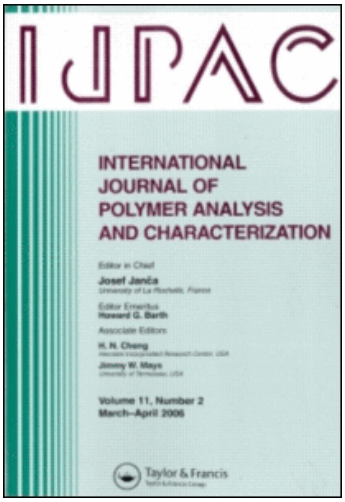

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NOVEL HYDROPHILIC POLY(VINYL ESTER RESIN-CO-ETHYLENE DIMETHACRYLATE) MONOLITH FOR BIOSEPARATION

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Poly(vinyl ester resin-co-ethylene dimethacrylate) monolithic columns have been prepared by in situ copolymerization of the monomers in the presence of a porogenic agent. The monolith was further derived by 3-glycidoxypropyltrimethoxysilane and then hydrolyzed in 0.25 mol/L sulfuric acid to prepare diol column. The chicken egg white (CEW) was separated by a column with a spacer arm and another without a spacer arm. The results showed that the derived monolith had a higher separation capacity. Moreover, the separations under different flow rates were compared. The separation could be carried out at the flow rate of 3.0 mL/min, and one separation takes place within 3 min. Finally, the maximum amount of adsorption of lysozyme on the monolith was obtained by the use of a pH and concentration gradient.

***Keywords*:** Bioseparation; Monolith; Novel; Poly(vinyl ester resin-co-ethylene dimethacrylate)

INTRODUCTION

Chromatography is widely used for the separation and analysis of proteins. Conventional columns for bioseparation are built up of uniform, spherical beads. The preparation of the columns involves many expensive and time-consuming steps. In the quest for enhanced and simple preparation of chromatographic separation media, researchers have found that a monolithic column could be a useful alternative to columns packed with particles. The use of such a monolith in chromatography would provide increased speed, capacity, and resolution.[1,2] The screening of a suitable matrix is very important in protein adsorption and separation. As a general observation, it has been found that proteins adsorb weakly to neutral hydrophilic surfaces, are attracted or repelled by charged surfaces, and adsorb strongly to hydrophobic surfaces.[3–5] The interactions of proteins with solids are often determined by the electrostatic and hydrophobic properties of the proteins and the solid surface.

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Hydrophobic interaction chromatography (HIC) has widely been employed in the separation of proteins. However, the use of an eluent containing a high concentration of salt is inconvenient, particularly in large-scale purification, and it is a major disadvantage of current HIC. So the ideal materials for adsorption and separation of protein are large network structures of high hydrophilicity, low irreversible adsorption of protein, high rigidity, and low cost. In order to allow a reversible adsorption, the chromatography material must be very hydrophilic.

Vinyl ester (VE) resin is a kind of thermosetting resin containing esters of acrylic or methacrylic acids and having double-bond and ester linkage sites only at the ends of the resin molecules. The two terminal free-radical polymerizable unsaturation sites enable the vinyl ester resin monomers to cross-link. Vinyl ester resins are used to make polymer matrix composites for military and commercial applications because of their high modulus, high strength, high glass transition temperature, low weight, and low cost.[6]

Lysozyme is a commercially important enzyme, currently used in food technology as a potent antibacterial agent in milk products and in wine production processes, as well as in pharmacological technology as a drug for treatment of ulcers and infections. Chicken egg white (CEW) is a mixture of proteins and has been routinely used as the source material of lysozyme. The content of lysozyme in CEW is about 3.4%. The classical lysozyme purification method requires several steps, such as precipitation, centrifugation, and adsorption.[7]

This article presents the preparation of hydrophilic polymer monoliths by co-polymerizing vinyl ester resin and ethylene dimethacrylate. An indirect method to gain insight into adsorption mechanisms is to study the protein-material interaction at variable pH or ionic strength conditions. Moreover, the effect of flow rate on the separation of CEW and the adsorption amount of lysozyme on the monolith has been studied.

EXPERIMENTAL SECTION

Materials

Bisphenol A diglycidyl ether (BADE) was purchased from Blue Star New Chemical Materials Co., Ltd. (Wuxi, China), ethylene glycol dimethacrylate (EDMA) was purchased from Acros (New Jersey, USA), and 2, 2′-azobisisobutyronitrile (AIBN) was produced by Shanghai Chemical Plant (Shanghai, China) and refined before use. Dodecanol and cyclohexanol were from Fuchen Chemical Plant (Tianjin, China). Tetrabutylammonium bromide was purchased from Xingfu Fine Chemicals Research Institute (Beijing, China), α-methacrylic acid was purchased from Jinshan Chemical Reagent Co., Ltd. (Chengdu, China). Dioxane was from Chemical Reagent Co., Ltd. (Beijing, China). Lys was bought from Sigma Chemical Co. (St Louis, Mo., USA), and 3-glycidoxypropyltrimethoxysilane was purchased from Yili Co. Ltd. (Beijing, China). All water was prepared from a Millipore-Q system and solutions were filtered through a 0.45 μm membrane before use.

Synthesis of Poly(Vinyl Ester Resin-co-Ethylene Dimethacrylate) Monolith

The monolithic column was prepared by an in situ polymerization within the confines of the stainless steel tube of a 10×4.6 mm i.d. chromatographic column.

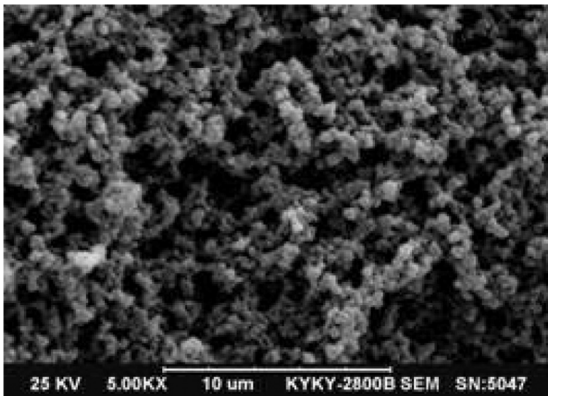

Figure 1. Scanning electron micrographs of the monolithic column.

First, a mixture consisting of 1.2 mL VE resin, 1.2 mL EDMA, 3 mL 1-dodecanol, and 0.09 g AIBN was purged with nitrogen for 10 min. A stainless steel tube sealed at the bottom was filled with the polymerization mixture and then sealed at the top. After the polymerization was allowed to proceed at 55°C for 24 h, the seals were removed from the tube and the column was provided with fittings, attached to the high-performance liquid chromatography (HPLC) system, and washed with methanol at a flow rate of 1 mL/min for 60 min to remove the alcohols and other soluble compounds present in the polymer rod after the polymerization was completed. Scanning electron micrographs of the monolithic column are shown in Figure 1.

Preparation of the Diol Monolith

The monolithic column and 1 mL 3-glycidoxypropyltrimethoxysilane were refluxed in 10 mL toluene for 12 h. After cooling, the monolithic column was washed with toluene and acetone. The epoxide monolithic column was dried and then hydrolyzed in 10 mL 0.25 mol/L sulfuric acid for 10 h at 60°C. After that, the monolithic column was washed with water until neutral pH was reached and dried, thus obtaining the hydrolyzed column.

Purification of Lysozyme from Egg White

The fresh hen eggs were purchased from local market. CEW was separated from fresh eggs and diluted to 50% (v/v) with phosphate buffer (50 mmol/L, pH 7.0). The diluted egg white was homogenized in an ice bath and centrifuged at 4°C at 10,000 rpm for 30 min. The supernatant fluid was used as the lysozyme source.

RESULTS AND DISCUSSION

Effect of pH

In the poly(vinyl ester resin-co-ethylene dimethacrylate) monolithic matrix, there exist the hydroxyl groups as the hydrophilic groups. On a hydrophilic solid

surface, the electrostatic attraction between a charged surface and an oppositely charged protein molecule was often the driving force for adsorption from solution on the solid surface. The amount adsorbed may then be determined by a balance between this electrostatic attraction and the electrostatic repulsion within the adsorbed layer.

The pH value was thought to affect protein adsorption in a more direct way by adjusting protein charge and altering its conformation, so lysozyme release from the monolith was studied at various pH conditions (4.5, 7.0, 7.6, and 8.0; the ionic strength of the phosphate buffer was kept constant at 10 mmol/L). The results showed that the amount of protein released from the membrane slightly increased when pH value increased, but an incomplete release of lysozyme was observed in all the cases. Because the isoelectric point (pI) of lysozyme was 11.2, in all the pH value conditions, lysozyme obtained a net positive charge while the monolithic column was negatively charged. So the electrostatic interaction between the protein and the hydrophilic groups influenced the amount of protein adsorbed on the monolithic materials. Since lysozyme was more stable at pH 8 than at pH 4.5, the difference in initial adsorption was dominated by the greater relative stability of lysozyme to denaturation at the higher pH. A change of pH from 8 to 4.5 reduced the stability of the protein to unfolding and resulted in more adsorption than when the pH was changed in the opposite direction. These results demonstrated that the contribution of the electrostatic interaction was dominant for protein adsorption on the monolithic materials.

Effect of Phosphate Buffer (PB) Concentration

Proteins with a high internal stability, such as lysozyme, do not adsorb to hydrophilic surfaces unless there is electrostatic attraction.[8] The primary effect of increasing the ionic strength of a protein solution is the reduction of the thickness of the electric double layer over charged surfaces or around charged protein molecules. The results in Figure 2 show that the binding amounts of lysozyme decreased as phosphate buffer (PB) concentration increased at pH 8, and the maximum release of the protein was observed when the concentration of PB was 0.2 mol/L, the reason being that salt molecules shielded the electrostatic interaction between proteins and monolithic column in this electrostatic-dominant adsorption effect.

Effect of Flow Rate on Separation of Lysozyme from Natural CEW Solution

In order to examine the separation capacity of the monolith, real protein mixtures were used. The effect of flow rate on the separation of lysozyme from natural CEW solution was investigated by increasing the volumetric flow rate from 1 to 3 mL/min. From Figure 3, it could be seen that the monolithic column allowed separations to be conducted at high flow rates, whereas the resolution was only slightly affected by the flow rate. This observation allows accelerating the separation of lysozyme from natural CEW solution.

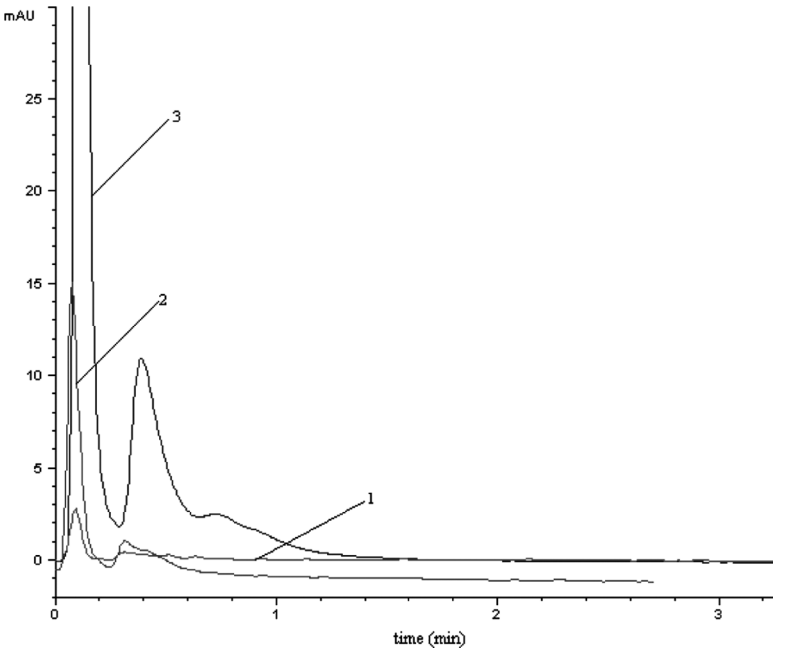

Figure 2. Effect of salt concentration on the elution of lysozyme. 1, 0.001 mol/L K_2HPO_4; 2, 0.01 mol/L K_2HPO_4; 3, 0.2 mol/L K_2HPO_4.

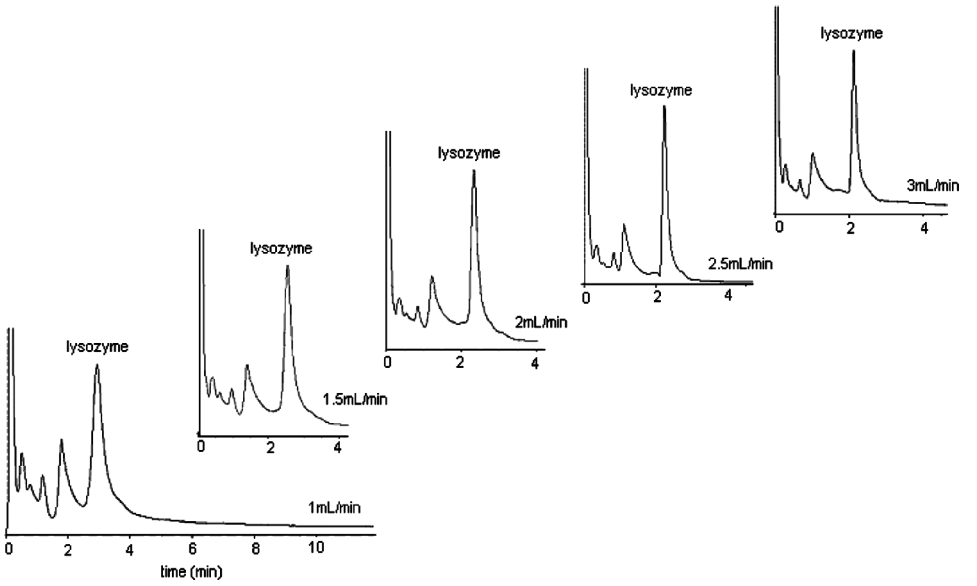

Figure 3. Effect of increasing flow rate on the separation of lysozyme from natural CEW solution. Gradient was carried out with buffers A (0.01 mol/L KH_2PO_4) and B (0.2 mol/L K_2HPO_4). Gradient was performed from 100% A to 80% A in 0.5 min, and then from 20% B to 100% B in 1.2 min and kept at buffers B.

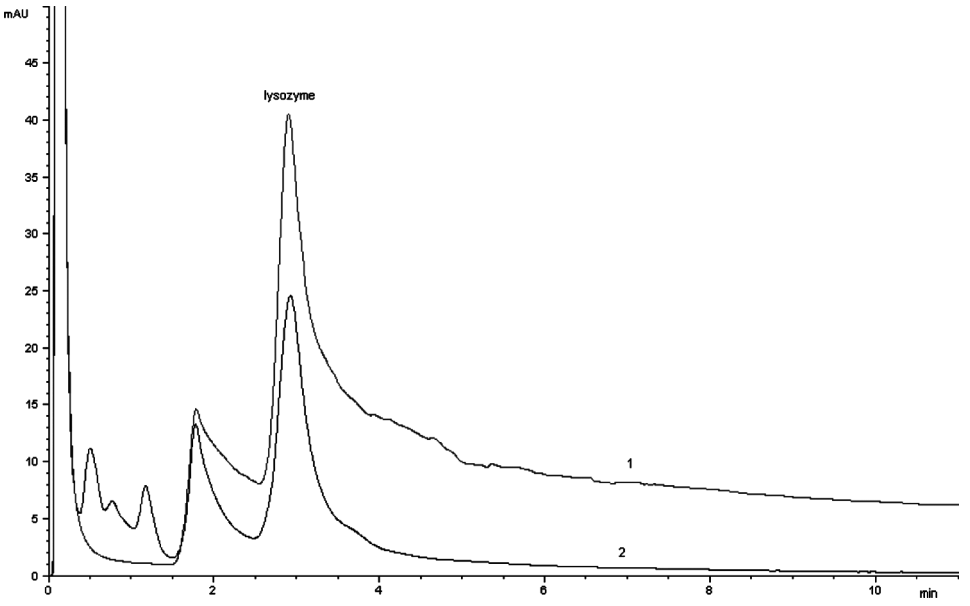

Figure 4. Chromatogram of CEW solution on the monolith by a spacer arm (2) and without spacer arm (1); chromatographic conditions as in Figure 3.

Influence of Spacer Arm on Separation of CEW

On the monolithic column surface, the electrostatic attraction between the hydroxyl groups and protein molecule was the driving force for adsorption from solution onto the solid surface. Because the hydroxyl groups are small molecules, steric hindrance would occur. Therefore, it was not easy to establish an interaction between the hydroxyl groups on the column and the protein in the medium. In order to overcome this problem, a spacer arm was often bound to the support. To investigate the effect of a spacer arm on separation ability, CEW solution was separated on the columns both with a spacer arm (diol monolith) and without a spacer arm (naked monolith) under the same conditions. The results showed (Figure 4) that the resolution of CEW solution on the monolith with a spacer arm was higher than that without a spacer arm.

Maximum Loading Capacity

The maximum loading experiment was conducted to investigate the adsorption performance of the 10 mm × 4.6 mm i.d. monolithic column for lysozyme. First, different amounts of lysozyme were injected into the monolithic column when 0.01 mol/L KH_2PO_4 was used as the mobile phase and the flow rate was 0.01 mL/min. Second, the column was eluted by 0.2 mol/L K_2HPO_4 at 1 mL/min. As long as no overloading of the column occurred, no lysozyme would be eluted when 0.01 mol/L KH_2PO_4 was used as the mobile phase at the flow rate of 0.01 mL/min. Moreover, the peak area increased when the injection amount increased before the monolithic column reached saturation. After the column

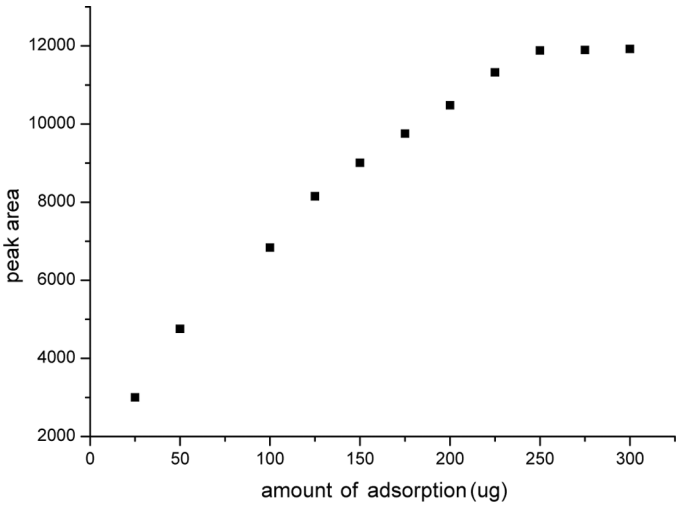

Figure 5. Peak area as functions of the loading of lysozyme to the monolith.

reached saturation, although more lysozyme was added to the column, only part of it was adsorbed on the column. So the peak area stayed unchanged when the injection amount increased. The result is shown in Figure 5. The maximum adsorptive amount of lysozyme under these conditions can be seen in Figure 4; it is 4.41 mg/g.

CONCLUSIONS

In the present study, poly(vinyl ester resin-co-ethylene dimethacrylate) monolith column was prepared and further modified using 3-glycidoxy-propyltrimethoxysilane as a spacer arm. By investigating the adsorption of lysozyme on the modified column at variable pH and ionic strength conditions, a conclusion was obtained that the dominant adsorption between proteins and monolithic column was electrostatic interaction. The effect of flow rate on the separation of lysozyme from natural CEW solution indicated that the separation could be operated under higher flow rates. Moreover, the influence of the spacer arm on the separation of CEW proved that the adsorption between the small groups and biomolecules was also affected by steric hindrance.

REFERENCES

1. Svec, F., and J. M. J. Fréchet. (1992). Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. *Anal. Chem.* **64**, 820–822.
2. Wang, Q. C., F. Svec, and J. M. J. Fréchet. (1995). Hydrophilization of porous polystyrene-based continuous rod column. *Anal. Chem.* **67**, 670–674.
3. Lee, J. H., K. W. Jung, I. K. Kang, and H. B. Lee. (1994). Cell behavior on polymer surfaces with different functional groups. *Biomaterials* **15**, 705–711.
4. Nakanishi, K., T. Sakiyama, and K. Imamura. (2001). On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. *J. Biosci. Bioeng.* **9**, 233–244.

5. Hammer, D. A., and M. Tirrell. (1996). Biological adhesion at interfaces. *Annu. Rev. Mater. Sci.* **26**, 651–691.
6. Malik, M., V. Choudhary, and I. K. Varma. (2000). Current status of unsaturated polyester resins. *J. Macromol. Sci. Rev. Macromol. Chem. Phys.* **40**, 139–165.
7. Senel, S., B. Elmas, T. C. Çamli, M. Andac, and A. Denizli. (2004). Poly(hydroxyethylmethacrylate-N-methacryloyl-(L)-histidine methylester) based metal-chelate affinity adsorbent for separation of lysozyme. *Sep. Sci. Technol.* **39**, 3783–3795.
8. Norde, W., and J. Lykleman. (1991). Why proteins prefer interfaces. *J. Biomater. Sci. Polym. Ed.* **2**, 183–202.