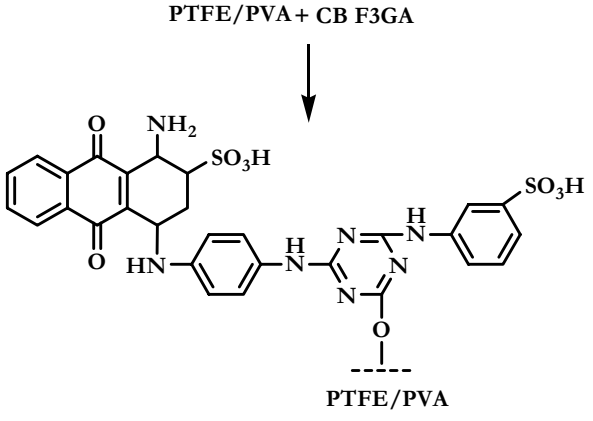
is displayed in TABLE 1.

The reaction between grafted PTFE membranous capillary and dye is as following:

In this study, in order to overcome the drawbacks of traditional affinity albumin sorbent, we attempted



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to prepare a novel affinity sorbent for albumin adsorption from aqueous solution and human plasma in a recirculation flow-injection system. CB F3GA was used as the affinity dye for binding of albumin molecules. This new sorbents has the advantage over both membrane and micro-column, and it has mass transfer of higher velocity, better adsorption capacity, less fouling and longer useful life.

MPTFE capillary was selected as the carrier matrix. MPTFE capillary is a new type of functional material, which has good chemical and mechanical stability, fairly large pore size on surface and large porosity as described in our previous papers[16]. MPTFE capillary has been used in the field of analysis[16,23]. As we know, PTFE is an extremely inert and hydrophobic matrix, which has poor blood compatibility, so it must be modified before used in purpose of biological or medical treatment. Modification of the MPTFE capillary is a very difficult job, not only because of its chemical stability, but also because of the shape of capillary. At present, the modification of PTFE has performed by methods of irradiation (i.e, γ-radial, laser and plasma)[24-26], but these methods need special equipments and destroy the surface structure completely. Now, it can result in a sufficient number of free functional groups without damaging the structural integrity of the PTFE polymer with wet chemical methods, LÖhbach first modified PTFE vessels with fresh piranha solution[18], Watanabe modified the PTFE capillary with NaOH solution[23]. However, it is not enough because the surface of PTFE remains hydrophobic and the active site is quite low. In this paper, the PVA was coupled to the surface of PTFE, which can be as an intermediate for further attachment to an immobilized molecule.

After this, CB F3GA was covalently coupled to the MPTFE capillary via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl groups of the PVA which coating on the capillary under alkaline conditions. Few of the contaminants will be immobilized on the supporting matrix and proper washing of the matrix can remove absorbed contaminants, so purification of the dye before immobilization is not necessary. The average density of CB F3GA attached on the capillary is 89.8 μmol/g polymer.

It is reported that CB F3GA has no adverse effect on biochemical systems[27]. Its specific structures (hence their specific affinities) and the dye contents are responsible for its adsorption capacity. CB F3GA has a structure similar to that of bilirubin, and can bind more tightly to the bilirubin sites of the HSA[11]. The studies of CB F3GA leakage from the modified membranous capillaries showed that there was no leakage in any of the media, which meant that the washing procedure was quite satisfactory for the removal of non-specific adsorbed dye molecules from the membranous capillaries.

Adsorption from aqueous solution

1. Effects of flow-rate on adsorption

In these experiments, the flow rate of the aqueous solution varied from 0.5 to 2.5 ml/min; other parameters were kept constant. The adsorption capacities at different flow-rates are given in figure 2.

With the increase of the flow-rate from 0.5 to 2.5 ml/min, the adsorption capacity decreased significantly from 81.2 to 23.6 HSA mg/g polymer. This change may be due to the decrease of residence time in the capillary, which does not give enough time for HSA to interact with the adsorbent. As we know, the specific interaction of proteins with immobilized

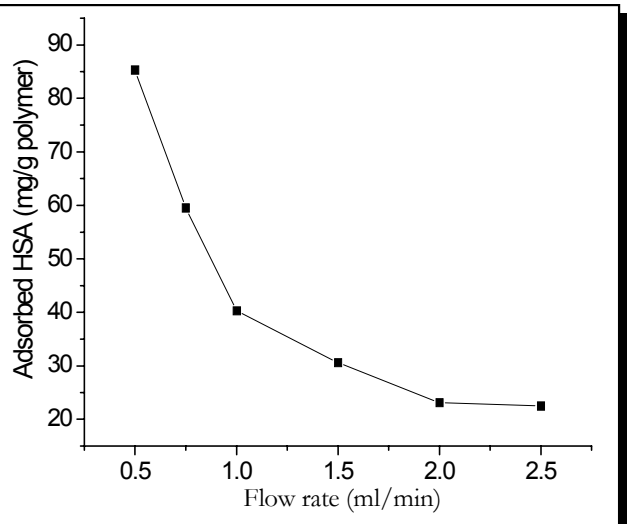

Figure 2: Effects of flow rate on HSA adsorption

HSA initial concentration: 5.0mg/ml; pH: 5.0; ionic strength: 0.01 (adjusted with NaCl); temperature: 25°C; and total solution volume: 50ml.

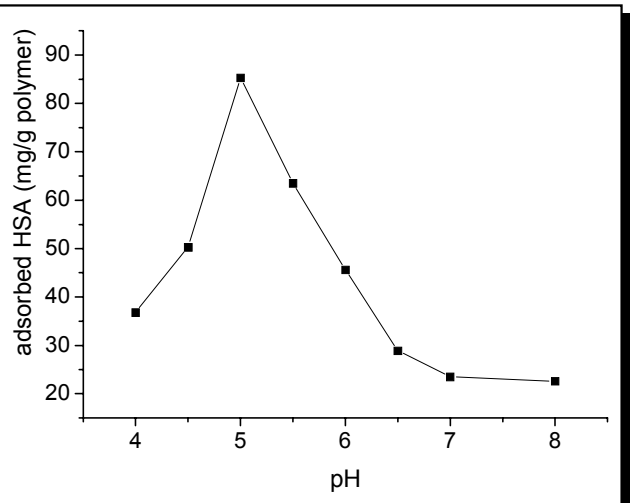

Figure 3: Effects of pH on HSA adsorption.

Flow rate: 0.5 ml/min; initial concentration of HSA: 5.0 mg/ml; ionic strength: 0.01 (adjusted with NaCl); temperature: 25 °C; and total solution volume: 50 ml.

small molecules often varies as protein surface topography, chemistries, and physical variables change. So the orientation effect is an important factor for the interaction between small molecule and affinity biomacromolecules (HSA). In other words, when the CB F3GA as substrate conjugated with HSA, the morphology of HSA must change in order to adapt the structure of CB F3GA. Whereas this process need some time, it may be a key to HSA adsorption because the greatest adsorption occurs only when their orientations are almost identical. So with the increase of the flow-rate, the efficiency of conjugation between CB F3GA and HSA decreased, and led up to a descent of adsorbed HSA. Thus low adsorption capacities were observed at high flow-rates. At the flow-rates lower than 0.5 ml/min we encountered some technical problems in our experiment; therefore we carried out all other adsorption tests at a flow-rate of 0.5 ml/min.

2. Effects of pH

Figure 3 provided the effects of pH (4-8) on HSA adsorption which is very significant. Since activity of HSA will be destroyed if pH exceeded 8, we did not try it at higher pH. At pH values lower and higher than pH 5.0, the adsorbed amount of HSA drastically decrease, and the maximum adsorption of HSA was observed at pH 5.0.

The HSA adsorption includes electrostatic and/or hydrophobic interactions between charged HSA molecules and immobilized CB F3GA. As pH of the protein solution (pH under isoelectric point) increases, the degree of protonation of these amino acid residues decreases and the negatively charge of CB F3GA increases. To view the results as a whole, the adsorption of HSA increases. At the isoelectric points, proteins have no net electrical charge and have the biggest affinity to the capillaries, so the maximum adsorption from aqueous solutions is usually observed at their isoelectric point[28]. The maximum adsorption was observed at pH 5.0 which is the isoelectric pH of HSA. CB F3GA is a monochlorotriazine dye and it contains three acidic sulfonate groups. These specific interactions may result from these groups on both the CBF3GA and amino acid side chains in the HSA structure, and from the conformational state of HSA molecules at this pH. In addition, pH increases (pH above isoelectric point), the adsorption of HSA decreases. This may be due to electrostatic repulsive effects on between HSA and the ligand molecule.

3. Effects of ionic strength

The effect of the ionic strength on HSA adsorption is presented in figure 4, which shows the adsorption capacity decreases with increasing NaCl concentration in the binding buffer (pH: 5 and 8). When the added concentration of NaCl changes from 0.0

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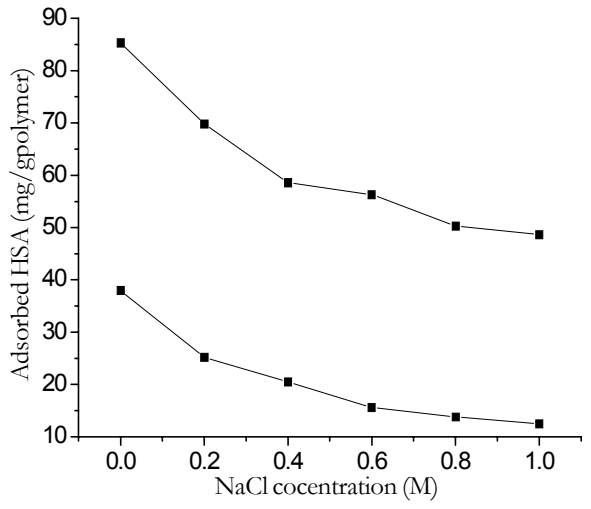

Figure 4: Effects of ionic strength on HSA adsorption

Flow rate: 0.5 ml/min; HSA initial concentration: 5.0 mg/ml; pH: 5 and 8; temperature: 25 °C; and total solution volume: 50 ml.

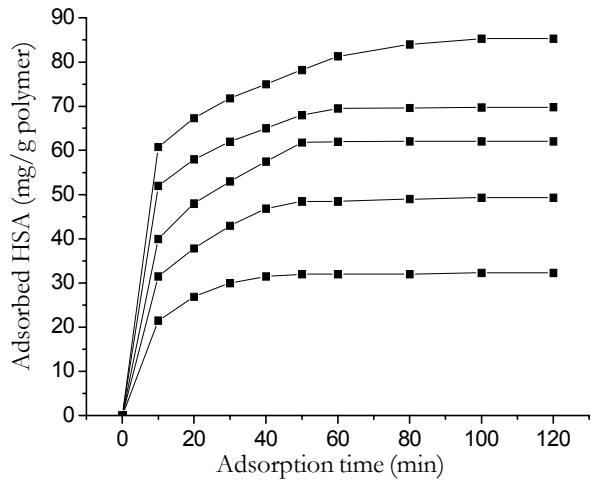

Figure 5: Effects of time on HSA adsorption at different its concentration

Flow rate: 0.5 ml/min; pH: 5; ionic strength: 0.01 (adjusted with NaCl); temperature: 25 °C; and total solution volume: 50 ml.

to 0.5 M, the adsorption of HSA decreases by ~ 44.2% (pH=5) and ~57.8% (pH=8). This is in agreement with the results reported in the literature[29]. This may be explained by the formation of more compact structures of the albumin molecules at high ionic strengths. More ions may also be attached to albumin molecules at high ionic strengths. This causes further stabilization of the protein molecules (higher solubility), which may lead to lower adsorption of albumin on the affinity capillaries.

4. Adsorption rate

Figure 5 showed the adsorption rate curves which were obtained by following the decrease of the concentration of HSA within the protein solution with time. As can be seen, the relatively faster adsorption rates were observed at the beginning of adsorption process, and then adsorption equilibriums would be achieved gradually in about 60 min. This may be due to the decrease in the HSA concentration with time by adsorption. The driving force decrease (i.e., the concentration difference between the aqueous solution and affinity) results in a drop in the adsorption rates when the HSA concentration in the mobile phase decreases. In addition, adsorption rates increased within increasing HSA concentration. This may be due to a high driving force, which is the HSA concentration difference the mobile phase (i.e., the protein solution) and the stationary phase (i.e., the affinity capillaries), in the case of high HSA concentration.

5. Effects of HSA initial concentration

Figure 6 showed the non-specific and specific adsorption of HSA onto the plain capillaries, dye immobilized capillaries, respectively. Note that one of the main requirements in the affinity system is the specificity of the carrier matrix and the molecules to be removed should be minimum in order to have

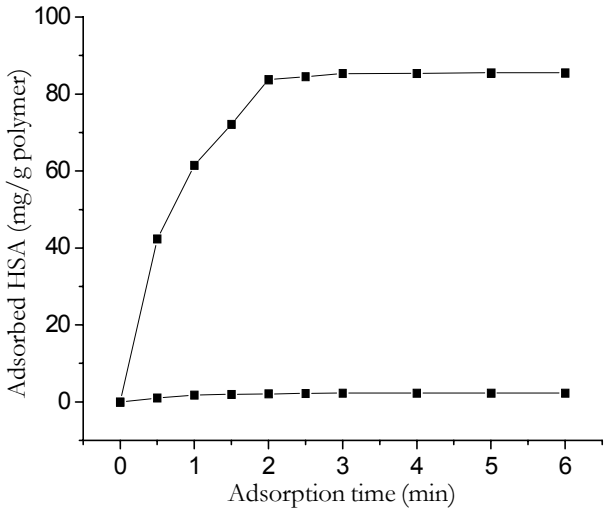

Figure 6: Effects of initial HSA concentration on HSA adsorption

Flow rate: 0.5 ml/min; pH: 5; ionic strength: 0.01 (adjusted with NaCl); temperature: 25 °C; and total solution volume: 50 ml.

a high specificity. As seen from here, the amount of HSA adsorption on the unmodified capillaries was quite low, which was about 2.3 mg /g polymer. With increasing HSA concentration in solution, the adsorption capacity of HSA increases. CB F3GA attached capillaries significantly increased the HSA adsorption capacity (up to 85.3 mg/g polymer), because of the specific interactions between albumin and CB F3GA molecules.

6. Electrostatic and hydrophobic interactions to protein adsorption on dye-ligand adsorbents

In spite of the extensive studies and applications of the dye-ligand adsorbent, the fundamental interactions between the reactive dye and proteins remain poorly understood because of the complexity of its affinity for proteins. CB F3GA, a so-called pseudoaffinity ligand, has a unique chemical structure that enables it to bind to a variety of proteins according to different mechanisms. First, the anthraquinone chromophore in CB F3GA molecules is hydrophobic, so it can interact with nonpolar surfaces of proteins; Second, CB F3GA has a conformation that minics the orientation and anionic groups characteristic of nicothinamideadenine dinucleotie and nicotinaonide adenine dinadeotide phosphate; Finally, because several of the aromatic rings in CB F3GA at substituted with negatively charged sulfonate groups, the dye can function as cation exchanger to bind proteins by electrostatic interactions[29]. In addition, for use in chromatography, the dye is always covalently coupled to an insoluble support matrix and can not move as freely as in solution. Thus, studying immobilized CB F3GA -protein bind mechanisms seems to be more difficult due to the steric effect and the interactions between the ligand and the solid matrix. However, in general, whether specific or nonspecific protein-dye binding, it can be described in term of electrostatic and/or hydrophobic interactions[30].

In this paper, the adsorption mechanism of HSA still can not clearly explained for the HSA is a complicated biomolecule and the factors influencing the interactions are numerous. However, it is not difficult to understand the important effects of coordination and hydrophobic force on HSA adsorption from the data in figure 2 and 3. So we conclude that the interaction of HSA with immobilized CB F3GA could be categorized into specific interactions and nonspecific interactions.

HSA adsorption from human plasma

The results of HSA adsorption from human plasma are summarized in TABLE 2. There was a low adsorption of HSA on the plain capillaries (the amount of HSA adsorbed is 2.3 mg/g polymer). And a higher adsorption (up to 85.3 mg/g polymer) was obtained when the affinity capillaries were used. As presented in this table, the adsorption of HSA onto affinity capillaries was higher (about 1.5 folds) than those obtained in aqueous solution. This may be due to the conformational structure of HSA molecules in human plasma being much more suitable for specific interaction with the affinity capillaries. In addition, the high HSA concentration (42.3 mg/ml) may also contribute to this high adsorption capacity. There is a competitive adsorption in human plasma, so other protein molecules may also be adsorbed. The experiment of competitive protein adsorption also carried out in these studies. As see from TABLE 2, the adsorption of other plasma proteins (i.e., fibrinogen and γ-globulin) on the affinity capillaries are low. It should be noted that HSA is the most abundant protein in plasma. It generally makes up more than half of the total plasma proteins. It may be resulted that this low adsorption of fibrinogen and γ-globulin is due to the high concentration of HSA.

Desorption of HSA

Desorption of HSA was carried out using 1.0 M NaSCN solution at pH 8. 25 ml of eluant were recirculated through the HSA adsorbed affinity capillar-

TABLE 2: HSA adsorption from the human plasma (healthy donor)

HSA concentration (mg/ml)	Amount of protein adsorbed (mg/g polymer)		
	Fibrinogen	γ-Globulin	HSA
2.6	0.50	0.62	56.8
5.3	0.80	1.25	72.3
10.5	1.15	1.85	82.6
21.2	1.56	2.31	98.5
42.3	2.05	2.88	125.8

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TABLE 3: Desorption of albumin form affinity capillaries from aqueous solution

Aqueous solution (mg/ml)	HSA adsorbed (mg/g)	Desorption ratio (%)
0.5	28.9	96.6
1.0	56.6	95.8
2.0	70.4	95.2
4.0	80.3	96.2
5.0	85.3	94.3

TABLE 4: Desorption of albumin form affinity capillaries from human plasma

Human plasma (mg/ml)	HSA adsorbed (mg/g)	Desorption ratio (%)	Purity (%)
2.6	56.8	94.5	93.6
5.3	72.3	94.3	92.8
10.5	82.1	92.8	93.5
21.2	98.6	93.5	94.3
42.3	125.8	93.2	91.9

ies for 30 min, at 25°C. As presented in TABLE 3 and TABLE 4, more than 92% of the adsorbed albumin molecules could be easily desorbed from the affinity capillaries. When the NaSCN is used, the specific interaction forces (such as electrostatic and hydrophobic forces) between the albumin molecules and the dye is broken, which forces desorption of albumin molecules from the solid matrix. In addition, SCN^- ions bind albumin molecules easily. This also affects the conformational structure of adsorbed albumin molecules. Furthermore, electrostatic interactions between the adsorbed protein and CB F3GA could be easily decreased because of the conformational changes of albumin molecules.

Reusability

The reusability is one of important advantages of this novel sorbents. In order to show the reusability of the affinity capillaries, the adsorption-desorption cycle of HSA was repeated ten times by using the same capillaries. As seen from figure 7, there was no remarkable reduction in the adsorption capacity of the affinity capillaries. The HSA adsorption capacity decreased only 3.9 % after ten cycles.

Estimate of adsorption capacities

Note that different sorbents with different adsorption capacities were reported in the literature for albumin adsorption. McCreath et al. have showed 9.7 mg/g HSA /ml equilibrium adsorption capacity with the PVA coated pariticulate perfluoropolymer containing anion exchange and cation exchange group[31]. Zeng et al. reported 10.2mg HSA/g adsorption capacities with cibacron blue F3GA attached polyethersulfone supported chitosan sorbent[10]. Li et al. used cibacron blue F3GA-attached poly (ethylene imine)-coated titania and achieved 4.4 mg HSA/g[32].

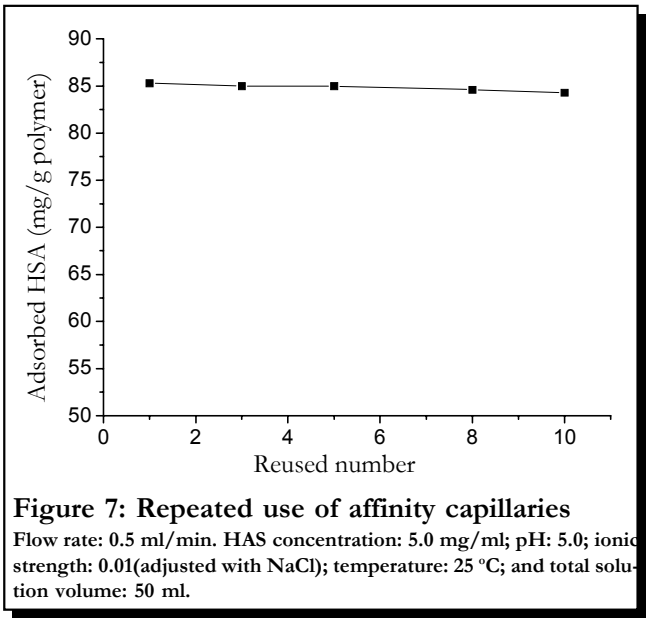

Figure 7: Repeated use of affinity capillaries
Flow rate: 0.5 ml/min. HAS concentration: 5.0 mg/ml; pH: 5.0; ionic strength: 0.01(adjusted with NaCl); temperature: 25 °C; and total solution volume: 50 ml.

Denizli et al.[33-34] obtained adsorption capacities of 35-215 mg/g polymer for HSA. Nash and Chase [35] presented adsorption capacities of 11.7-27 mg HSA/g. The maximum HSA adsorption that we achieved with the sorbent system developed in this study was 85.3 mg/g polymer, which was quite comparable with the related literature.

CONCLUSION

Mechanically stable, microporous PTFE membrane capillary has been modified by PVA. The capillaries carrying the PVA were incorporated with cibacron blue F3GA. The dye/PVA attached capillaries have a high adsorption capacity, a shorter time of saturation adsorption and eluting, and desorption ratio (%) is not less than 95%. The adsorption under different pHs and ionic strengths and the desorption with different eluants have revealed the high selec-

tivity of cibacron blue F3GA membranous capillary for HSA. The interaction of HSA with immobilized CB F3GA could be categorized into specific interactions and nonspecific interactions.

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