Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Denzl, Adil , Tuncel, Ali , Kozluca, Ahmet , Ecevt, Kutay and Pşkn, Erhan(1997) 'Cibacron Blue F3G-A Attached Poly(Vinyl Alcohol) Particles for Specific Albumin Adsorption', Separation Science and Technology, 32: 5, 1003 — 1015
To link to this Article: DOI: 10.1080/01496399708000942
URL: http://dx.doi.org/10.1080/01496399708000942

SEPARATION SCIENCE AND TECHNOLOGY, 32(5), pp. 1003–1015, 1997

Cibacron Blue F3G-A Attached Poly(Vinyl Alcohol) Particles for Specific Albumin Adsorption

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ABSTRACT

Poly(vinyl alcohol) (PVAL) particles (average size: 50 μm) were prepared in the present study by chemical crosslinking of PVAL with glutaraldehyde in an organic dispersion oil phase. Cibacron Blue F3G-A was attached to these hydrophilic PVAL particles. Bovine serum albumin (BSA) adsorption onto these dye-attached PVAL particles from aqueous solutions containing different amounts of BSA in three different salts (i.e., NaCl, $CaCl_2$, and NaSCN) at different pH and ionic strengths was investigated in batch reactors. The maximum adsorption capacity (about 35 mg BSA/g dye-attached PVAL) was observed around pH 6.0 in a medium containing NaCl with an ionic strength of 0.01. Nonspecific BSA adsorption on plain PVAL particles was almost zero. About 90% of the adsorbed BSA was desorbed by using a 0.5 M NaSCN desorption medium for 1 hour.

INTRODUCTION

The interest in and demand for proteins with high purity in biotechnology, biochemistry, and medicine have contributed to an increased exploitation of affinity chromatography. Most emerging affinity techniques have attempted to use biospecific sorbents in which proteins specifically and reversibly interact with the biological ligands on these sorbents (1–3). Biological ligands are highly specific. However, the preparation of sor-

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bents carrying biological ligands is usually very expensive because the ligands themselves often require extensive purification and it is difficult to immobilize them on the carrier matrix with retention of their biological activity. As an alternative to their natural biological counterparts, the reactive triazinyl dyes have been investigated as ligands for protein affinity separation (4–6). These dyes are able to bind proteins in a remarkably specific manner. They are inexpensive, readily available, biologically and chemically inert, and are easily coupled to support materials. Cibacron Blue F3G-A and many other reactive dyes have been coupled to a variety of supports including agarose, cellulose, polyacrylamide, sephadex, silica, and glass (7–12). Dye-ligand chromatography has permitted the purification of a wide range of proteins (e.g., lactate hydrogenase, alcohol dehydrogenase, hexokinase, carboxyl peptidase, etc.) (4–12).

We recently prepared monosize dye-attached polyvinyl alcohol (PVAL) coated polystyrene (PS) microbeads (i.e., monosize with a diameter of 4 μm) and investigated reversible albumin adsorption on these sorbents (13–16). In this paper we present the results of another alternative affinity sorbent system in which we changed only the carrier. Instead of PVAL-coated PS particles we used PVAL particles of irregular shape (average particle size: 50 μm) which were prepared in our laboratories. The ligand was the same dye, i.e., Cibacron Blue F3G-A. Similarly, we studied albumin adsorption/desorption at different medium conditions. Here, we present the data obtained in these studies and compare the results with those obtained in the previous investigation.

EXPERIMENTAL METHODS

Preparation of PVAL Particles

Polyvinyl alcohol (PVAL) particles were prepared by chemical crosslinking of PVAL (Aldrich Chemical Co., USA, MW: 50.000) within an organic dispersion phase. The dispersion medium was prepared by adding 20 mL of 1-butanol (Merck A.G., Germany) into 80 mL of corn oil (Livio, Unilever, Turkey) containing 20 mg/mL Tween-20 (Sigma Chemical Co., USA) at a stirring rate of 700 rpm. Five milliliters of an aqueous solution of PVAL (9.0% w/v) was dispersed within the dispersion medium. Glutaraldehyde solution (0.8 mL, 25% w/v, Sigma Chemical Co., USA) and 0.4 mL of concentrated HCl (37.5%, Merck A.G., Germany) were added to the final mixture. The temperature was raised to 50°C and the medium was stirred for 1 hour at 700 rpm. The resulting crosslinked PVAL microspheres were separated from the reaction medium by decantation and were first washed with 50% aqueous ethanol (TEKEL, Turkey) solution,

then several times with hot and cold distilled water, and dried at vacuo at room temperature.

Swelling

In order to determine the swelling behavior of PVAL particles, initially dry particles were placed in distilled water and kept at a constant temperature of 25 ± 0.5°C. Swollen particles were periodically removed and weighted by an electronic balance (Shimadzu, Japan, EB.280 ± 1×10^{-3} g). The water contents of the swollen particles, i.e., the so-called "swelling ratios," were calculated by using the following expression:

$$\text{Swelling ratio } \% = [(w_s - w_0)/w_0] \times 100 \quad (1)$$

where w_0 and w_s are the weights of the PVAL particles before and after swelling, respectively.

Determination of Average Size

The washed PVAL particles were spread on a glass slide, and photographs of these swollen particles were taken at 100× magnification under an optical microscope (Nikon, Alphaphot YS, Japan). The average sizes of the PVAL particles were determined from these optical micrographs.

Dye Attachment to PVAL Particles

Three hundred milligrams of the dye, i.e., Cibacron Blue F3G-A (Polyscience Inc., USA) was dissolved in 10 mL of distilled water. This solution was then added to an aqueous dispersion prepared by dispersing 3.0 g of PVAL particles in 90 mL of distilled water and then adding 4 g of NaOH. The medium was heated to 80°C in a sealed reactor for 4 hours at a stirring rate of 400 rpm. The particles were filtered and washed with distilled water and methanol several times until all the unbound Cibacron Blue F3GA was removed. The particles carrying the Cibacron Blue F3GA-attached PVAL particles were then used in the adsorption/desorption studies described below.

Albumin Adsorption/Desorption

Bovine serum albumin (BSA, lyophilized, Fraction V, Sigma Chemical Co., USA) was selected as a model protein. BSA adsorption onto the plain and dye-attached PVAL particles was studied. First, BSA adsorption isotherms were derived in the experiments in which the initial BSA

concentration was changed to between 0.5 and 4.0 mg/mL. The pH of the medium was 5.0 (i.e., the isoelectric point of BSA). The ionic strength of these BSA solutions was adjusted to 0.01 by using NaCl.

In the second group of adsorption experiments the effects of pH, ionic strength, and the existence of coions on the BSA adsorption capacity of the sorbent was investigated. The medium pH was changed to between 4 and 8 by using different buffer systems (i.e., CH_3COONa–CH_3COOH for pH 4–6; K_2HPO_4–KH_2PO_4 for pH 7; NH_4OH–NH_4Cl for pH 8). The ionic strength of these media was 0.01. These experiments were repeated by using different salts, namely, NaCl, $CaCl_2$, and NaSCN. The effects of ionic strength on albumin adsorption was studied in another set of experiments. In these experiments the ionic strength of the adsorption media was adjusted to 0.01 and 0.1 by using NaCl. The pH of these media was 5.0.

In a typical adsorption experiment, BSA was dissolved in 25 mL of buffer solution containing the salt at a certain ionic strength, and 500 mg of particles was added to this medium. The adsorption experiments were conducted for 2 hours at 25°C at a stirring rate of 100 rpm by using a magnetic stirrer. At the end of the equilibrium period (i.e., 2 hours), the particles were separated from the solution by centrifugation. The concentration of albumin left in the medium was then measured spectrophotometrically at 280 nm (17–19).

BSA desorption experiments were performed in a buffer solution containing 0.5 M NaSCN at pH 8.0. The BSA-adsorbed particles were placed in the desorption medium. The medium was stirred for 1 hour at 25°C. Then the final BSA concentration was determined spectrophotometrically at 280 nm. The desorption ratio was calculated by using the amount of BSA adsorbed on the particles and the final BSA concentration in the desorption medium.

RESULTS AND DISCUSSION

Poly(vinyl alcohol) (PVAL) is a water-soluble polymer. However, when PVAL molecules are crosslinked, a hydrogel structure is achieved. This form is no longer soluble in water but does swell to a certain extent, mainly depending on the crosslinking density. PVAL gels can be crosslinked by several methods, including chemical crosslinking where formaldehyde or glutaraldehyde is the common type of crosslinker used for crosslinking (20, 21). PVAL hydrogels have been extensively used in the biomedical area, mainly due to their inertness (1, 2, 22). There is a very low (even zero) protein adsorption on PVAL surfaces (23). This was the main reason why we selected PVAL particles as the carrier matrix in our biospecific

sorbent system. In addition, PVAL has a very strong affinity to the dye, i.e., Cibacron Blue F3G-A, used as the ligand in our previous study (14–16).

Characterization of PVAL Particles

An optical micrograph of the swollen PVAL particles obtained by dispersion crosslinking is given in Fig. 1. As seen here, these particles were irregular in shape and had an average diameter of 50 μm. In this study we considered this irregularity in shape as an advantage because the outer surface area per unit weight of these particles is probably much higher than those of spherical shape with the same average size. Note that a higher outer surface area means a higher adsorption capacity.

The variation of swelling ratio of the PVAL particles with time is given in Fig. 2. As seen in this figure, the PVAL particles first swell rapidly and then gradually reach the equilibrium swelling value in 1 hour. The equilibrium swelling value of the PVAL particles obtained in this study

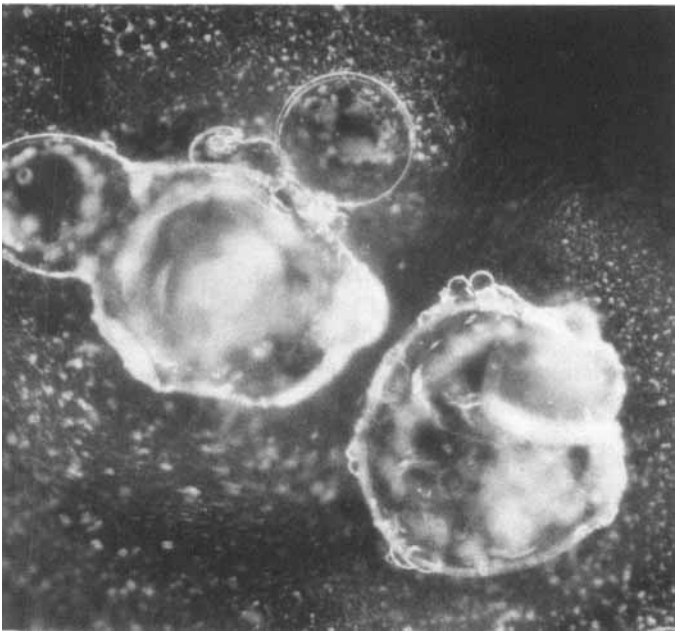

FIG. 1 Optical photograph of PVAL particles.

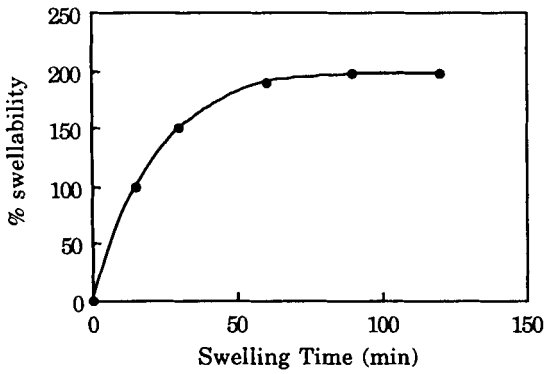

FIG. 2 Swelling behavior of PVAL particles.

by using the recipe given in the previous section was about 200% on a weight basis. Note that, as mentioned above, the swelling ratio can easily be changed by changing the amount of crosslinker (i.e., glutaraldehyde). Higher amounts of crosslinker mean a higher crosslinking density and a less swellable matrix.

The blue color of Cibacron Blue F3G-A-attached PVAL particles clearly indicates the existence of dye on the surfaces of the PVAL particles. After the extensive washing steps described above, the color of the particles stayed stable, which means there was no more dye release.

BSA Adsorption/Desorption Studies

BSA Adsorption

Effects of BSA Initial Concentration. In the first group of experiments the initial BSA concentration was changed to between 0.5 and 4.0 mg/mL for the derivation of the adsorption isotherms at 25°C with an adsorption equilibrium time of 2 hours. BSA was adsorbed onto the dye-attached PVAL and plain PVAL particles from an aqueous medium having a pH of 5.0 and containing NaCl at an ionic strength of 0.01. The variation of the BSA adsorption capacity with the initial BSA concentration is given in Fig. 3. As seen in this figure, no nonspecific adsorption of BSA onto the plain PVAL particles was observed. On the other hand, remarkably higher adsorption capacities due to the specific interaction between the dye and albumin molecules were obtained with the Cibacron Blue F3G-A-attached PVAL particles. The BSA adsorption capacity of these dye-attached PVAL particles first increased with an increase in the initial BSA

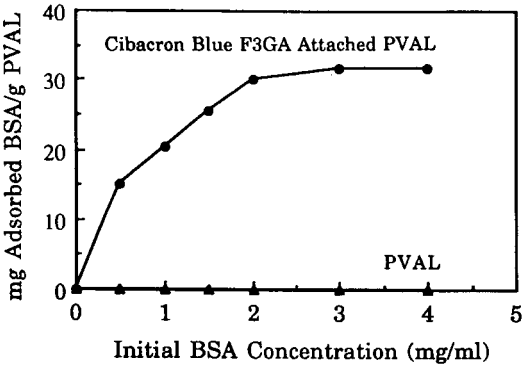

FIG. 3 BSA adsorption on PVAL particles.

concentration, and then reached a plateau value (i.e., about 30 mg BSA per gram of the PVAL particles, which corresponds with the maximum capacity) at about 2 mg/mL of BSA initial concentration, as was expected (24–26).

Effects of pH. The effects of pH on BSA adsorption onto the dye-attached PVAL particles were investigated by changing the pH of the medium to between 4 and 8 by using different buffer systems (i.e., CH_3COONa–CH_3COOH for pH 4–6; K_2HPO_4–KH_2PO_4 for pH 7; NH_4OH–NH_4Cl for pH 8). The ionic strength of these media was 0.01 (adjusted with NaCl). The initial concentration of BSA in the medium was 2.0 mg/mL.

Figure 4 shows the pH effect. It is usually expected that the maximum adsorption of BSA will be observed around the isoelectric point of the protein, which is approximately pH 5.0 (27). However, the maximum adsorption in our experiments was observed around pH 6.0. The shift observed here may originate from conformational changes in the albumin molecules adsorbed on the PVAL particles and from dye attachment. Note that HCl and NaOH were used in the preparation of PVAL particles. Of course, most of the HCl and NaOH was removed during the extensive washing steps. However, there may be some small amount entrapped within the bulk of the matrices. These entrapped HCl and NaOH molecules in the PVAL particle matrices can create a local environment which cannot be easily controlled by buffering the adsorption medium. Most probably due to the effects of this local environment, BSA molecules sitting on the particles may change their conformations, which may cause

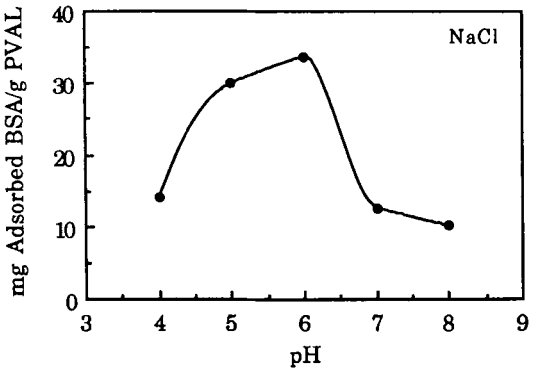

FIG. 4 Effects of pH on BSA adsorption.

the shift of the isoelectric point of BSA from 5.0 to 6.0, or may be better for interactions with dye molecules.

Effects of Cations. In order to obtain the effects of cations on BSA adsorption, the adsorption experiments described in the previous section were repeated by using $CaCl_2$ instead of NaCl. The other experimental conditions were exactly the same.

Figure 5 shows the cation effect. As seen in this figure, higher BSA adsorption capacities were obtained with Na^+ relative to those observed with Ca^{2+} at the same pH. This may be due to higher binding affinity between the albumin molecules and Ca^{2+} ions, especially above the isoelectric point of BSA. Binding of Ca^{2+} ions may cause conformational changes in the albumin molecules, which, in turn, leads to less adsorption, as also discussed in the related literature (28–30). It should also be noted that monovalent cations such as Na^+ hardly bind to albumin molecules (31).

Effects of Anions. The effects of anions on BSA adsorption were evaluated by repeating the adsorption experiments described above but by using NaSCN instead of NaCl. All other experimental parameters were exactly the same.

Figure 6 shows the anion effect. As seen in this figure, higher BSA adsorption capacities were obtained with Cl^- relative to those observed with SCN^- at constant pH values. The relatively lower BSA adsorption capacities in the presence of SCN^- ions may be attributed to the higher binding affinity of these anions to the BSA molecules, which may cause conformational changes leading to smaller adsorption values, as also reported by others (32, 33).

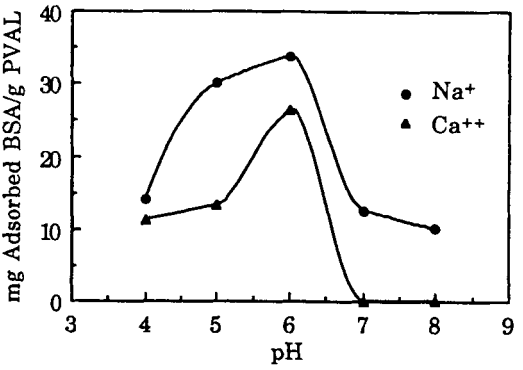

FIG. 5 Effects of cations on BSA adsorption.

Effects of Ionic Strength. The effect of ionic strength was studied by repeating the adsorption experiments described above at two different ionic strengths, i.e., 0.01 and 0.10, adjusted with NaCl.

Figure 7 shows the ionic strength effect. The amount of BSA adsorbed decreased with an increase in the ionic strength of the medium. A similar tendency is reported in the literature (34, 35). This may be explained by the adsorption of more ions to the BSA molecules at higher ionic strengths. These bindings than cause conformational changes in the protein structure, thus leading to less adsorption.

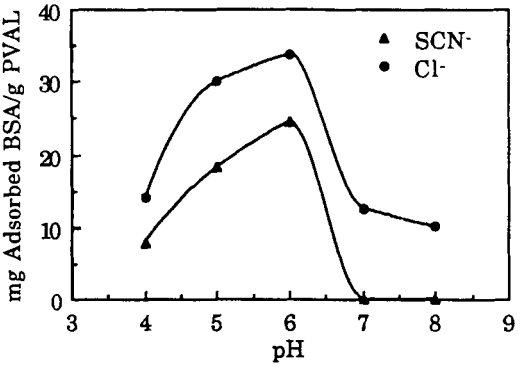

FIG. 6 Effects of anions on BSA adsorption.

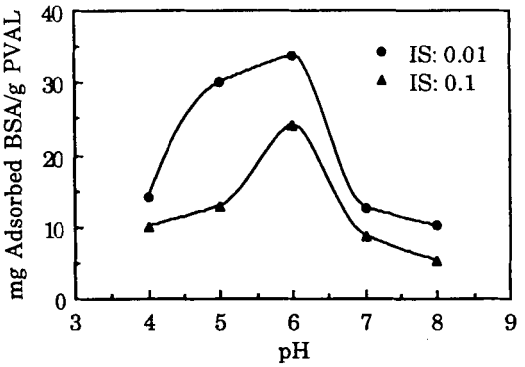

FIG. 7 Effects of ionic strength on BSA adsorption.

Desorption

Desorption of the adsorbed BSA from the Cibacron Blue F3G-A-attached PVAL particles was studied in a batch experimental setup. Dye-attached PVAL particles loaded with different amounts of BSA were placed within a desorption medium containing 0.5 M NaSCN at pH 8.0, and the amount of BSA released in 1 hour was determined. The desorption ratio was then calculated by using the following expression:

TABLE 1
BSA Desorption Ratios: (desorption medium: 0.5 M NaSCN, pH 8.0, 25°C)

Initial BSA concentration (mg/mL)	Desorption ratio (%)	
	I[a]	II[b]
0.5	83.31	91.27
1.0	82.50	87.65
2.0	94.28	89.41
3.0	83.65	94.69
4.0	83.99	85.21
5.0	83.49	88.39

[a] Albumin adsorption conditions: Ionic strength = 0.01 (adjusted with NaCl), pH 5.0.

[b] Albumin adsorption conditions: Ionic strength = 0.1 (adjusted with NaCl), pH 5.0.

$$\text{Desorption ratio} = \frac{\text{the amount of BSA released to the desorption medium}}{\text{the amount of BSA adsorbed on the particles}} \times 100$$

Table 1 gives desorption data obtained in this group of experiments. As seen in this table, we were able to remove about 83–95% of the BSA adsorbed on the particles, which showed the reusability of our dye-attached PVAL particles in protein affinity separation.

CONCLUSION

As reported in our previous paper, very high adsorption capacities (up to 60 mg BSA adsorbed per gram of the sorbent) were obtained in which the dye (i.e., Cibacron Blue F3G-A) molecules were attached on the PVAL-coated monosize PS microspheres of 4 μm diameter (14–17). Since these particles were monosized, we were able to calculate the adsorption capacities in the units of mg BSA/m^2 of the sorbent surface area. The maximum adsorption capacity of the PVAL/PS monosize particles was 40 mg BSA/m^2, which corresponds to 60 mg BSA/g PS. The maximum adsorption capacity of the dye-attached PVAL particles prepared in this study was about 32 mg BSA/g PVAL. Since the PVAL particles are irregular in shape and they exhibit a size distribution, it was not possible to determine the exact adsorption capacities in units of BSA mg/m^2. However, by taking the density of the particles as 1 g/mL and assuming the particles were spherical in shape with a diameter of 50 μm (which is their average diameter), we were very roughly able to determine their adsorption capacities based on unit surface area. We obtained the maximum BSA adsorption capacity of the dye-attached PVAL particles as 264 mg BSA/m^2, which was surprisingly higher than the value we obtained for dye-attached PVAL-coated PS microspheres. We thought that this was not correct. The main error may come from the assumptions we made. We assumed that the PVAL particles were spherical and that they were monosized with a diameter of 50 μm, which are not correct. The particles we produced were very irregular in shape and exhibited a size distribution. Therefore, they must have a much higher surface area than what we calculated based on the assumptions mentioned above. This is a common mistake made in related literature. We believe that if the sorbent particles are not spherical and monosized, we should present the adsorption data in units of mg/g, as we did in this paper.

By considering the adsorption capacities based on unit weight of the sorbent, we concluded the following: The monosize dye-attached PVAL/PS microspheres have higher adsorption capacities for BSA. However,

due to their very small size, they cannot be used in fixed-bed adsorption columns. Due to very high flow resistance in these columns, high-pressure column systems are needed. They can be used in fluidized-bed columns, which is very common in chemical engineering technology but not in affinity chromatography systems. However, the dye-attached PVAL particles are bigger in size and can be easily used in fixed-bed column chromatography systems. Therefore, they may be considered as alternative affinity sorbents in protein separation and purification.

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Received by editor August 11, 1994