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Poly [(chloromethyl) styrene-co-divinylbenzene] Continuous Rod Column of Weak Cation Exchange Chromatography and its Applications in the Separation of Biopolymers

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Abstract: Macroporous poly [(chloromethyl) styrene-co-divinylbenzene] continuous rod was prepared by direct polymerization of the monomers in the presence of a porogenic diluent inside an empty chromatographic column. A new "*in-situ*" technique was used to modify the synthesized polymer rod for a weak cation exchanger and it has been used successfully for the separation of biopolymers. It was found that the back pressure of the continuous rod column was much lower and its surface was proved to be modified well.

Keywords: Liquid chromatography, weak cation exchange, polymer, continuous rod, modification, biopolymer, separation.

Introduction

For decades, macroporous polymeric beads have been widely employed as a kind of chromatographic matrix for the analysis and separation of proteins. However, some inherent drawbacks, such as the complicated synthetic process or the lower occupied space inside a chromatographic column, *etc*, have limited their applications. The continuous rod column (continuous rod), which has been recently explored, could be used to solve these problems[1-3]. Since poly [(chloromethyl) styrene-co-divinylbenzene] is very difficult to be modified, the continuous rod as a kind of separation media for biopolymers has not been reported. This paper presents a new chemically modified method for poly [(chloromethyl) styrene-co-divinylbenzene] continuous rod column. The characteristic of the new column were tested by the separation of biopolymers, hydrophobicity, column loading, and back pressure.

Experimental

The mixure of initiator , monomer (chloromethyl) styrene and divinylbenzene, as well as the porogenic solvent was purged with nitrogen for 15 min. After that, an empty stainless steel column (50 mm×8 mm I.D.) was filled with the mixture. The polymerization was carried out at 70℃ for 24 hours.

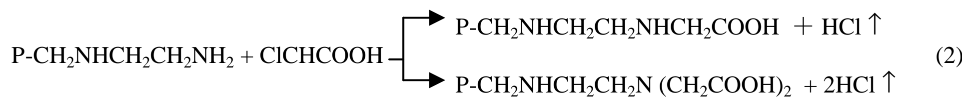
A weak cation exchange column could be obtained after a two-step modification process involving reaction with ethylenediamine, then with chloroacetic acid.

Results and discussion:

"In-situ" Modification

The application of the continuous rod of the "molded" macroporous poly [(chloromethyl) styrene-co-divinylbenzene] in the separation of biopolymers was limited because of its strong hydrophobic surface. With the modification of the hydrophobic surface by using a hydrophilic reagent, the irreversible adsorption on the surface of the continuous rod and conformation changes of protein molecules could be avoided. **Figure 1** shows the "*in-situ*" modification scheme designed in this paper. The "P" in the scheme denotes the polymer frame. The modification process would be completed only after the reagent solution was pumped through the continuous rod column for enough time.

Figure 1. Scheme of the "*in-situ*" modification process for poly [(chloromethyl)styrene-co-divinylbenzene]

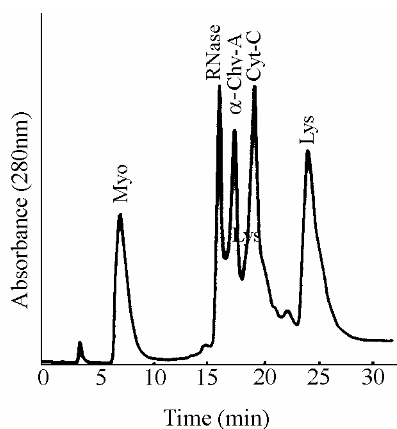


The Separation of Biopolymers by the Continuous Rods

The chromatogram of five kinds of proteins on the continuous rod is shown in **Figure 2**. It can be seen that good separation efficiency was obtained from the continuous rod. The experiment results indicate that the resolution decreases as the injected amount increases. This is a normal phenomenon in high performance liquid chromatography. The resolution of ribonucleotide A (RNase-A) and cytochrome C (Cyt-C) decreased nearly to one-half when the injected amount approached to 1.5 mg.

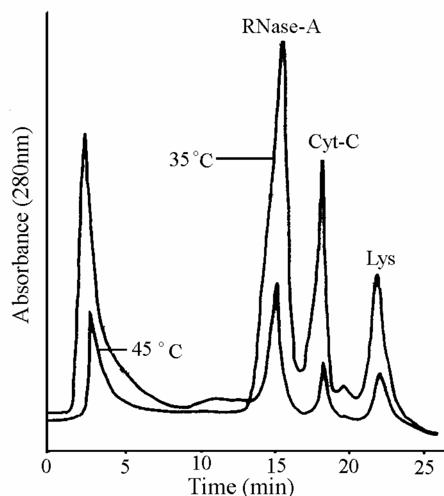
The effect of hydrophobic on the biopolymer retention was investigated by adding 5% (V/V) 2-propanol into the mobile phase. It was found that the retention time of myoglobin (Myo), RNase-A, α -chyotrypsin A (α -Chy-A), Cyt-C, and lysozyme (Lys) only shortened by about 10%, compared with that in the absence of any organic solvent in the mobile phase. Because the presence of the organic solvent may affect the partition coefficient of proteins in ion-exchange chromatography, this foregoing fact indicates the hydrophobicity of the continuous rod to be very weak. When the same five proteins were separated with the same continuous rod but by using the elution mode of hydrophobic interaction chromatography (HIC), *i.e*, 3.0mol/L $\mathbf{(NH_4)_2SO_4}$+0.05mol/L phosphate buffer solution (**PBS**) and 0.05mol/L **PBS** as mobile phases (pH 7.0) A and B, respectively, the result indicates that RNase-A, and Cyt-C were not retained, while the five kinds of proteins would be strongly retained and separated very well by usual HIC column. This further confirms that the hydrophobicity of the synthesized rod is really very weak. The hydrophilicity of the polymer was proved to increase rapidly after chemical modification. Therefore, the three dimensional structure of the separated protein molecules in the circumstance should not be changed. This point was also proved by the high bioactive recovery being 103% for Lys.

Figure 2. Separation of proteins by ion-exchange chromatography on the continuous rod column. conditions: column 50×8 mm I.D.; mobile phase A: (0.02mol/L Tris + H_2O, pH=8.0). mobile phase B: (0.02mol/L Tris +1.0mol/L NaCl, pH=8.0). linear gradient from 100%mobile phase A to 50%mobile phase B within 30 min. flow rate 1.0 mL/min; injection of RNase-A 25 mg, injections of the other four kinds of proteins 10 mg. UV detection at 280nm.



Effect of Temperature on the Protein Retention

Figure 3. Comparison of the chromatogram of Rnase-A, Cyt-C, Lys from the continuous rod between 35℃ and 45℃. conditions are the same as described in **Fiure 2.**



The change in the retention times of proteins with temperature in LC is usually taken as one of the criteria to judge whether the retention mechanism of a column to be hydrophobic or not. A series of chromatograms of the three proteins, RNase-A, Cyt-C, and Lys ,were obtained at column temperatures of 0℃, 10℃, 25℃, 35℃, and 45℃. It was found that the retention time of the three proteins did not change in the temperature range from 0℃ to 45℃. This fact again indicates the hydrophobicity of the rod column being

very weak. Otherwise the retention time of the three proteins would increase with rising temperature. However, as shown in **Figure 3**, the peak area of each protein at 45 ℃ is much smaller than that at 35 ℃ even though the injected amount of these proteins is same. This phenomenon could be explained as that the molecular conformations of the three proteins had been changed at 45 ℃. It might be partially denatured also[4]. As a result, the peak areas of these natural proteins were decreased. Each peak area of the three proteins was not found to change when the column temperature was in the range from 0 ℃ to 35 ℃.

Flow Rate

The back pressure of the continuous rod is only about 2 MPa when the flow rate reaches 10 mL/min. It shows the back pressure of the rod column to be much lower than that of the normal packed column with the same size. In addition, the dependence of the back pressure on the flow rate is directly proportional to the flow rate of the mobile phase in the range from 1.0 mL/min to 10 mL/min which is the maximum flow rate of the pump used in this work. This should be attributed to structural characteristic of the synthesized rod material. The largest "channels" in the polymer could even reach 1000nm. This could not only reduce the back pressure greatly, but also accelerate up the separation process. The band broadening of proteins inside the rod column was greatly reduced because of the fast convection mass transfer. This could also confirm the fact that the macroporous structure of the polymer was not seriously influenced by the foregoing modification with two steps.

Acknowledgment

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