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Coating Gigaporous Polystyrene Microspheres with Cross-linked Poly(vinyl alcohol) Hydrogel as a Rapid Protein Chromatography Matrix

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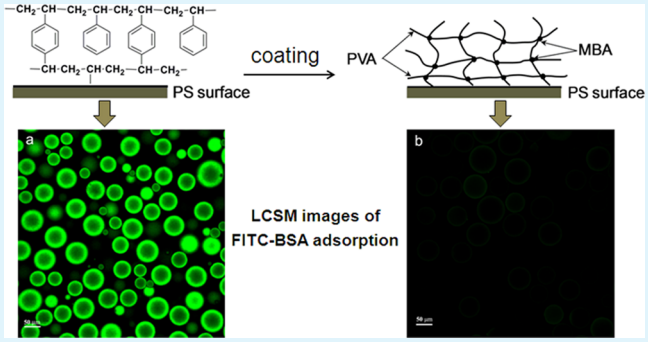
Supporting Information

ABSTRACT: Gigaporous polystyrene (PS) microspheres were hydrophilized by *in situ* polymerization to give a stable cross-linked poly(vinyl alcohol) (PVA) hydrogel coating, which can shield proteins from the hydrophobic PS surface underneath. The amination of microspheres (PS-NH_2) was first carried out through acetylization, oximation and reduction, and then 4,4′-azobis (4-cyanovaleric acid) (ACV), a polymerization initiator, was covalently immobilized on PS-NH_2 through amide bond formation, and the cross-linked poly(vinyl acetate) (PVAc) was prepared by radical polymerization at the surfaces of ACV-immobilized PS microspheres (PS-ACV). Finally, the cross-linked PVA hydrogel coated gigaporous PS microspheres (PS-PVA) was easily achieved through alcoholysis of PVAc. Results suggested that the PS microspheres were effectively coated with cross-linked PVA hydrogel, where the gigaporrous structure remained under optimal conditions. After hydrophilic modification (PS-PVA), the protein-resistant ability of microspheres was greatly improved. The hydroxyl-rich PS-PVA surface can be easily derivatized by classical chemical methods. Performance advantages of the PS-PVA column in flow experiment include good permeability, low backpressure, and mechanical stability. These results indicated that PS-PVA should be promising in rapid protein chromatography.



KEYWORDS: *gigaporous, coating, polystyrene particles, cross-linked poly(vinyl alcohol) hydrogel, protein adsorption*

1. INTRODUCTION

The isolation and purification of biological macromolecules lies at the heart of downstream processing in modern biotechnology. Up to now, liquid chromatography has been the most important technique at all scales of protein purification taking advantage of its high resolution and mild separation conditions.[1] Modern chromatography media have developed over the years, providing improved binding capacity, faster mass transfer, better chemical resistance, and greater selectivity. Compared with silica and polysaccharide media (e.g., dextran and agarose), PS microspheres with high cross-linking degree have evident advantages as chromatographic supports for biomacromolecules, which possess excellent mechanical and chemical properties.[2,3] However, the pore size of conventional porous microspheres is in the range of 10−30 nm, which leads to the slow mass transfer rate and consequently restricts their application in biomacromolecule separation.[4] It is imperative to develop efficient separation media with high-resolution, high-speed, and high-capacity for a broad range of applications including pharmaceuticals, nutrition and health products, bioenergy, and environmental protection.

POROS (perfusion adsorbent) opened a new era of fast and efficient protein separation.[4−9] In perfusion adsorbent, the size of flow-through pores ranges from 600 to 800 nm and the size of diffusive pores is in 80−150 nm. The connection of diffusive pores by flow-through pores creates finite convection into individual particle. The application of POROS in protein purification is largely hinderedbecause of their complicated preparation and the difficulty in controlling the size of the two sets of pores. Recently, gigaporous PS microspheres with a pore diameter ranging from 300 to 500 nm have been prepared by a surfactant reverse micelles swelling method.[10] These microspheres are very promising in high-speed protein chromatography.[11,12]

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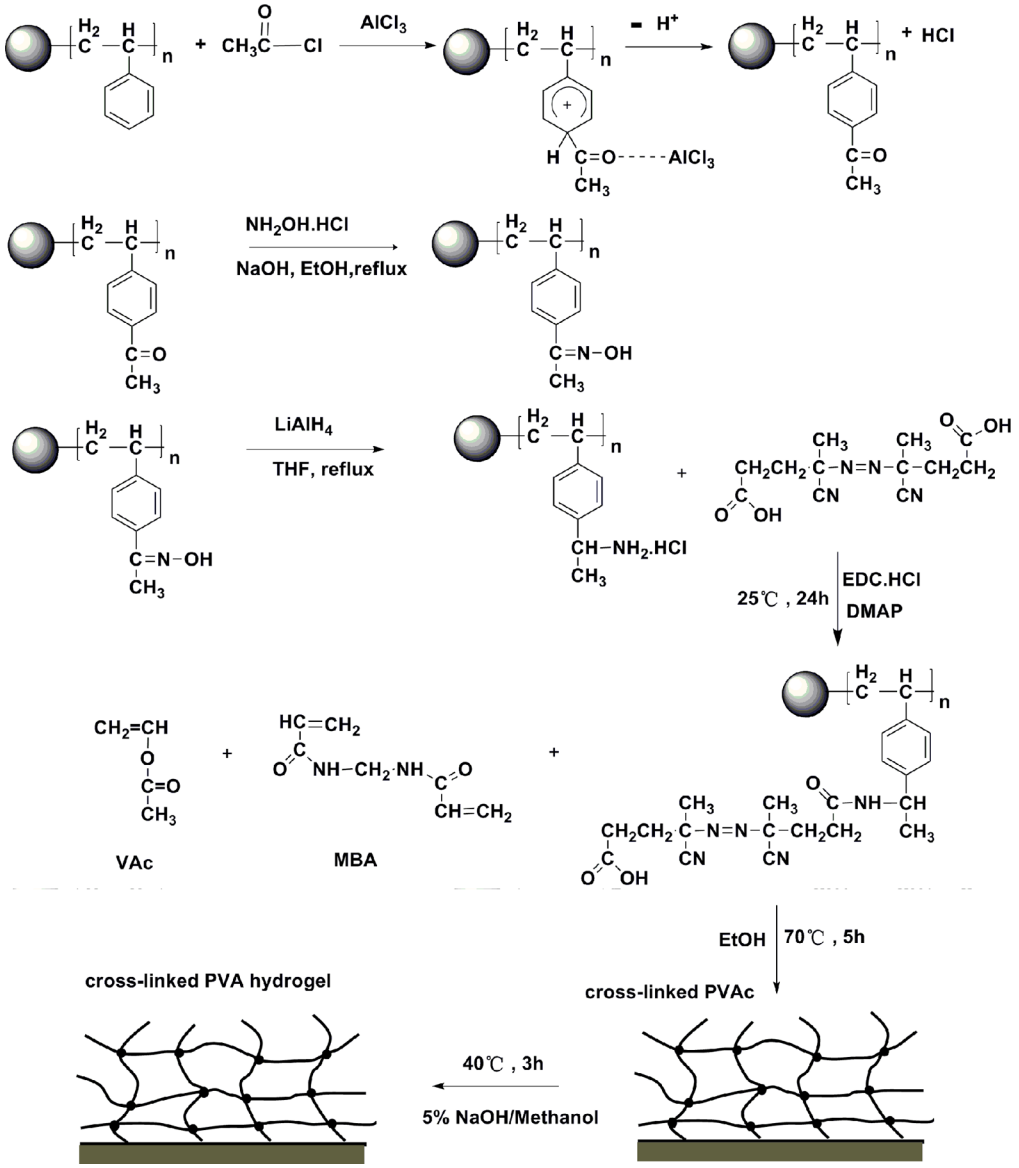
Scheme 1. Preparation of Cross-linked PVA Hydrogel-Coated Gigaporous PS Microspheres



Unfortunately, hydrophobic interactions between PS particles and proteins are considerable strong, which will cause nonspecific adsorption and denaturation of proteins. To overcome this drawback, both physical adsorption and covalent grafting of hydrophilic polymers on PS surfaces are testified to be efficient ways.[13−20] In line with this, we have also explored several systems.[21−23] Among them, physical adsorption of phenoxyl agarose exhibited excellent hydrophilic coating on gigaporous PS microspheres surfaces.[11,23] However, the desorption of coating is the main drawback of physical adsorption over time. For covalent grafting, the hydrophobic surface of PS particles may not be shielded enough because the grafting density is hard to control. In the present study, gigaporous PS microspheres have been coated with cross-linked PVA hydrogel through radical polymerization *in situ*, which is expected to provide a stable hydrophilic coating with efficient masking and long lifetime. The particles before and after modification were characterized in detail to test the possibility of PS-PVA as a rapid protein chromatography base support.

2. EXPERIMENTAL SECTION

2.1. Materials. The 55 μm diameter of gigaporous PS microspheres were prepared through the method we reported previously (average pore size 280 nm, surface area 22.69 m^2/g) .[10]

4,4′-Azobis(4-cyanovaleric acid) (ACV, 98%), 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC.HCl, 98%) and fluorescein isothiocyanate (FITC, 95%) were ordered from Alfa Aesar (UK); Bovine Serum Albumin (BSA) was purchased from Amresco (USA); vinyl acetate (VAc, AR) was from Shanghai Shanpu Chemical Co. Ltd. (China) and purified by distillation. *N,N′*-Methylene bis(acrylamide) (MBA, AR) was from Chengdu Xiya Chemical Reagent Co. Ltd. (China) and purified by recrystallization from acetone and dried at 25 °C in vacuum. Anhydrous aluminum chloride ($AlCl_3$, AR), carbon disulfide (CS_2, AR), lithium aluminum hydride ($LiAlH_4$, 97%), triethylamine (AR, 99%) and hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, AR) were from Sinopharm Chemical Reagent Co. Ltd. (China); acetyl chloride (CP) was from Tianjin Kemiou Chemical Reagent Co. Ltd. (China). Other reagents were of analytical grade from local sources.

2.2. Preparation of PS Microspheres Coupled with ACV (PS-ACV). As shown in Scheme1, the initiator coupled gigaporous PS microspheres were prepared by a four-step reaction. Briefly, C═O

(connecting with benzene ring) groups were first introduced through Friedel–Crafts acetylation onto the gigaporous PS microspheres. The acetylated microspheres were then reacted with hydroxylamine hydrochloride to introduce hydroxyimino groups (C=N–OH). Aminated PS microspheres were then prepared by reduction of the hydroxyimino groups.[24] Further preparation detail can be found in the Supporting Information.

The preparation of PS-ACV was carried out by amidation. Typically, ACV (8.89 g, 31.7 mmol) and EDC.HCl (6.08 g, 31.7 mmol), an initiator and a condensing agent respectively, were dissolved in 90 mL of various solvent mixtures in a flask. Aminated PS microspheres (5 g) were then immersed in the solution and triethylamine (4.81 g, 47.5 mmol) added. The reaction was carried out at 25 °C overnight. The resulting PS-ACV was washed with DMF twice and ethanol three times and then dried at 25 °C under vacuum. The amount of ACV coupled onto aminated PS microspheres was determined by eq 1.

$$z\% = \frac{x\% + 56y/1000}{1 - 36.5x\%/14 + 262.28y/1000} \quad (1)$$

where x% and z% are the nitrogen content of aminated PS microspheres and PS-ACV measured by total nitrogen and sulfur analyzer (ANTEK 9000NS, USA) respectively (g/g), y is the coupling amount of ACV (mmol/g), 56 is the mass of nitrogen element in per mol ACV, 36.5 is the molar mass of lost HCl when aminated PS microspheres reacted with triethylamine, 14 and 262.28 are the molar masses of N and ACV. The PS-ACV prepared at the optimal condition (DMF:THF = 2:1, the amount of coupling ACV is 0.12 mmol/g) was chosen for the further coating process.

2.3. Preparation of PVA Hydrogel-Coated PS Microspheres (PS-PVA). The cross-linked PVAc at the surface of PS-ACV was prepared by radical polymerization of VAc and MBA (see Scheme 1). Typically, VAc (4.65 g, 54 mmol) and MBA (0.17 g, 1.10 mmol) were dissolved in 30 mL of ethanol in a flask. PS-ACV (2 g) was added to the monomer solution. The reaction mixture was then bubbled with nitrogen for 0.5 h, and polymerization was carried out at 70 °C for 12 h under nitrogen atmosphere. PVAc-modified PS microspheres were filtered and washed with chloroform three times to remove any unattached PVAc. The microspheres were then washed twice with ethanol and dried at 40 °C under a vacuum.

Amount of cross-linked PVAc grafted on PS-ACV was calculated from eq 2.

$$M = \frac{W_2 - W_1}{W_2}1000 \quad (2)$$

where W_1 and W_2 are the dry weights of PS-ACV before and after reaction (g), and M is the amount of cross-linked PVAc grafted on PS microspheres (mg/g).

Cross-linked PVA hydrogel coated PS microspheres were obtained through alcoholysis of the PVAc on the outer surface of the PS microspheres. Briefly, 1 g of PVAc immobilized PS microspheres were immersed in 20 mL of methanol solution of NaOH (1.25 M) in a flask. The alcoholysis was carried out at 40 °C for 3 h. The particles were filtered and washed with deionized water thoroughly and dried in vacuum.

If we suppose the alcoholysis degree of PVAc is 100%, the amount of grafted net PVA hydrogel can also be evaluated by gravimetric analysis from eq 3.

$$M_{PVA} = \frac{(W_2 - W_1)44}{42W_2}1000 \quad (3)$$

where W_1 and W_2 are the dry weights of PS-PVAc before and after alcoholysis (g), 42 is the lost molar mass of PVAc after alcoholysis, 44 is molar mass of PVA unit.

2.4. Composition and Morphology Analysis. The chemical compositions of PS microspheres, PS-ACV, PS-PVAc and PS-PVA were characterized by X-ray photoelectron spectroscopy (XPS) (VG Scientific ESCALab220i-XL) and Fourier transform infrared spectroscopy (FTIR) (Nicolet6700, USA), respectively. Surface morphology of gigaporous PS microspheres and PS-PVAc were observed using scanning electron microscopy (SEM) (S-4800, Hitachi, Japan).

2.5. Measurement of Microsphere Pore Diameter and Specific Surface Area. The porosity of PS microspheres and PS-PVA was measured by mercury porosimetry (Micrometrics AutoPore IV 9500, USA). The specific surface areas were measured using a Micrometrics (ASAP 2020, USA) apparatus. Prior to analysis, samples were degassed under vacuum at 70 °C for 8 h.

2.6. Column Compressibility and Permeability. Columns of native PS microspheres and PS-PVA (stainless steel column, 100 mm × 4.6 mm I.D.) were packed with 20% aqueous ethanol solution using a slurry packing technique at a Waters Alliance system (Waters 2695, USA). The column compressibility and permeability was tested by the relationship between flow rate and column backpressure at 25 °C. The bed permeability (K) can be calculated by Darcy's law.[25]

$$K = \frac{\mu u L}{\Delta P} \quad (4)$$

where u is the superficial velocity (cm/s), μ the viscosity of the mobile phase (Pa s), ΔP the column pressure-drop (Pa), and L the length of column (cm).

2.7. Evaluations of Surface Hydrophilicity. Hydrophilicity of particles was defined as the ratio of the water content of the wet particles relative to the original weight. The surface hydrophilic property of gigaporous PS microspheres after coating was also evaluated by nonspecific protein adsorption using BSA as model protein.[26] In addition, An A1 laser scanning confocal microscopy (LSCM) (Nikon, Japan) was used to visualize the nonspecific adsorption of FITC-labeled BSA (FITC-BSA) on particles. The detailed experimental processes are described in our previous work.[21]

3. RESULTS AND DISCUSSION

3.1. Effect of Solvent on the Amount of ACV Coupled on PS Microspheres. The amount of azo initiator, ACV, introduced to the PS microspheres determined the uniformity and extent of coating and is affected by the solvent. Figure 1 shows the effect of solvent composition on the coupling amount of ACV. Compared with pure DMF, the addition of more weakly polar THF into the solvent can significantly increase the coupling amount of ACV. However, the coupling amount of ACV decreased again when the ratio of THF to DMF is larger than 0.5. Excess THF in the solvent will lead to a lower solubility of ACV and the limit of a heterogeneous system is evidently disadvantageous to the reaction.

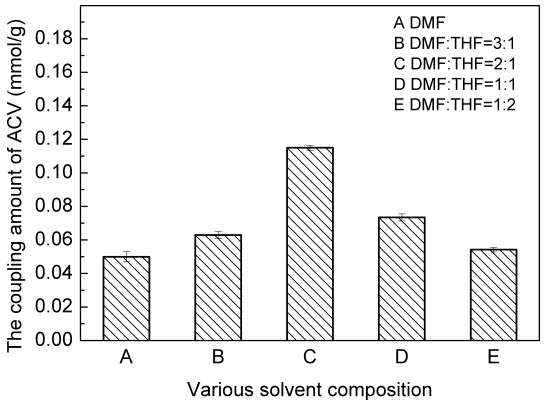


Figure 1. Effect of solvent on the amount of ACV coupled on PS microspheres.

3.2. Effect of Monomer Ratio on the Amount of Coating Grafted on PS Microspheres. Figure 2 shows the amount of cross-linked PVAc and net PVA hydrogel grafted on

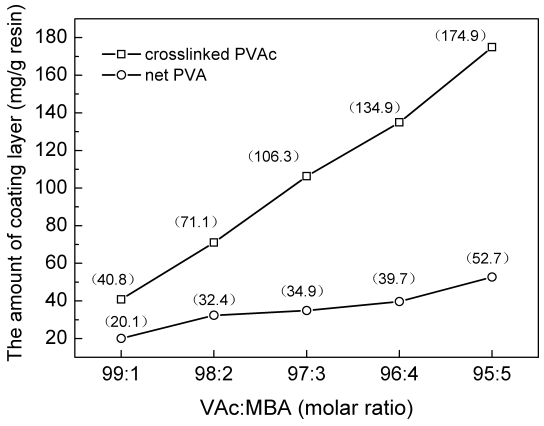


Figure 2. Amount of coating hydrogel grafted on gigaporous PS microspheres under various monomer ratios (the numbers in the parentheses are the amount of coating hydrogel).

gigaporous PS microspheres under various monomer ratios. Both of them increased almost linearly with the decrease in VAc:MBA (molar ratio), which means the coating on the hydrophobic surfaces of PS microspheres became thicker and denser, and this is advantageous to shield the hydrophobic surfaces of PS particles. As can also be seen from Figure 2, the amount of cross-linked PVAc increased far more rapidly than that of the average molecular weight of monomer mixture with the decrease of VAc:MBA (the average molecular weight of VAc:BMA (99:1), VAc:BMA (98:2), VAc:BMA (97:3), VAc:BMA (96:4) and VAc:BMA (95:5) are 86.68, 87.36, 88.04, 88.72 and 89.4, respectively). This is because the monomer reactivity of MBA is much higher than that of VAc. The amount of net PVA hydrogel-grafted PS microspheres increased slightly with the decrease of VAc:MBA. We think that an increasing concentration of MBA maybe facilitate the polymerization of VAc to some extent. It is also noteworthy that the amount of coating must be proper because excess

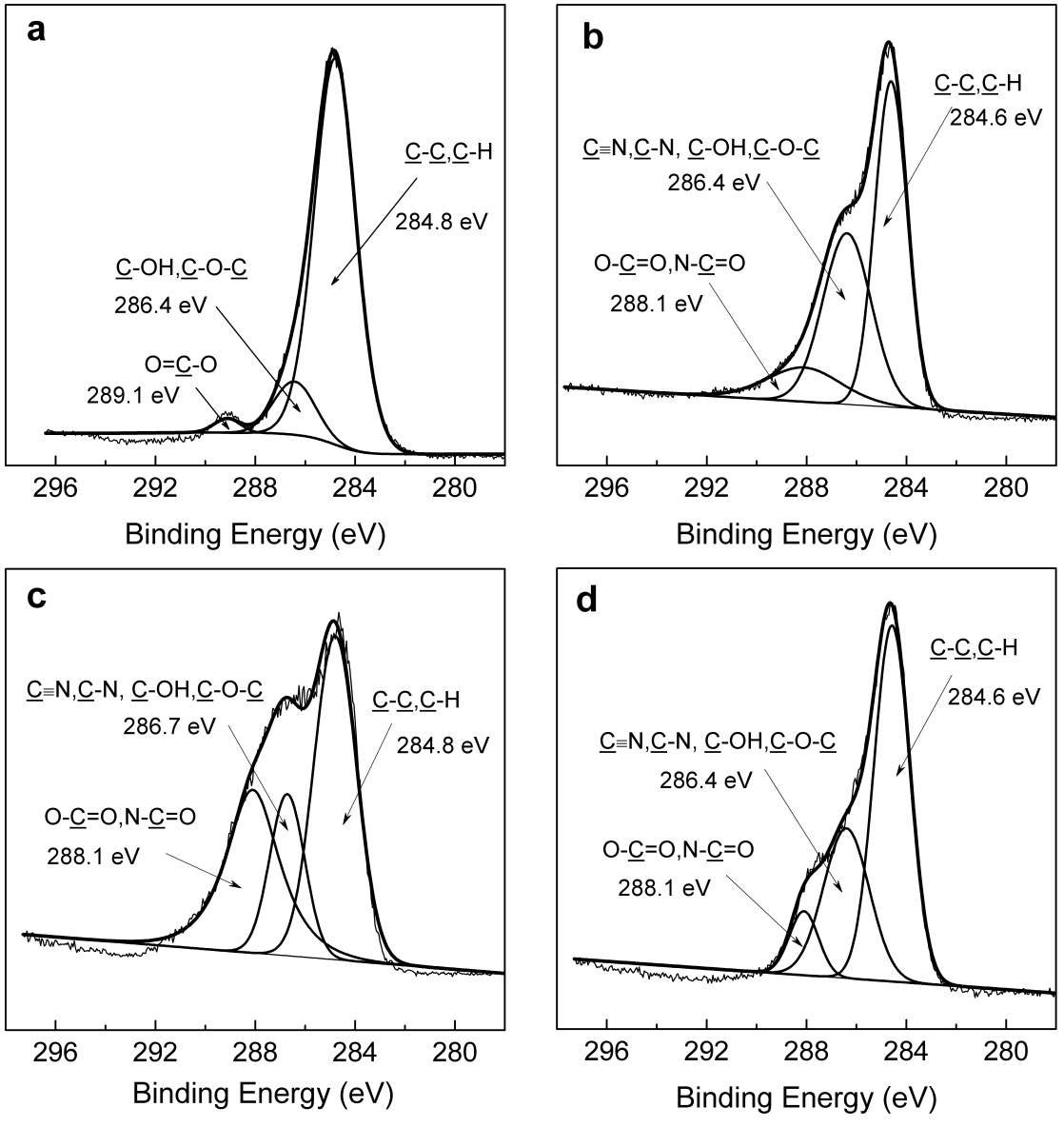


Figure 3. Typical C 1s XPS spectra of (a) PS microspheres, (b) PS-ACV, (c) PS-PVAc (96:4), and (d) PS-PVA (96:4) surface.

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Table 1. Peak Area Ratio of C_{1s} Spectra for PS Microspheres, PS-ACV, PS-PVAc (96:4), and PS-PVA (96:4)

	C_{1s}			peak area ratio	
sample	peak 1	peak 2	peak 3	peak 2/peak 1	peak 3/peak 1
PS	C−C, C−H	C−OH, C−O−C	O−C═O	0.135	0.023
PS-ACV	C−C, C−H	C≡N, C−N, C−OH, C−O−C	O−C═O, N−C═O	0.791	0.247
PS-PVAc	C−C, C−H	C≡N, C−N, C−OH, C−O−C	O−C═O, N−C═O	0.413	0.705
PS−PVA	C−C, C−H	C≡N, C−N, C−OH, C−O−C	O−C═O, N−C═O	0.535	0.144

cross-linked PVA hydrogel will block the gigapores of PS microspheres.

3.3. Composition Verification of the Microspheres. The C_{1s} spectra of PS microspheres, PS-ACV, PS-PVAc (96:4) and PS-PVA (96:4) are presented in Figure 3. Herein the number in the brackets behind PS-PVAc or PS-PVA presents the preparing monomer molar ratio (VAc:MBA) of the sample. The C_{1s} spectra of all samples exhibit three peaks with different attributions and peak area ratios (see Table 1). Figure 3a shows that XPS C_{1s} spectrum of PS microspheres presents three peaks at 284.8, 286.4, and 289.1 eV, which are attributed to C−C/C−H, C−O−C/C−OH, and O−C═O bonds, respectively. The presence of C−O−C/C−OH and O−C═O bonds in PS microspheres can be assigned to the use of sorbitan monooleate (Span80) during the preparation of microspheres, which was partly integrated into the particles and cannot be extracted by solvent. In the C_{1s} spectrum of PS-ACV (Figure 3b), the introduction of C≡N, C−N and N−C═O bonds after coupling ACV greatly increased both the peak area ratios of Peak 2 (286.4 eV)/Peak 1 (284.6 eV) and Peak 3 (288.1 eV)/Peak 1. After polymerization of VAc and MBA *in situ*, the peak area ratio of Peak3 (288.1 eV)/Peak1(284.8 eV) in the C_{1s} spectrum of PS-PVAc (96:4) (Figure 3c) further increased to 0.705 whereas that of Peak2 (286.7 eV)/Peak1 (284.8 eV) declined to 0.413. This can be ascribed to the O−C═O and N−C═O bonds contained in the VAc and MBA, respectively. When it comes to C_{1s} spectrum of PS-PVA, the amount of C−OH groups increased further owing to the alcoholysis of PVAc. Correspondingly, the peak area ratio of Peak 2 (286.4 eV)/Peak 1 (284.6 eV) increased to 0.535, and the peak area ratio of Peak 3 (288.1 eV)/Peak 1 decreased to 0.144.

The composition changes of PS, acetylated PS, oximated PS and aminated PS microspheres were characterized by FT-IR spectroscopy (see details in Supporting Information, Figure S1). Figure 4 shows the spectra of PS microspheres, PS-ACV, PS-PVAc (96:4) and PS-PVA (96:4). In the spectrum of PS-ACV, the medium absorption peaks at 3514 and 3317 cm^{-1} suggests the N−H and O−H stretching vibration of amino and carboxyl groups, respectively. The adsorption peak at 1737 cm^{-1} was ascribed to the C═O stretching vibration of carboxyl groups. After coated with cross-linked PVAc, the intensity of peaks at 1669 and 1734 cm^{-1} in the spectrum of PS-PVAc (96:4) increased significantly due to the introduction of amide and ester groups. In the spectrum of PS-PVA (96:4), the adsorption peak at 1734 cm^{-1} nearly disappeared after alcoholysis of PVAc, and the intensity of peak at 3417 cm^{-1} was further increased. The spectra changes described above also indicate that gigaporous PS microspheres were successfully coated with cross-linked PVA hydrogel.

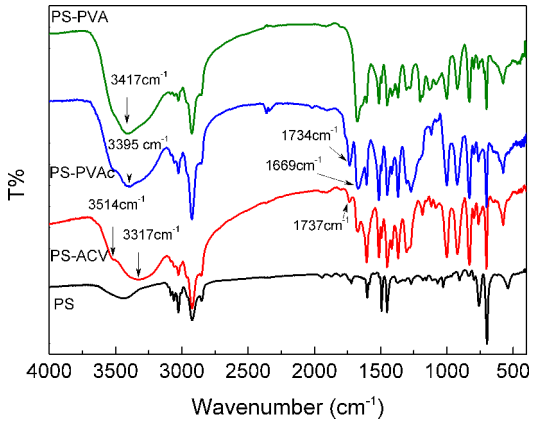

Figure 4. FT-IR spectra of PS microspheres, PS-ACV, PS-PVAc (96:4), and PS-PVA (96:4).

3.4. Physical Properties of the Microspheres before and after Coating. SEM images of the PS microspheres and PS-PVAc prepared under various monomer ratios are shown in Figure 5. As mentioned above, the amount of cross-linked PVAc increased with the decrease of VAc:MBA (molar ratio). Therefore, the amount of coating layer must be proper to ensure the gigaporous structure of PS microspheres was well maintained. As can be seen from Figure 5, when the molar ratio of VAc:MBA is larger than 96:4, little morphology changes between PS microspheres and PS-PVAc can be found. When the molar ratio of VAc:MBA further decreased (Figure 5f), the coating layer was nonhomogeneous and cross-linked PVAc had evidently plug the gigapores of PS microspheres, which is disadvantageous to chromatographic packing materials.

Figure 6 shows the pore diameter distributions of gigaporous PS microspheres and PS-PVA (96:4) determined by mercury porosimetry. It is clearly that the superpores ranging from 100 to 700 nm contributed to most of pore volumes, and the largest incremental pore volume was assigned to the pores with diameter of about 280 nm. As can also be seen from Figure 6, there are little differences between PS microspheres and PS-PVA (96:4) in the pore diameter distributions, confirming the gigaporous structure of PS microspheres was robustly maintained after coating.

Compared with native gigaporous PS microspheres, the specific surface area of PS-PVA samples decreased with the increasing amount of coating hydrogel (see details in Supporting Information, Figure S2). It is a remarkable fact that the lower surface area of particles in dry state cannot stand for the real loading capacity of particles in wet swollen state.[27]

3.5. Column Compressibility and Permeability. Column compressibility and permeability is an important feature for chromatographic packing materials. As shown in Figure 7, a good linear relationship between flow velocity and column backpressure was obtained for both PS and PS-PVA (96:4) columns. The backpressure of PS and PS-PVA (96:4) columns under 3612 cm/h was only 0.83 and 0.98 MPa, respectively. The bed permeability (K) calculated from eq 4 for PS and PS-PVA (96:4) column was 2.71×10^{-10} m^2 and 2.05×10^{-10} m^2, which indicates that the micropores of PS microspheres were

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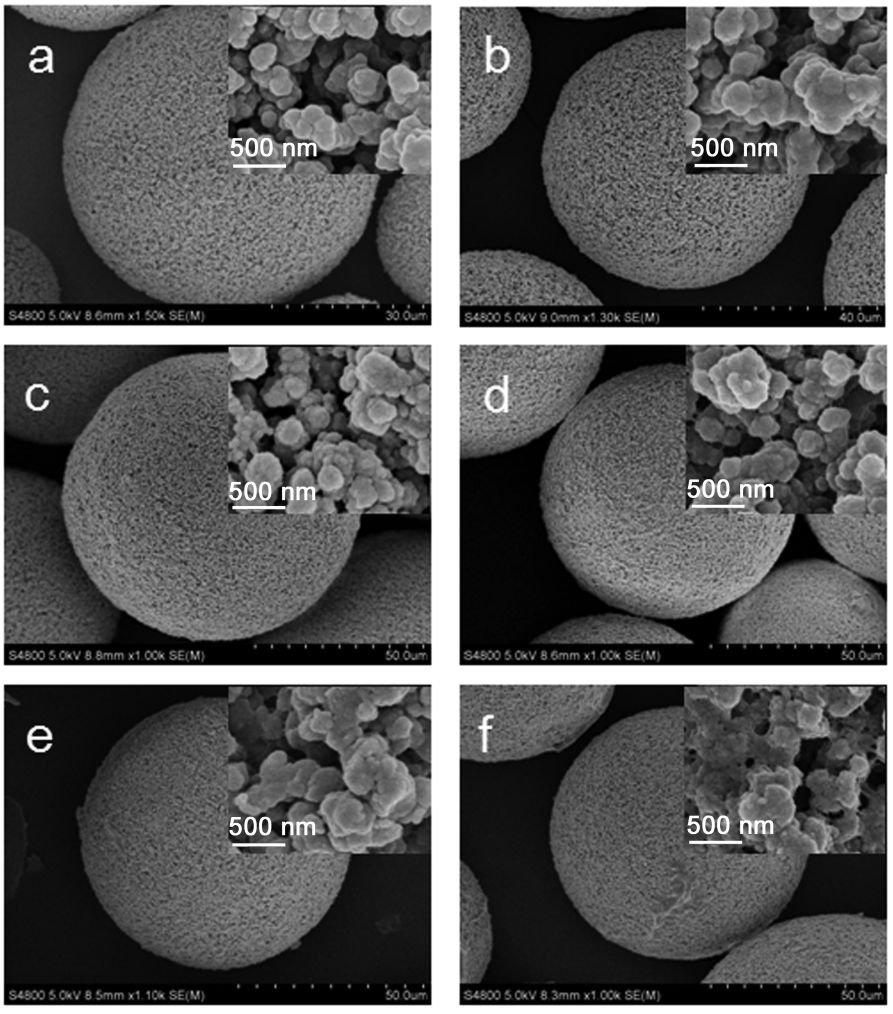

Figure 5. SEM images of gigaporous PS microspheres and PS-PVAc. (a) PS microspheres. (b–f) PS-PVAc prepared under various monomer ratios (the molar ratios of VAc to MBA were 99:1, 98:2, 97:3, 96:4, and 95:5, respectively).

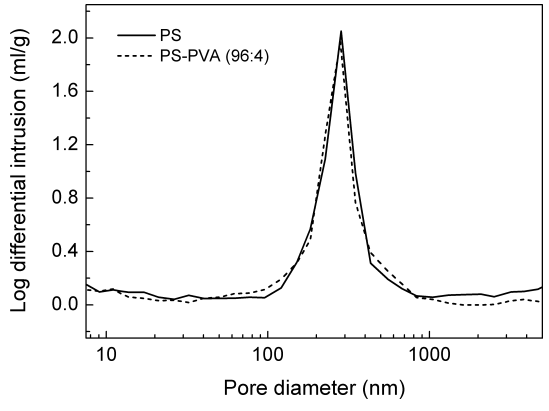

Figure 6. Pore size distributions of gigaporous PS microspheres and PS-PVA (96:4).

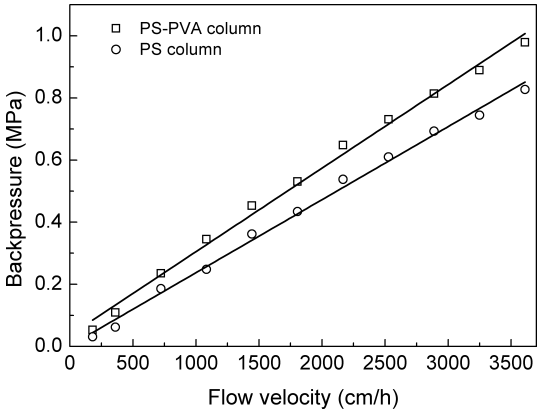

Figure 7. Effect of flow velocity on backpressure of PS and PS-PVA columns. Column, 100× 4.6 mm i.d.; mobile phase, 20% aqueous ethanol solution.

blocked slightly after coating with cross-linked PVA hydrogel. In a previous study, we have testified that the presence of flow-through pores, which can reduce the flow resistance significantly by forming connective channels, would contribute to the low backpressure and high bed permeability of columns.[11] A similar conclusion was also made by Sun et al.[28,29]

3.6. Surface Hydrophilicity of the Microspheres after Coating. The surface hydrophilicity of PS microspheres and PS-PVA are given in Figure 8. Compared with native PS microspheres, the surface hydrophlicity of PS-PVA improved significantly due to the incorporation of cross-linked PVA hydrogel on particles surface. It can be seen that the hydrophilicity of PS-PVA increased with the decrease of monomer molar ratio (VAc:MBA). This is because the amount of cross-linked PVA hydrogel increased with the decrease of

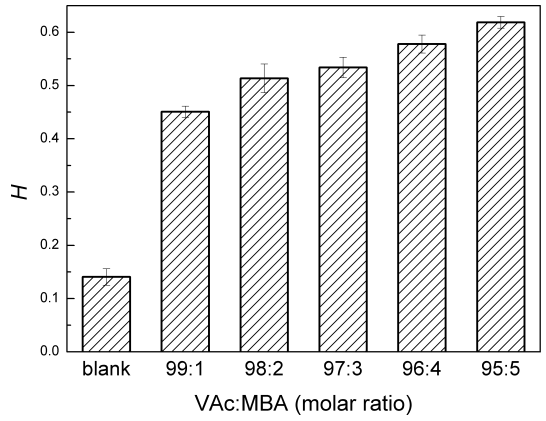

Figure 8. Hydrophilicity of gigaporous PS microspheres and PS-PVA prepared under various monomer ratios.

VAc:MBA. The average H of PS-PVA increased to nearly four times as compared with PS microspheres. The low hydrophilicity of PS microspheres is attributed to their hydrophobic surface, which makes the water can hardly diffuse into internal pores of the particles.

3.7. Adsorption Isotherms of Protein on the Microspheres. Figure 9 shows protein adsorption isotherms of PS

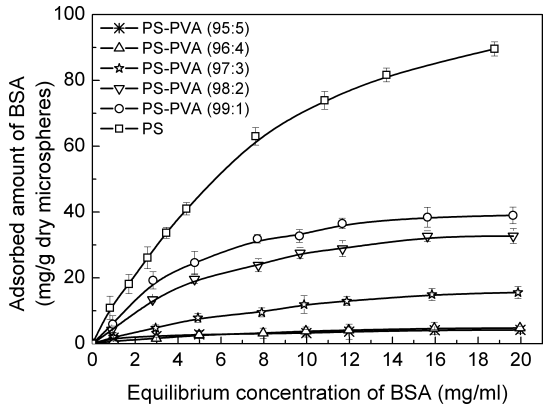

Figure 9. Adsorption isotherms of BSA on PS microspheres and PS-PVA prepared under various monomer ratios in 0.05 M phosphate buffer (pH 7.4, 25 °C). The numbers in the parentheses of legends present the preparing molar ratios of VAc:MBA.

microspheres and PS-PVA at 25 °C. After coating with cross-linked PVA hydrogel, the nonspecific adsorption amount of BSA on PS particles decreased significantly, which is attributed to the hydrophobic PS microspheres surfaces were well shielded by cross-linked PVA hydrogel. It can be seen that the saturated adsorption amount of BSA on PS microspheres (89.55 mg/g dry microspheres) was 18.9-fold as compared with PS -PVA (96:4) (4.74 mg/g dry microspheres), moreover, the nonspecific adsorption amount of BSA on PS-PVA decreased gradually with the increase of coating amount. It can also be seen from Figure 9 that the saturated adsorption amount of BSA on PS-PVA (95:5) (4.31 mg/g dry microspheres) was nearly the same as that on PS-PVA (96:4), which indicates that the amount of cross-linked PVA hydrogel on PS-PVA (96:4) is enough to mask the hydrophobic PS microspheres surfaces. Excess grafting amount of cross-linked PVA hydrogel (PS-PVA (95:5)) is unnecessary and can block the gigapores of PS microspheres. Compared with PS microspheres modified with linear PVA chains (7.72 mg BSA/g dry microspheres),[21] the cross-linked PVA hydrogel grafted on PS microspheres surfaces is more effective in rejecting protein adsorption. The adsorption amount of BSA on PS-PVA did not fall to zero, we suppose the hydrogen bonds formed between amide group (cross-linked PVA hydrogel) and carboxyl/amino group (BSA) can facilitate the adsorption of BSA on PS-PVA. Although the adsorption amount of BSA on PS-PVA is still larger than that on PS microspheres with agarose physical coating (1.18 mg/g),[23] the cross-linked PVA hydrogel grafted on PS microspheres surfaces can effectively avoid the problem of desorption of physical coating over time, which is advantageous to the long-term use of chromatographic supports.

3.8. Visual Observation of FITC-BSA Adsorbed on Particles. LSCM images can give a straight insight on the incremental hydrophilicity of PS microspheres after coating. Figure 10 illustrates that strong fluorescence from FITC-BSA can be visualized on PS microspheres, whereas weak fluorescence was observed in PS-PVA (96:4). This also indicates that nonspecific adsorption of BSA on PS micro-

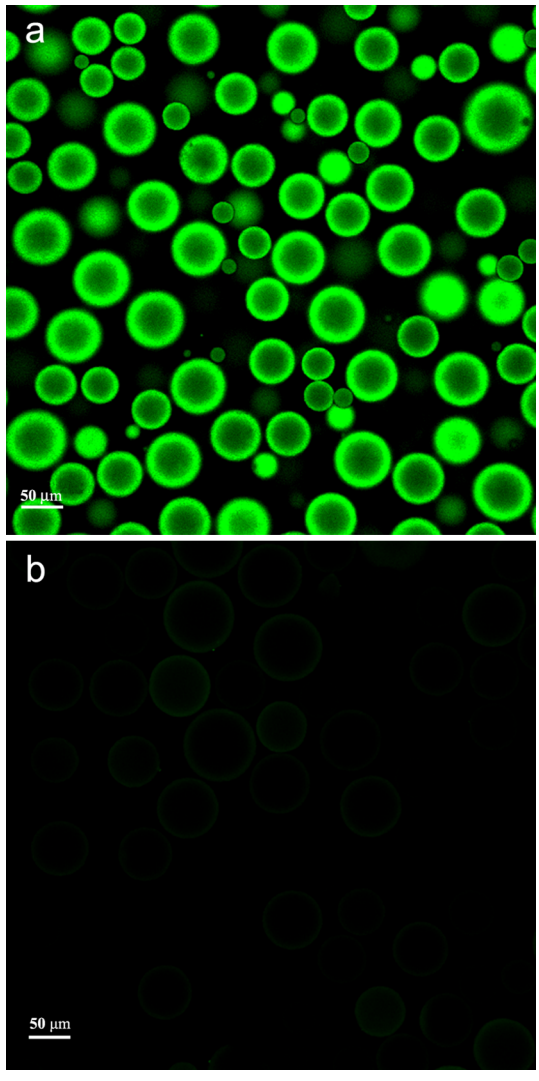

Figure 10. LSCM images of (a) PS microspheres and (b) PS-PVA (96:4) after being incubated with FITC-BSA in 0.05 M phosphate buffer (pH7.4, 25 °C).

spheres decreased significantly after grafting cross-linked PVA hydrogel, i.e., the hydrophilicity of PS-PVA improved markedly.

4. CONCLUSIONS

A promising chromatographic packing material has been prepared by *in situ* polymerization of VAc and MBA on gigaporous PS microspheres. The cross-linked polymer gave a dense covalent coating on PS microspheres, where the gigaporous structure of microspheres remained well. After alcoholysis of PVAc, both the hydrophilicity and biocompatibility of PS-PVA improved significantly. A column packed with PS-PVA exhibited low backpressure and high permeability up to 3612 cm/h because flow resistance was reduced greatly by the flow-through pores of particles. Compared with phenoxyl agarose modified PS microspheres through physical adsorption in our previous study, the cross-linked PVA hydrogel grafted on PS microspheres is promising in long-term stability and reusability of PS-PVA as a base support. The hydrophilic gigaporous matrix should have great potentials in rapid protein chromatography because the hydroxyl-rich PVA coating can be derivatized easily by classical chemical methods.

■ ASSOCIATED CONTENT

Supporting Information

Preparation detail of PS-ACV, FT-IR spectra of gigaporous PS microspheres before and after amination, the specific surface area of PS microspheres before and after coating, and relevant discussions. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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