Preparation and Characterization of a Novel Porous Cation-Exchange Polymeric Monolith by Atom Transfer Radical Polymerization

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A porous cation-exchange polymeric monolith with uniform structure has been prepared by atom transfer radical polymerization. Methyl methacrylate was used as the monomer; ferrous chloride was used as the catalyst; sodium bisulfate was first used both as inorganic moderator and co-initiator together with CCl₄ to control the concentration and activity of the free radical in the process of polymerization. The characters such as morphology, pore size distribution, mechanical stability and permeability of the polymer were investigated. It revealed good chromatographic characters. The polymer was used as stationary phase of high performance liquid chromatography to separate lysozyme from chicken egg white. Besides, the mixture of Lys, papain and bovine serum albumin had been separated by the polymeric monolith with good resolution and reproducibility.

Key Words: Atom transfer radical polymerization, Methyl methacrylate, Cation-exchange, HPLC.

INTRODUCTION

Polymers being used in chromatographic domain, such as polymeric monoliths, are often synthesized by classical *in situ* radical polymerization. But it has non-uniform structure by the *in situ* radical polymerization because of the disadvantages of slowly initiation, fast increase, easily chain transfer and quickly chain termination.

In order to avoid the disadvantages, living/control polymerization technique has been used in the preparation of chromatographic polymeric monoliths¹⁻⁴. All potentially growing polymer chains grow at the same speed in living/control polymerization technique. As a result, polymers with similar chain length are formed⁵⁻⁷. So a more uniform structure will be obtained.

In the present work, the porous cation-exchange polymer has been synthesized by a living/control polymerization that is atom transfer radical polymerization⁸. Sodium bisulfate and carbon tetrachloride were used as co-initiators and ferrous chloride as catalyst. Besides, Sodium bisulfate was also used as inorganic moderator to control the concentration and activity of the free radical in the process.

EXPERIMENTAL

Lysozyme, papain and BSA were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ethylene glycol

dimethacrylate was produced by Anxin Chemical Reagents Co. (Liaoning, China). Methyl methacrylate was purchased from Shanghai Chemical Reagents Co. (Shanghai, China). All of these Chemicals were analytical reagent grade. Ultrapure water was prepared from a Millipore-Q water-purification system (Taiwan, China) and all of the solutions were filtered through a 0.45 μm membrane.

Preparation of the polymeric monolith: Methyl methacrylate (0.5 mL), ethylene glycol dimethacrylate (0.3 mL), CCl₄ (0.05 mL), FeCl₂ (0.003 g) methanol A (0.1 mL) and dodecyl alcohol (1.0 mL) were added into a dry ampule. Then 0.01 g NaHSO₃ (solved in 0.1 mL of water) was poured into the ampule. The ampule was sealed with a rubber septum and degassed. Then the reaction mixture was added into the stainless chromatographic column (50 mm × 4.6 mm i.d). The polymerization was allowed to proceed at 70 °C for 24 h. At last, methanol was pumped through the monolith to wash the porogenic solvents and other soluble compounds in HPLC system (1100 system from Agilent Technologies).

Characters of the polymeric monolith

The polymeric monolith was assayed by Fourier-transfer IR (FTIR-8400S, Shimadzu Co., Japan). Morphology of the polymeric monoliths were studied by scanning electron microscopy (SEM) (KYKY 1000B, Chinese Academy of Sciences Scientific Instrument Co. Ltd., China).

Fig. 1. Scanning electron microscopy of samples; a: SEM of M_a ; b: SEM of M_b ; c: SEM of M_c .

Separation of proteins by the polymeric monolith:

Lysozyme source was prepared as described previously⁹. The chromatographic separation was performed by using the following gradient: water for the first 3 min, 0.025 mol L⁻¹ of Na₂HPO₄ aqueous solution (pH = 10) for the next 3 min and 0.05 mol L⁻¹ of Na₂HPO₄ aqueous solution (pH = 10) for the last 3 min.

The chromatographic conditions for the separation of the three protein mixture (lysozyme, papain and BSA) were as follow: The gradient changed linearly from 100 % A to 100 % B in 10 min. (A was 0.05 mol L⁻¹ of NaH₂PO₄ and B was 0.05 mol L⁻¹ of Na₂HPO₄ aqueous solution.)

Determination of content of sulfonic groups on the polymeric monolith: In order to determine the content of sulfonic groups of the polymeric monolith, the following experiment has been carried out. The polymeric monolith was grinded and then about 0.1 g of it was weighed accurately and put into a beaker. 20 mL of water was added into the beaker and the beaker was put into an ultrasonicator to obtain a uniform mixture. Excess NaOH (0.1 mol L⁻¹ aqueous solution) was added quantitatively at 25 °C. Then the excess NaOH solution was titrated with HCl aqueous solution (0.1 mol L⁻¹).

Dihydroxyphthalophenone was used as the indicator. An average of three measures was the final content of sulfonic groups of the monolith.

Determination of binding capacity of the polymeric monolith for lysozyme: To determine the dynamic binding capacity of the polymeric monolith for lysozyme, frontal analysis of the column was carried out with 2 mg mL⁻¹ lysozyme in the mobile phase of 0.025 mol L⁻¹ Na₂HPO₄ aqueous solution (pH = 10). The binding capacity (Q) was calculated by eqn. (1).

$$Q = \frac{(V_{HB} - V_0)c}{m} \quad (1)$$

wherein, V_{HB} (mL) was the half breakthrough volume of lysozyme, V_0 (mL) was the dead volume of the column, c (mg mL⁻¹) was the concentration of lysozyme in the mobile phase and m (g) was the dry weight of the polymer monolith.

RESULTS AND DISCUSSION

Effect of monomer and cross-linking agent on the monolith: The effect of monomer and cross-linking agent was investigated. Compare to the previous work¹⁰, the more rate cross-linker led the smaller pore size. The optimized condition was that the volume ratio of methyl methacrylate and ethylene glycol dimethacrylate was 5:3 (v/v).

Effect of temperature on the polymerization: The preparation of polymeric monolith was thermally initiated and so temperature was a particularly effective factor. The effect of temperature could be explained in terms of the nucleation rates, at higher temperature, the polymerization reaction was very fast and more growing chains were transformed into individual globules rather than being captured by the primary nuclei. These globules were small and more small pores were produced and their surface was larger. The temperatures of 40, 50, 60, 70 and 80 °C were studied, respectively. The result showed that the polymerization could not be induced when temperature were 40 and 50 °C. The polymerization could be realized when the temperature were 60, 70 and 80 °C. But the polymer had bad mechanical function when the temperature was 60 °C and had high backpressure when the temperature was 80 °C. When the temperature was 70 °C, the polymer had good mechanical function and good permeability. So, 70 °C was chosen as the temperature of polymerization.

Effect of porogen on the polymerization: Dodecyl alcohol and isopropyl alcohol were investigated as porogens, respectively. The polymeric monolith prepared with isopropyl alcohol being used as the porogen had much higher back-pressure and worse permeability than that of dodecyl alcohol. The reason was that dodecyl alcohol was a poorer solvent than isopropyl alcohol. In the process of pore-forming, larger pores would be obtained with a poor solvent due to an earlier onset of phase separation.

SEM figures of the polymeric monoliths: The morphologies of the polymeric monoliths were investigated by SEM and the results were shown in Fig. 1. The figures showed that M_a had a more uniform structure than M_b . M_c had smaller pore size than that of M_a and M_b . The pore diameter of M_a was about 1 μm that was large enough for the separation of macromolecules.

Mechanical stability and permeability of M_a : To determine the mechanical stability and permeability of the monolith, the back-pressure drop at several flow rates were investigated. Fig. 2 showed the effect of the flow rate on the back pressure of M_a with water (b) and methanol (a) as the mobile phases, respectively. Good linear relations had been obtained ($r_a = 0.9991$, $r_b = 0.9990$). These results clearly indicated that the internal structures of the monoliths were not damaged and were suitable for high-throughput elution.

Pore size distribution: The pore size distribution of M_a was characterized by mercury intrusion porosimetry and the result was shown in Fig. 3. The result indicated that the general

Fig. 2. Back-pressures of the polymeric monolith at different flow rates; a: methanol was used as the mobile phase; b: water was used as the mobile phase

Fig. 3. Pore size distribution profiles for the monolith (Ma) by mercury intrusion porosimetry

pore volume, average pore diameter and internal porosity were 1.25 mL g^{-1} , $1.09 \mu\text{m}$ and 64.74% , respectively.

IR spectra of M_a : The IR spectra of monolith were shown in Fig. 4. The absorption at 2990 cm^{-1} and 2950 cm^{-1} C-H were due to the stretching in C-H of an aromatic group or alkenes. The clear adsorption peak at 1725 cm^{-1} was due to C=O; the peak at 1480 cm^{-1} showed the presence of the C-O-C group. The absorptions observed at 1250 cm^{-1} and 1150 cm^{-1} were caused by $-\text{HSO}_3$. This indicated that the cation exchanger ($-\text{HSO}_3$) had been connected onto the monolith successfully.

Content of sulfonic group of M_a : The content of sulfonic group was 0.29 mmol g^{-1} that was calculated by eqn. (2).

$$x = \frac{(V_0 - V_1)C}{1000W} \quad (2)$$

where, V_0 : the volume of NaOH solution (mL); V_1 : the volume of HCl solution (mL); C : the concentration of standard solution (mol L^{-1}); W : the quality of sample (g).

Fig. 4. FT-IR spectra of M_a

Chromatographic characters of the polymeric monoliths: Fig. 5 (a, b and c) showed the separations of lysozyme from chicken egg by M_a , M_b and M_c , respectively. There were three distinct peaks in (a) and the second one was due to lysozyme. There was only one peak in (b) that suggested M_b had no ability to separate lysozyme from chicken egg white. The reasons were that M_a and M_c had negatively charged sulfonic groups that were cation exchangers. When pH of mobile phase was lower than the pI of lysozyme (about 11), lysozyme was positive charged. Then there would be cation-exchange between lysozyme and polymeric monolith. So lysozyme would be remained by the polymeric monolith in the first 3 min when water was used as the mobile phase. When the pH of mobile phase was near to 11, there was seldom positive charge in lysozyme and then lysozyme was eluted from the polymeric monolith in the next 3 min when Na_2HPO_4 aqueous solution (0.025 mol L^{-1} , $\text{pH} = 10$) was used as the mobile phase.

Fig. 5. Separation of lysozyme from chicken egg white; a: Chromatogram by the M_a ; B: Chromatogram by the M_b ; c: Chromatogram by the M_c ; Chromatographic conditions: the prepared polymeric monoliths, $50 \text{ mm} \times 4.6 \text{ mm i.d.}$, respectively; injection volume: $3.0 \mu\text{L}$; flow rate: 1.0 mL min^{-1} ; the gradient: water for the first 3 min, 0.025 mol L^{-1} of Na_2HPO_4 aqueous solution ($\text{pH} = 10$) for the next 3 min and 0.05 mol L^{-1} of Na_2HPO_4 aqueous solution ($\text{pH} = 10$) for the last 3 min

There were three peaks in (c), but the efficiency and resolution of monolith were low. The results suggested that proper pore size and uniform structure were important chromatographic characters that affected the separation directly.

M_a revealed good stability in separating lysozyme from chicken egg white. Under the given conditions, the RSD of retention time and peak area was 0.20 % ($n = 11$) and 0.98 % ($n = 11$), respectively.

Dynamic binding capacity of M_a for lysozyme: According to the process being described in section 2.3.5, the binding capacity of M_a for lysozyme was 3.53 mg g⁻¹.

Conclusion

A novel porous cation-exchange polymeric monolith with uniform structure has been polymerized by atom transfer radical polymerization. Sodium bisulfate was first used both as co-initiator to provide sulfonic group and inorganic moderator to control the concentration and activity of the free radicals, respectively. The polymeric monolith had high permeability and low back pressure. Moreover, the monolith was used as HPLC stationary phase to separate the mixture of lysozyme, papain and BSA successfully. Moreover, lysozyme has been separated successfully from chicken egg white by the polymeric monolith. The results suggested that this kind of polymeric monolith could be used as an effective HPLC stationary phase to separate certain proteins.

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