Preparation of an Anion-Exchange Adsorbent by the Radiation-Induced Grafting of Vinylbenzyltrimethylammonium Chloride Onto Cotton Cellulose and Its Application for Protein Adsorption

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ABSTRACT: Poly(vinylbenzyltrimethylammonium chloride)-*graft*-cotton cellulose, an anion-exchange matrix, was synthesized by a mutual radiation-induced grafting technique with a ^{60}Co γ-radiation source. The grafted matrix was characterized by grafting yield estimation, elemental analysis, Fourier transform infrared spectroscopy, and scanning electron microscopy. The grafting yield decreased with the increase in the dose rate. However, the grafting yield and nitrogen content of grafted samples increased almost linearly with an increase in the total irradiation dose. To evaluate the performance of the grafted anion-exchange matrix, the protein adsorption and elution behavior were investigated in a continuous column process under various experimental conditions, with bovine serum albumin used as a model protein. The binding and elution behavior of the anion-exchange matrix depended on different experimental parameters, such as the grafting yield, ionic strength, pH of the medium, and amount of protein loaded. From a breakthrough curve, the equilibrium binding capacity and elution percentage of the grafted anion-exchange matrix were estimated to be 40 mg/g and 94%, respectively. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 5512–5521, 2006

Key words: adsorption; FTIR; graft copolymers; ion exchanges; proteins; radiation

INTRODUCTION

Proteins are natural polymers that play essential roles in the structures and functions of all living organisms. In recent years, the separation and purification of proteins have gained economic significance as a number of proteins are now being used in the pharmaceutical and food industries.[1] Furthermore, studies of molecular properties of individual proteins require their isolation and purification, which have always been a challenge in proteonics. The rapid development of biotechnology and the pharmaceutical potential of proteins are refueling the demand for reliable and efficient protein separation, recovery, and purification methods.[1] Among the various separation techniques, chromatography has emerged as a powerful and versatile technique for protein purification in which various functional moieties such as ion-exchange, hydrophobic, and affinity groups immobilized onto solid matrices have been used to achieve the separation. Ion-exchange chromatography is one of the important techniques in the protein purification process, as it can be used for a wide variety of proteins. Also, the high binding capacity (BC) of ion exchangers suggests their use in the separation of proteins from concentrated mixtures, regardless of the initial volume to be loaded for separation.

Several methods have been employed to prepare functional polymer adsorbents for the immobilization, recovery, separation, and filtration of biomolecules such as proteins, enzymes, and DNA in the biotechnology and medical industries.[2–16] These methods include the graft polymerization of various functional monomers onto different types of polymeric substrates by chemical-redox-initiated grafting,[2–4] photoinitiated grafting,[5] plasma-induced grafting,[6–10] γ-radiation-induced grafting,[11–13] and electron-beam-induced grafting.[1,14] Different kinds of functional polymer matrices have been obtained by the grafting of glycidyl methacrylate (GMA) and its subsequent chemical modifications into desired functional groups such as amine, hydroxylamine, polyol,[14,15] sulfonic acid,[14] and phosphoric acid[16] by the chemical treatment of the GMA grafted matrix.

The radiation-induced grafting technique is an easy and highly efficient technique for incorporating

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desired chemical groups onto existing polymer substrates. Radiation grafting leads to highly functional polymer carriers, which have many applications; the high-performance separation and immobilization of proteins is one of them.[17] Cellulose has been used most often as a support in ion-exchange and affinity chromatography because of its easy accessibility, low cost, hydrophilic character, and easy chemical modification.[18] Furthermore, hydroxyl groups present on the cellulose matrix reduce the irreversible nonselective adsorption of proteins.[19] Radiation-induced grafted polymer adsorbents fulfill most of the desired criteria (e.g., fast uptake, good uptake capacity, and repeated use) of an efficient recovery system.[18] Moreover, uniformly functionalized polymer brushes prepared by radiation-induced grafting methods do not suffer from the diffusional mass-transfer resistance of proteins, unlike conventional gel-bead packed columns. Proteins are captured in a multilayer by the ion-exchange-group-containing grafted brushes because of the formation of a three-dimensional space for protein binding via the interchain electrostatic repulsion between the grafted brushes, which leads to a high uptake capacity of the adsorbent.[14,20]

In this work, an anion-exchange adsorbent was prepared for the recovery of proteins by mutual irradiation grafting of poly(vinylbenzyltrimethylammonium chloride) (PVBT) onto a cotton cellulose matrix with a ^{60}Co γ-radiation source. The anion-exchange quaternary ammonium group was incorporated in a single-step process, unlike earlier reports in which the first step is the grafting of a monomer such as GMA and the second step is a chemical reaction of an epoxy group with different chemicals such as diethylamine and trimethylamine to modify the grafted chains for conversion into the required ion-exchange groups.[11,14,16] To evaluate the performance of the grafted anion-exchange matrix [poly(vinylbenzyltrimethylammonium chloride)-*graft*-cotton cellulose (PVBT-*g*-cotton cellulose)], the protein adsorption and elution behavior was investigated in a column process under various experimental conditions, with bovine serum albumin (BSA) used as a model protein.

EXPERIMENTAL

Materials

Vinylbenzyltrimethylammonium chloride (VBT; molecular weight = 211.74), a mixture of 3-vinyl and 4-vinyl isomers obtained in a powder form from Fluka Chemika (St. Gallen, Switzerland), and BSA (A4378; molecular weight $\sim$ 66 kD) from Sigma Chemical Co. (St. Louis, MO) were used as received. Analar-grade chemicals, namely, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), acetic acid, sodium acetate, boric acid, and sodium borate, supplied by S.D. Fine Chemicals (Mumbai, India), were used to prepare buffer solutions of different pHs. Cotton cellulose fabric in a woven form, procured from a local supplier, was treated with a 1% NaOH solution for 1 h, washed in ethanol and water, dried at 50°C, and stored in a desiccator for the rest of the studies. Double-distilled water was used for the preparation of all the solutions.

Preparation of PVBT-*g*-cotton: Radiation grafting

Grafting PVBT onto the cotton cellulose backbone was carried out by a mutual irradiation grafting technique with a ^{60}Co γ-radiation source. A washed cotton cellulose sample was completely immersed in a known volume of a 20% aqueous solution of VBT in a stoppered glass bottle and left for an hour to facilitate the swelling of the cotton fabric in the monomer solution. The bottles containing the cotton samples and monomer solution were then irradiated in a ^{60}Co γ-chamber (GC 5000, Brit, India) for various total irradiation doses in the range of 0.3–3.0 kGy at three different dose rates (2.0, 4.0, and 8.0 kGy/h). The homopolymer attached to the grafted sample was removed by 8 h of extraction in a Soxhlet extraction assembly with water as an extractant. The grafted samples were vacuum-dried at 50°C and stored in a desiccator for the rest of the studies.

Characterization of the grafted samples

Grafting yield (GY)

The PVBT-*g*-cotton samples were characterized by the estimation of GY determined gravimetrically with the following relation:

$$\text{GY}\ (\%) = [(\text{Weight after grafting} - \text{Initial weight})/\text{Initial weight}] \times 100 \quad (1)$$

Elemental analysis

The nitrogen content of the PVBT-*g*-cotton cellulose matrix was determined with a Flash EA (112 series) elemental analyzer from Thermofinnigan Italian Co. calibrated against cysteine.

IR analysis

Fourier transform infrared (FTIR) spectroscopy measurements were performed on an FTIR spectrophotometer (FT/IR-660) from Jasco (Japan). The samples were thoroughly ground at the temperature of liquid nitrogen and mixed with KBr to prepare discs by compression *in vacuo*. FTIR spectra were obtained from 400 to 4000 cm^{-1} in the transmittance mode with a resolution of 4 cm^{-1} and averaged over 25 scans.

Scanning electron microscopy (SEM)

The morphology of the control and PVBT-*g*-cotton fibrils was investigated by SEM with a VEGA

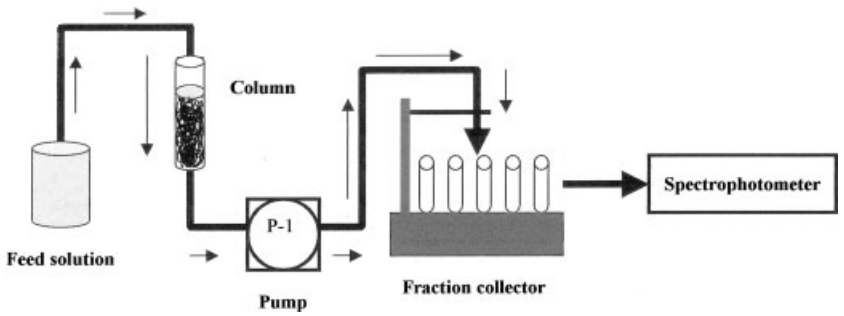

Figure 1 Schematic representation of the experimental setup for protein adsorption data collection in the column process.

MV2300T/40 (TS 5130 MM) microscope (Tescan). SEM micrographs of cotton fibers were taken after coating with gold *in vacuo* and pasted onto a conducing surface by carbon paste.

Adsorption of BSA

All the protein-binding experiments were carried out at 4°C in a cold room, most biological applications are generally carried out under low-temperature conditions to avoid the degradation of biomolecules. A schematic representation of the experimental setup for the protein adsorption data collection in the column process is shown in Figure 1. A PVBT-*g*-cotton cellulose matrix was packed in a glass column (Pharmacia, Sweden) 50 mm long with a 10-mm i.d. The column volume for all the experiments was kept at 3.0 mL. The column was equilibrated with a buffer solution. The column was attached to a pump (P-1, Pharmacia) connected to the fraction collector (Pharmacia), in which the effluent passing through the anion-exchange PVBT-*g*-cotton adsorbent was continuously sampled. All experiments were performed at a flow rate of $\sim$ 0.5 mL/min. A protein solution of a known concentration was loaded into the column, which was connected to the buffer solution for the washing of unbound protein. After washing, the column was switched to a buffer solution containing a 1*M* NaCl solution for the elution of bound protein. The protein concentration in different fractions was estimated by the monitoring of the optical densities of protein solutions at 280 nm referenced with a calibration plot with an ultraviolet–visible spectrophotometer (UV-Vis 2500, Chemito, India). Solutions of different pHs were prepared with buffer solutions [pH $\sim$ 4.0 (10 m*M* acetate buffer), pH $\sim$ 5.6 (10 m*M* acetate buffer), pH $\sim$ 7.0 (10 m*M* phosphate buffer), and pH $\sim$ 8.6 (10 m*M* borate buffer)].

Binding capacity (BC) and the elution percentage (EP) were defined as follows:

$$\text{BC (mg/g)} = (\text{Protein loaded} - \text{Protein unadsorbed})/\text{Weight of the dry adsorbent} \quad (2)$$

$$\text{EP (\%)} = (\text{Amount of protein eluted}/\text{Amount of protein adsorbed}) \times 100 \quad (3)$$

The breakthrough curve was obtained by the feeding of a dilute aqueous solution (0.4 mg/mL) of protein through the column at a flow rate of 0.5 mL/min. The relative concentration of the protein in the effluent was plotted against the effluent volume.

The equilibrium binding capacity (EBC) of the anion-exchange grafted matrix was estimated from a breakthrough curve with the following relation:

$$\text{EBC (mg/g)} = \sum_{i=1}^{s} (C_0 - C_i) V_i / W \quad (4)$$

where C_0 and C_i are the protein concentrations (mg/mL) in the feed and the ith fraction of the effluent, respectively; V_i is the volume (mL) of the ith fraction of the effluent; and W is the weight (g) of the dry anion-exchange adsorbent ($i = s$ when C_i reaches C_0).

RESULTS AND DISCUSSION

Radiation grafting of PVBT

The number of grafted chains and the chain lengths produced in the mutual irradiation grafting method are functions of the total irradiation dose and the dose rate.[21] The total irradiation dose is a measure of the total number of free radicals generated on the trunk polymer, whereas the dose rate determines the rate of initiation of polymerization. The effects of the radiation dose and dose rate on GY were investigated in a 20% aqueous solution of VBT irradiated with various doses at different dose rates.

Figure 2 shows GY as a function of the dose rate at various total doses. GY decreases with an increase in the dose rate. The lower GY values at higher dose rates can be attributed to energy deposition taking place predominantly in the bulk of the solution, favoring homopolymerization. Irradiation at higher dose rates generates higher radical density, which causes a greater extent of homopolymerization and

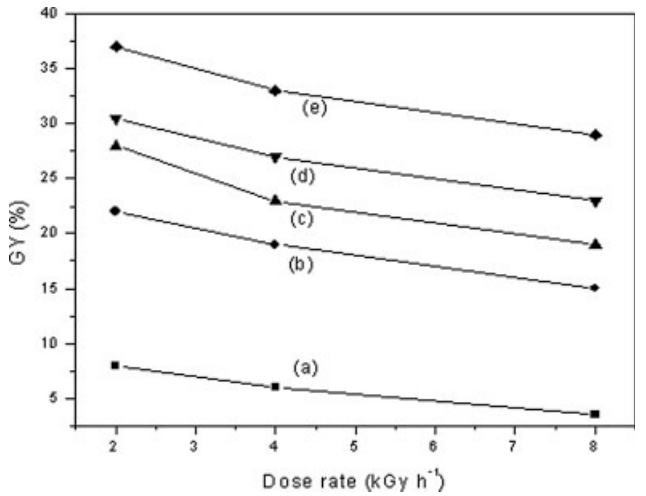

Figure 2 Effect of the dose rate on GY at different irradiation doses ([VBT] = 20 wt % in water, aerated solution): (a) 0.3, (b) 1.0, (c) 1.5, (d) 2.0, and (e) 3.0 kGy.

subsequent gelation. The homopolymerization suppresses the extent of grafting in two ways: (1) an increase in the bulk viscosity, which hinders the diffusion of the monomer from the bulk to the reactive site and growing chains at the trunk polymer, and (2) the consumption of the monomer in homopolymer formation, so less monomer is available for the grafting reaction. Figure 3 shows GY at different total radiation doses delivered at dose rate of 2.0 kGy/h. GY increases almost linearly with the γ-irradiation dose imparted. As the total irradiation dose is a measure of the total number of free radicals generated on the trunk polymer, the greater the dose is, the more grafted chains grow on the backbone, and this results in higher GY.

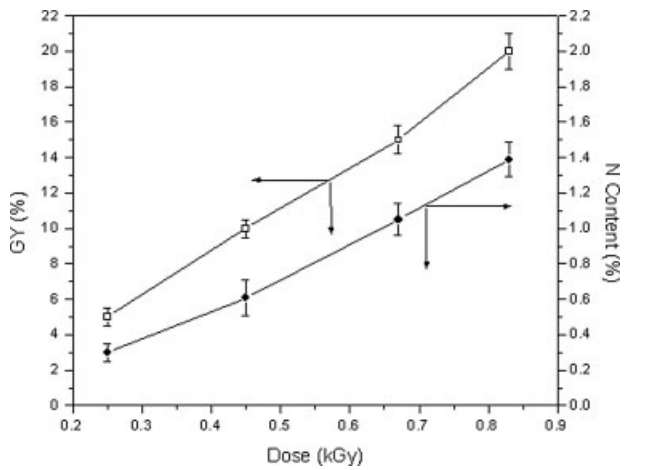

Figure 3 Effect of the irradiation dose on GY and the nitrogen content (dose rate = 2.0 kGy/h, [VBT] = 20 wt % in water, aerated solution).

Elemental analysis

The results of elemental analysis are also shown in Figure 3. The nitrogen content of the grafted matrix reflects the extent of incorporation of PVBT onto the cotton backbone. The nitrogen content in the grafted substrate increases almost linearly with the irradiation dose because the incorporation of PVBT increases with the radiation dose. The results from elemental analysis are in good agreement with GY estimated gravimetrically with relation (1).

FTIR analysis

Figure 4 shows IR spectra of the control cotton cellulose and PVBT-*g*-cotton cellulose samples. For the PVBT-*g*-cotton cellulose sample, additional peaks were obtained at 1428, 1488, and 890 cm^{-1}. The peaks at 1428 and 1488 cm^{-1} belong to the C—H bending of methyl groups and scissoring of methylene groups, respectively, whereas 890 cm^{-1} corresponds to out-of-plane bending of aromatic-ring C—H bonds. These peaks confirm the grafting of vinylbenzyltrimethylammonium groups in the cotton cellulose structure.

SEM

The change in the morphology of the cotton cellulose fibrils was investigated with SEM micrographs of grafted and control cotton fibrils, as shown in Figure 5. There was a marked difference in the morphology of the cotton fibrils before and after the grafting of PVBT. The PVBT-*g*-cotton fibrils became thicker (24 μm) in comparison with the control cotton fibrils (16 μm) because of the incorporation of grafted PVBT chains into the cotton backbone.

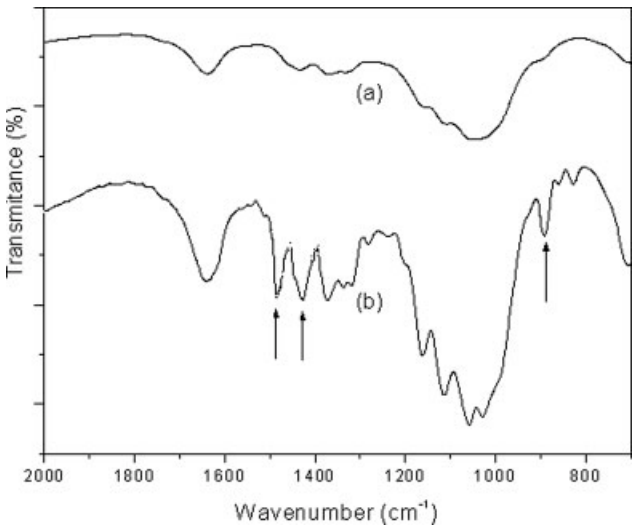

Figure 4 FTIR spectra of (a) control cotton and (b) PVBT-*g*-cotton cellulose samples.

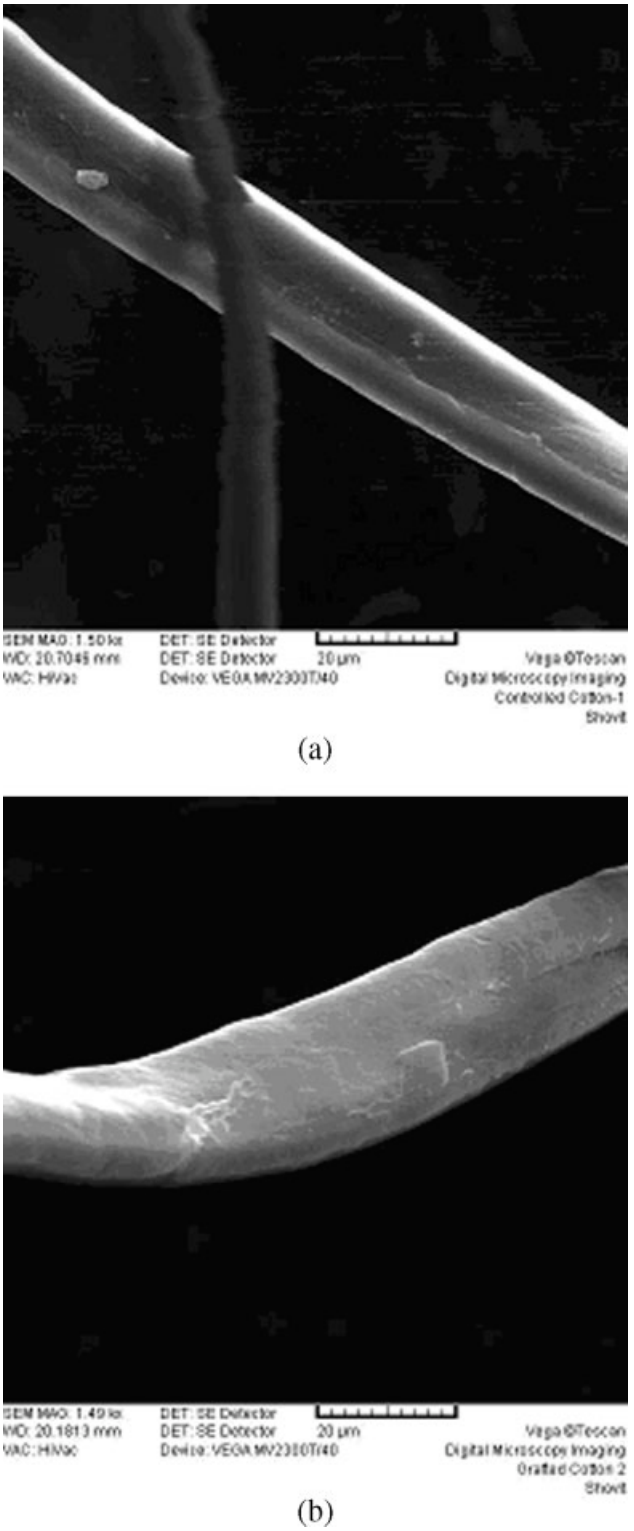

Figure 5 SEM micrographs of cotton fibrils: (a) control cotton fibrils and (b) PVBT-*g*-cotton cellulose fibrils with GY = 20% (magnification = 1500×).

Protein binding studies

A schematic representation of the multilayer binding of proteins by grafted PVBT polymer brushes of a PVBT-*g*-cotton cellulose matrix is shown in Figure 6(a). The brushlike structure of the ion-exchange grafted chains facilitates the uptake of protein molecules and improves BC and the kinetics of uptake.[1] Figure 6(b) shows a schematic representation of the configuration of grafted PVBT polymer chains under lower and higher ionic strength conditions to explain the decrease in BC under higher ionic strength conditions.

Effect of GY on the protein adsorption

The protein BC of the anion-exchange adsorbent depends on the loading of ion-exchange groups (VBT) onto the backbone matrix. The more ion-exchange groups there are, the greater BC is of the adsorbent. Figure 7 shows the BSA adsorption behavior of an anion-exchange matrix with different GYs. The first peak is the washing peak and represents the unadsorbed protein, whereas the second peak is the elution peak and represents the protein eluted in 1*M* NaCl. Figure 7 shows that the height of the washing peak decreases, whereas that of the elution peak increases, with an increase in GY. Figure 8 demonstrates BC of the grafted anion-exchange matrix and EP of the bound protein as functions of GY. As expected, the protein BC of the adsorbent increases with an increase in GY. According to the elution profile, with up to 10% GY, only about 60% of the bound protein can be eluted. However, for an adsorbent with GY ≥ 15%, EP reaches the saturation value (∼ 95%).

GY represents the concentration percentage of anion-exchange groups grafted to the base matrix. Grafting PVBT onto the cellulose matrix may alter the hydrophilicity and hydrophobicity of the backbone matrix and subsequently may affect the interaction between protein molecules and the ion-exchange groups. PVBT is an amphiphilic polymer containing both hydrophobic groups (backbone aliphatic carbon chain with a pendant aromatic ring) and hydrophilic groups (positively charged trimethylammonium group) in every monomer unit. Therefore, the grafting of PVBT introduces hydrophilic and hydrophobic properties into the backbone matrix. From the results of protein elution shown in Figure 8, it can be said that there is a competition between the hydrophilic and hydrophobic interactions for the binding of protein to the grafted matrix. Up to GY = 10%, the hydrophobic interaction seems to be significant, but for a grafted matrix with GY ≥ 15%, the hydrophilic character of the grafted matrix dominates. Therefore, the protein molecules are bound by an ionic interaction to the matrix with GY ≥ 15% and so are efficiently eluted in a 1*M* NaCl solution. On the other hand, for lower GY values, because some protein molecules might be adsorbed to the matrix by hydrophobic interactions, they cannot be eluted in a NaCl solution, and this results in lower EP. Taking these factors in to

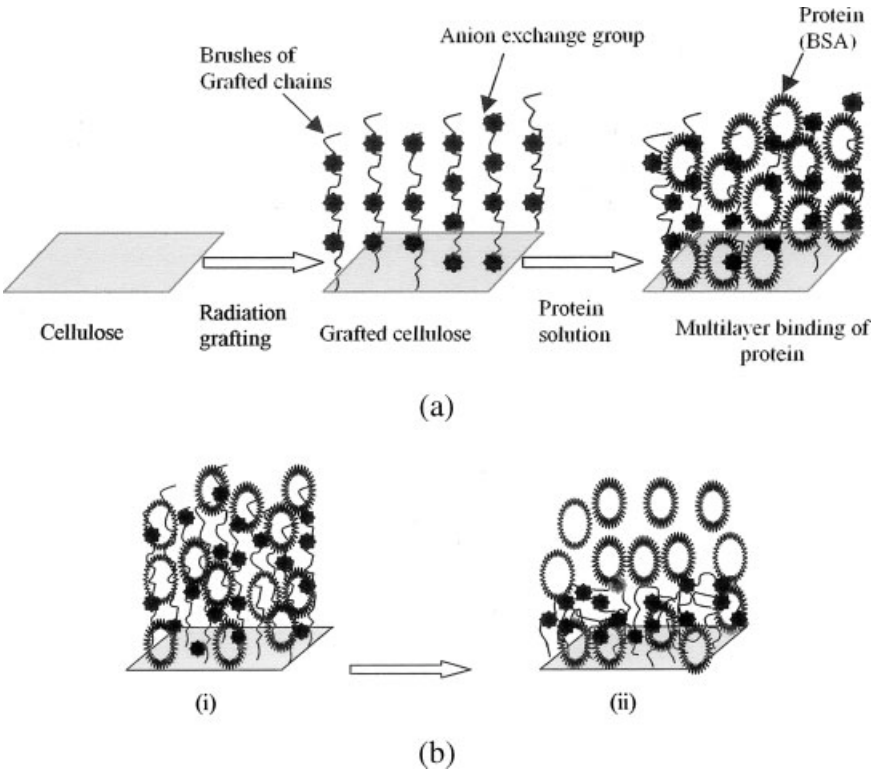

Figure 6 (a) Schematic representation of the structural arrangement of PVBT-grafted chains and protein binding by a radiation-grafted anion-exchange cellulose matrix and (b) proposed configuration of grafted PVBT polymer chains under different solution conditions: (i) the extended conformation under the lower ionic strength condition and (ii) the shrinking conformation under the higher ionic strength condition.

account, we fixed the optimum GY value for the rest of the study at 20%.

Effect of the ionic strength on the protein adsorption

The adsorption behavior of a PVBT-*g*-cotton cellulose adsorbent with GY = 20% toward BSA as a function of the concentration of NaCl is shown in Figure 9. The height of the washing peak increased and the height of the elution peak decreased with an increasing concentration of NaCl, and this indicated that the amount of protein bound to the anion-exchange ma-

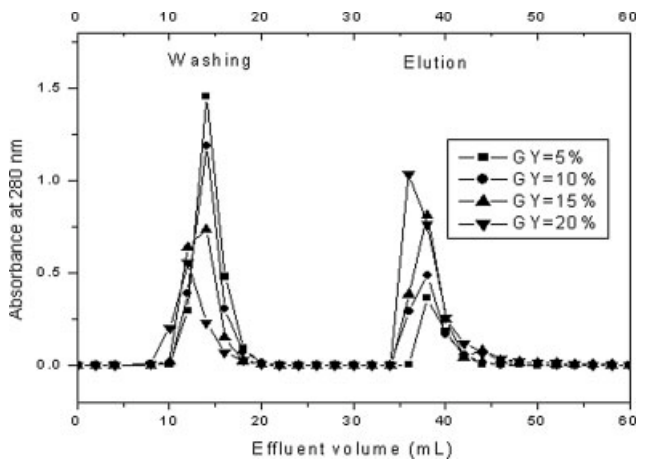

Figure 7 Adsorption behavior of PVBT-*g*-cotton cellulose for BSA as a function of GY (pH ~ 7.0 in a 10 m*M* phosphate buffer, protein load = 8.9 mg, flow rate = 0.5 mL/min).

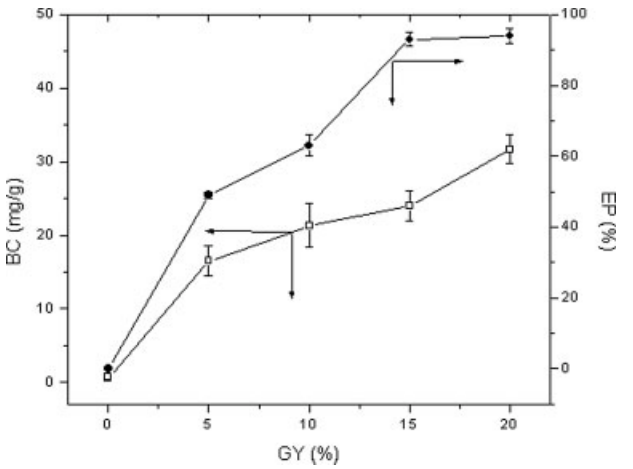

Figure 8 BC and EP of PVBT-*g*-cotton cellulose as a function of GY (pH ~ 7.0 in a 10 m*M* phosphate buffer, protein load = 8.9 mg, flow rate = 0.5 mL/min). Data are expressed as the mean plus or minus the standard deviation ($n = 3$).

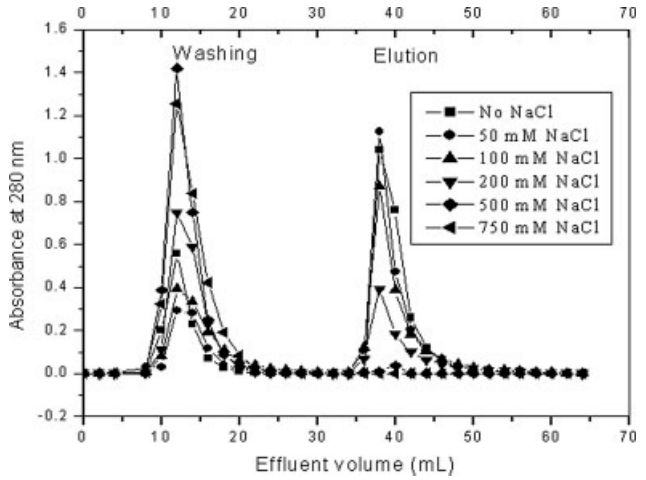

Figure 9 Adsorption behavior of PVBT-*g*-cotton cellulose with GY = 20% toward BSA as a function of the ionic strength (pH ~ 7.0 in a 10 m*M* phosphate buffer, protein load = 8.9 mg, flow rate = 0.5 mL/min).

trix decreased with an increase in the ionic strength of the medium. Figure 10 demonstrates BC and EP for a grafted matrix as a function of the ionic strength of the medium. As the ionic strength of the medium was increased, there was a gradual decrease in BC up to 300 m*M* NaCl, above which there was a drastic reduction in the binding of the protein to the grafted adsorbent. At a NaCl concentration of 750 m*M*, the protein BC of the matrix was negligible (~ 2 mg/g), and this showed that the adsorption of BSA onto PVBT-*g*-cotton cellulose with GY = 20% took place predominantly via an electrostatic interaction, and nonselective adsorption of the protein onto the polymer brushes was negligible. The effect of the ionic strength on the protein adsorption on ion-exchange

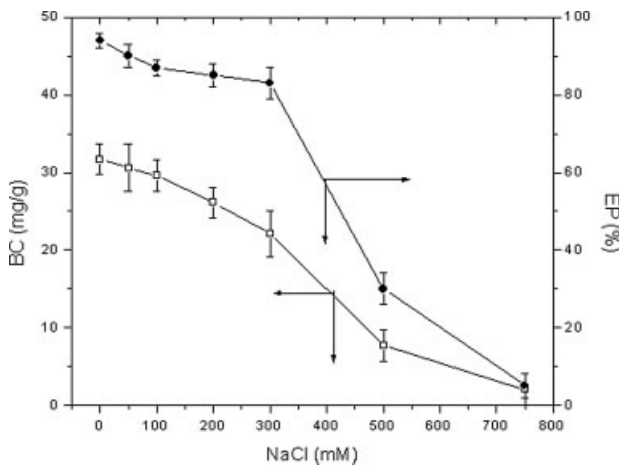

Figure 10 BC and EP of PVBT-*g*-cotton cellulose (GY = 20%) as a function of the ionic strength of the solution (pH ~ 7.0 in a 10 m*M* phosphate buffer, protein load = 8.9 mg, flow rate = 0.5 mL/min). Data are expressed as the mean plus or minus the standard deviation ($n = 3$).

adsorbents is a complex phenomenon. Ionic interactions are significantly affected by the ionic strength because of the Debye–Huckel screening of electrostatic interactions. Polyelectrolytes acquire a fully extended state (rodlike structure) in aqueous solutions because of electrostatic repulsion between its similarly charged chain segments. The presence of low-molecular-weight electrolytes in the solution can cause a change in the conformation of polyelectrolytes from a flexible, extended chain to a coiled or compact structure because of the screening of intramolecular electrostatic repulsive forces. At high electrolyte concentrations, the suppression of segment–segment repulsion suffices for some polyelectrolytes to transform a good solvent into one of marginal or poor quality, and so the polyelectrolyte chains acquire a compact structure.[22] It has also been reported that a high salt concentration increases the surface tension of an aqueous solution and therefore increases the hydrophobic interactions and stabilizes the folded or collapsed protein conformation.[23–25] The decrease in the protein BC of the grafted matrix in the presence of NaCl can be attributed to the following factors: (1) screening of the ionic interaction between the cationic group of the adsorbent and the anionic group of protein; (2) competition between salt counterions and protein for anion-exchange groups; (3) an increase in the ionic strength causing the grafted ion-exchange chains to shrink because of screening of the repulsion between the cationic charges on the grafted chains, resulting in lower accessibility of the ionic groups of the grafted chain for interaction with protein molecules[26] [presented in Fig. 6(b)]; and (4) screening of intramolecular repulsive electrostatic interactions of

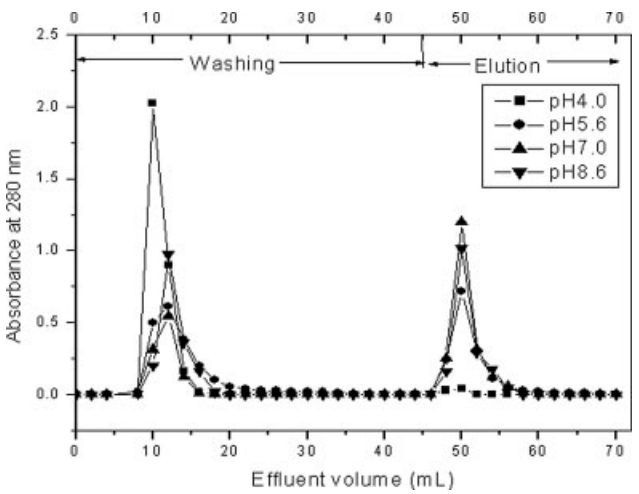

Figure 11 Adsorption behavior of PVBT-*g*-cotton cellulose with GY = 20% toward BSA as a function of pH (protein load = 8.9 mg, flow rate = 0.5 mL/min, pH ~ 4.0 in a 10 m*M* acetate buffer, pH ~ 5.6 in a 10 m*M* acetate buffer, pH ~ 7.0 in a 10 m*M* phosphate buffer, and pH ~ 8.6 in a 10 m*M* borate buffer).

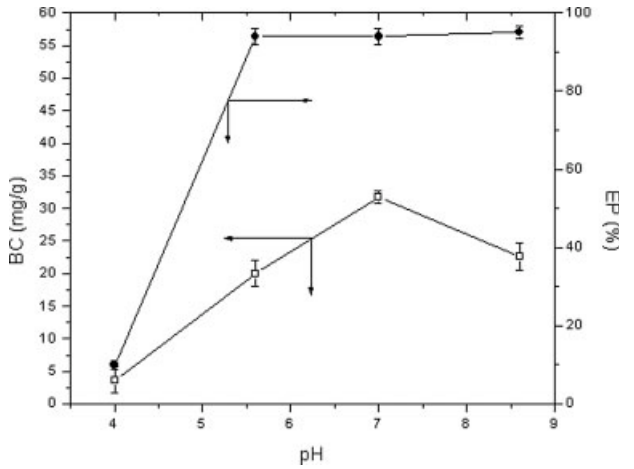

Figure 12 BC and EP of PVBT-*g*-cotton cellulose (GY = 20%) as a function of pH of the solution. Data are expressed as the mean plus or minus the standard deviation ($n = 3$).

charges on protein molecule leading to the folded conformation and hence lower accessibility of the anionic charges on the protein for interaction with the anion-exchange groups on the grafted chains. The effect of the ionic strength on EP was not very significant up to a salt concentration of 300 m*M*, above which there was a sudden decrease in EP. Low EP values at higher ionic strengths were attributed to very low amounts of the bound protein, which mainly adsorbed via hydrophobic interactions and could not be eluted.

Effect of pH on the protein adsorption

The influence of pH on the adsorption of proteins onto ionic adsorbents is of prime significance as the variation of pH of the system affects the charge profile and configuration of the protein as well as the adsorbent, thereby influencing the adsorption behavior of the adsorbent. The effect of pH on the adsorption behavior of the PVBT-*g*-cotton cellulose matrix (GY = 20%) was investigated in the pH range of 4.0–8.6 with a 10 m*M* concentration of different buffer systems, and the results are shown in Figure 11. At pH 4.0, most of the protein remained unadsorbed, and the protein content in the eluted fraction was negligible. BC and EP of the anion-exchange cellulose matrix for BSA are demonstrated as functions of pH in Figure 12. BC was minimum at pH 4.0 because BSA was in a cationic ($—NH_3^+$) form at pH 4.0 [<pI of BSA (4.9)]; therefore, BSA molecules experienced repulsion from a similar (positive) charge of grafted PVBT groups, and this resulted in a negligible uptake of BSA. At pHs higher than the isoelectric point, the protein being in an anionic form bonded easily to the cationic matrix. The binding was maximum at the neutral pH and decreased at pHs of ~ 5.6 and 8.6. One of the reasons for lower binding at pHs 5.6 and 8.6 was the conformational change of the grafted chains from an open structure to a shrunken structure at pHs 5.6 and 8.6. This assumption was supported by our earlier work, in which the swelling of the PVBT copolymer gels decreased on either side of the neutral pH.[27] A similar observation for the adsorption of Urokinase by a polypropylene film containing anion-exchange groups was reported by Lee et al.[11] A low EP value was obtained at pH 4.0 because the protein was most likely adsorbed mainly by hydrophobic interactions and could not be eluted in a NaCl solution. EP of the bound protein remained almost unaffected in the pH range of 5.6–8.6.

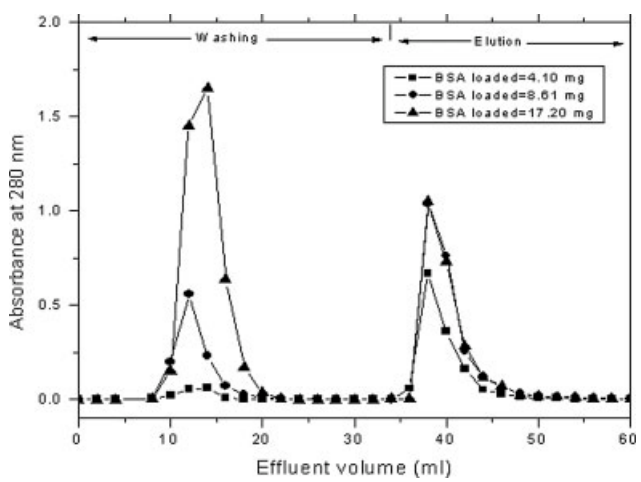

Figure 13 Adsorption behavior of PVBT-*g*-cotton cellulose with GY = 20% toward BSA as a function of amount of the BSA loaded (pH ~ 7.0 in a 10 m*M* phosphate buffer, flow rate = 0.5 mL/min).

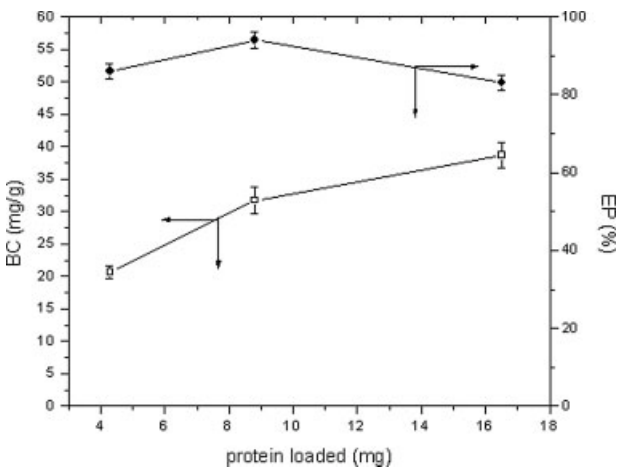

Figure 14 BC and EP of PVBT-*g*-cotton cellulose (GY = 20%) as a function of the amount of protein loaded (pH ~ 7.0 in a 10 m*M* phosphate buffer, flow rate = 0.5 mL/min). Data are expressed as the mean plus or minus the standard deviation ($n = 3$).

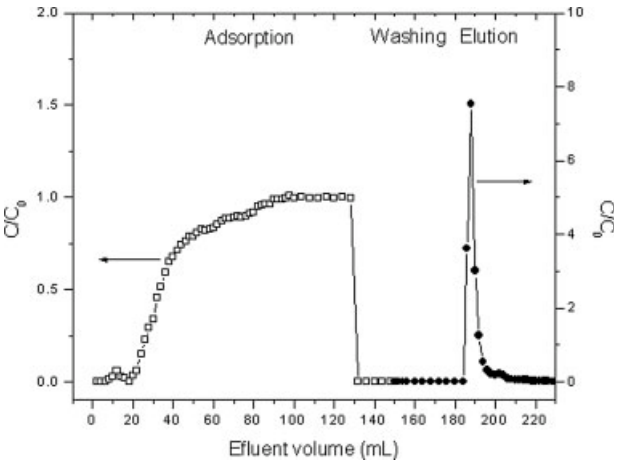

Figure 15 Breakthrough curve for the adsorption of BSA on an anion-exchange PVBT-*g*-cotton cellulose adsorbent with GY = 20% (BSA concentration in the feed solution = 0.4 mg/mL in a 10 m*M* phosphate buffer with pH ~ 7.0, flow rate = 0.5 mL/min, weight of dry anion-exchange matrix = 0.2 g).

Effect of the amount of protein loaded on the protein adsorption

Figure 13 shows the adsorption behavior of the matrix as a function of the amount of protein loaded to the column. As the amount of protein loaded to the column increases, the heights of the washing peak and elution peak also increase. BC and EP as functions of the amount of protein are shown in Figure 14. BC increases with an increase in the amount of protein. However, EP is not affected significantly by the amount of protein loaded to the column. In the column process, the binding of the protein is a dynamic phenomenon as the protein molecules are being carried by the buffer solution down through the column, and unlike in a batch process, the interaction time may not be sufficient for equilibration. BC increases with an increase in the amount of protein because for the same interaction time, more protein molecules are available for binding with grafted anion-exchange VBT groups. This suggests that for a lower protein concentration, a low flow rate will facilitate the binding of the protein.

Breakthrough curve

The design of a fixed-bed adsorber and prediction of the length of the adsorption cycle between regeneration require knowledge of the approach to saturation at the breakthrough point. The breakthrough curve of BSA for an anion-exchange PVBT-*g*-cotton cellulose matrix (GY = 20%), that is, a relative change in the protein concentration of the effluent as a function of the effluent volume, is shown in Figure 15. Data presented in Figure 15 show that the protein content in the unadsorbed fraction increased gradually with the increase in the effluent volume and finally reached the concentration of the feed solution, that is, the point of equilibrium at about 100 mL of the effluent. The equilibrated column was then washed, and the bound protein was eluted in a 1*M* NaCl solution. The adsorbed protein could be effectively eluted in a small volume (~ 10 mL) of the NaCl solution. EBC and EP of the PVBT-*g*-cotton cellulose anion-exchange adsorbent, estimated from the breakthrough curve, were 40.0 mg/g and 94%, respectively. The results of the breakthrough analysis of the PVBT-*g*-cotton cellulose anion-exchange adsorbent suggested its possible application in the concentration, purification, and recovery of biomolecules from dilute solutions. EBC of radiation-grafted PVBT-*g*-cotton cellulose with only a 20% GY was comparable to that of other anion-exchange, microporous, hollow fiber membranes with respect to the concentration of ion-exchanger groups such as amino, ethylamino, diethylamino, and 2-hydroxyethylamino groups, as reported by earlier workers.[1,28]

CONCLUSIONS

An anion-exchange PVBT-*g*-cotton cellulose adsorbent was successfully prepared in a single step by the mutual γ-irradiation grafting technique. The adsorption and elution behavior of BSA with the radiation-grafted anion-exchange adsorbent depended on experimental conditions such as the pH, ionic strength, GY, and amount of protein loaded. The grafted adsorbent with merely a 20% GY showed an excellent BC value of 40 mg/g, which could be further enhanced by an increase in the grafting extent. In view of the increasing technological importance of biomolecular separation and recovery in the field of modern biotechnology, this novel grafted adsorbent could play an important role.

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