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Preparation of a novel hybrid organic–inorganic monolith for the separation of lysozyme by high performance liquid chromatography

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ABSTRACT

A novel hybrid organic–inorganic monolith for high performance liquid chromatography (HPLC) was firstly developed by atom transfer radical polymerization (ATRP) by a simple and rapid method, in which vinyl ester resin was used as the monomer, natrium bisulfurosum was used both as organic adjunct and coadunate initiator to alter the activity of the free radical in the process of polymerization and then to control the molecular mass. The conditions of polymerization were optimized. The chemical group of the monolith was assayed by infrared spectra method, the morphology of monolithic material was studied by scanning electron microscopy (SEM) and the pore size distribution was determined by a mercury porosimeter. Finally, the monolith was used to separate lysozyme (Lys) from chicken egg white with good resolution and reproducibility that were obtained in a short time (10 min) by HPLC. In addition, the influences of buffer concentration and pH value on elution have been investigated and the hybrid monolith was used to separate benzene and its homologs from the mixture.

1. Introduction

In the past two decades, monolithic materials have been attracting attention as alternative stationary phases for high performance liquid chromatography (HPLC), due to their fast dynamic transport and time-saving process [1–4]. Basically, monolithic column are divided into two groups: rigid organic polymer-based monoliths and silica-based monoliths. The two kinds of monoliths now have been used in HPLC [5,6], solid phase extraction (SPE) [7–9] and capillary electrophoresis (CE) [10,11]. The drawback of the silica-based monolith is that they are subject to an insufficient hydrolytic of the Si–O–C linkage, especially under moderately acidic or slightly alkaline conditions. Nevertheless, the preparation of silica-based monolithic columns is not only time-consuming but also difficult to control the entire process, which leads to the problems in reproducibility. Though organic polymer monolith shows stability within the entire range of pH and exhibited excellent biocompatibility. They suffer from shrinking or swelling under the influence of temperature or organic solvents.

As an alternative, the hybrid organic–inorganic materials have been found to be highly advantageous as they exhibit flexibility, low density and long shelf-life with excellent biocompatibility and mechanical properties. The hybrid organic–inorganic monolith is often reported in silica-based materials in CE and solid phase extraction [12–15] and the sol–gel technique is often used for forming organic–inorganic materials in which alkoxysilane monomers are commonly used as inorganic network forming reagents [16–18].

Vinyl ester resin is the addition product of an epoxy resin and an unsaturated carboxylic acid. It based on the bisphenol A epoxy resin that exhibits easy handing properties. With mechanical and thermal properties, vinyl ester resin has good resistance to most chemical agents. This kind of resin has superior properties relative to unsaturated polyester systems [19]. Originally developed for their high corrosion resistance performance, vinyl ester resin has been used in a wide range of applications due to their inherent physical and mechanical properties [20,21]. Moreover, there are two double bonds in the molecule of vinyl ester resin that has been used both as monomer and cross linking agent in the process of polymerization in our previous work [22–24]. The polymer was prepared by in situ free radical polymerization. There is no ectopower in the in situ radical process, and the pores are induced by the phase separation. The characters of in situ radical polymerization process are slowly initiate, fast increase, easily chain transfer and quickly chain termination which lead an un-uniform structure with pores being created by accumulation of particles [25–27]. The un-uniform structure leads large eddy diffusion, low permeability and compressibility at high-pressure drops. Besides, the in situ polymeric monoliths need to be chemical modified when being used

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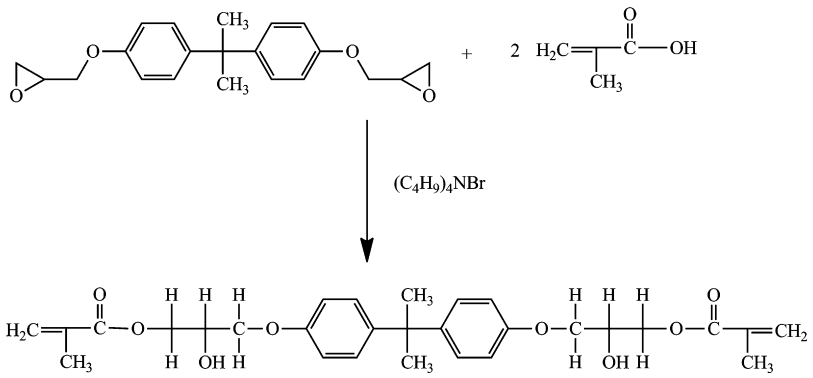

Fig. 1. The synthesis process of vinyl ester resin.

in separation as HPLC stationary phase. It is a time-consuming and tedious work.

Atom transfer radical polymerization (ATRP) is a widely studied controlled radical polymerization techniques with predictable molecular weight and narrow polydispersity [28,29]. The ATRP process uses an alkyl halide as initiator and a metal in its lower oxidation state with complexing ligands. The process involves the successive transfer of the halide from the dormant polymer chain to the ligated metal complex, thus establishing a dynamic equilibrium between active and dormant species. Nitrogenous compounds such as 1-hydroxybenzotriazole are often used as complexing ligands of metal in the process of ATRP.

In order to avoid the problems mentioned above, in this work natrium bisulfurosum and poly(vinyl ester resin) were used for the first time as inorganic and organic materials, respectively, to prepare a hybrid HPLC monolith by ATRP without complexing ligand being used. The results showed that the conjunction of natrium bisulfurosum, CCl_4, $FeCl_2$ and poly(vinyl ester resin) can lead a skeleton structure monolith. Moreover, the monolith being prepared in this way can be directly used as HPLC stationary phase in separating protein without being chemical modified.

2. Experimental

2.1. Chemicals

Lysozyme was obtained from Sigma Chemical Co. (St Louis, MO, USA). Vinyl ester resin was synthesized from bisphenol A diglycidyl ether (BADE). Dodecyl alcohol was obtained from China Medicament Co. Ltd. (Beijing, China). Carbon tetrachloride, ferrous chloride, natrium bisulfurosum were obtained from Yili Co. Ltd. (Beijing, China). All of these Chemicals were analytical reagent grade. Triplex distilled water was used for all experiments.

2.2. Preparation of the monolith

2.2.1. Synthesis of the vinyl ester resin

Vinyl ester resin was prepared according to the procedure described previously [30]. The process was that: 10 g of BADE, 0.2 g of tetrabutyl ammonium bromide and 10 mL of 1,4-dioxane were put into a three-necked flask which was heated in a hot up set. 4.3 mL of methacrylic acid was dropped in when the temperature was up to 80 °C. Then the temperature was heated up to 90 °C kept for 4.5 h. The vinyl ester resin was synthesized. The process was shown in Fig. 1. The chemical groups on the vinyl ester resin were assayed by infrared (IR) spectrometer, and the IR spectrogram was shown in Fig. 2a.

2.2.2. The process of the polymerization (Mf) [31]

The preparation of hybrid monolith (Mf) was as follows: 1.0 mL of vinyl ester resin, 0.05 mL of CCl_4, 1.0 mL of dodecyl alcohol, 0.1 mL of methanol and 0.05 g $FeCl_2$ were added to a dry ampule. Then, 0.05 g of $NaHSO_3$ was added to the ampule after being dissolved in 0.1 mL of water. The solution was dissolved to transparent and degassed with an ultrasonator. And then the solution was poured into a stainless-steel tube of a 30 mm × 4.6 mm i.d. chromatographic column, which was sealed at both ends with close column heads. The polymerization was allowed to proceed at 70 °C for 24 h. The monolith with the steel-tube was washed by methanol online for 12 h at a flow rate of 0.1 mL min^{-1} to remove all of dodecyl alcohol and other soluble compounds present in the polymer rod.

Chemical group of the monolith was detected by infrared spectra method and was shown in Fig. 2. Morphology of the monolithic materials was studied by scanning electron microscopy (SEM) and was shown in Fig. 3. Moreover, a mercury porosimeter (AutoPore II 9220 V3.04, Micromeritics Instrument Co., Atlanta, GA) was used to determine pore size distribution and the result was shown in Fig. 4.

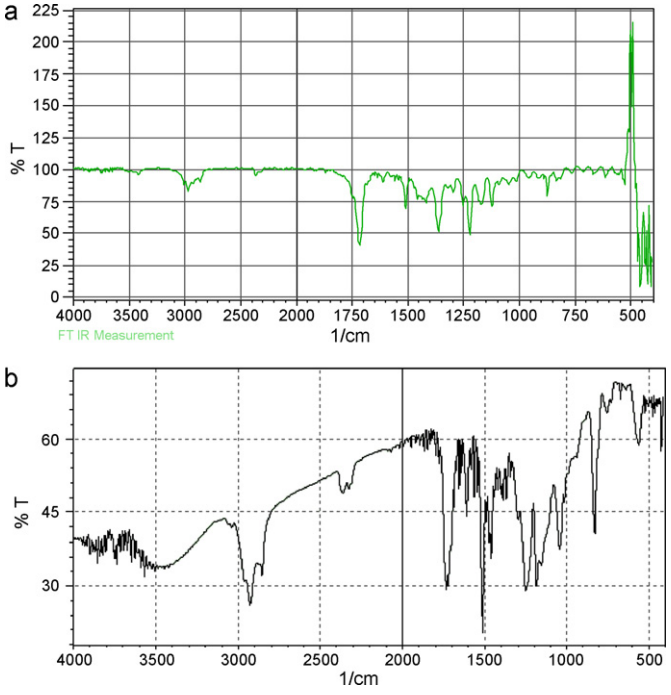

Fig. 2. The FT-IR spectrum of the hybrid monolith.

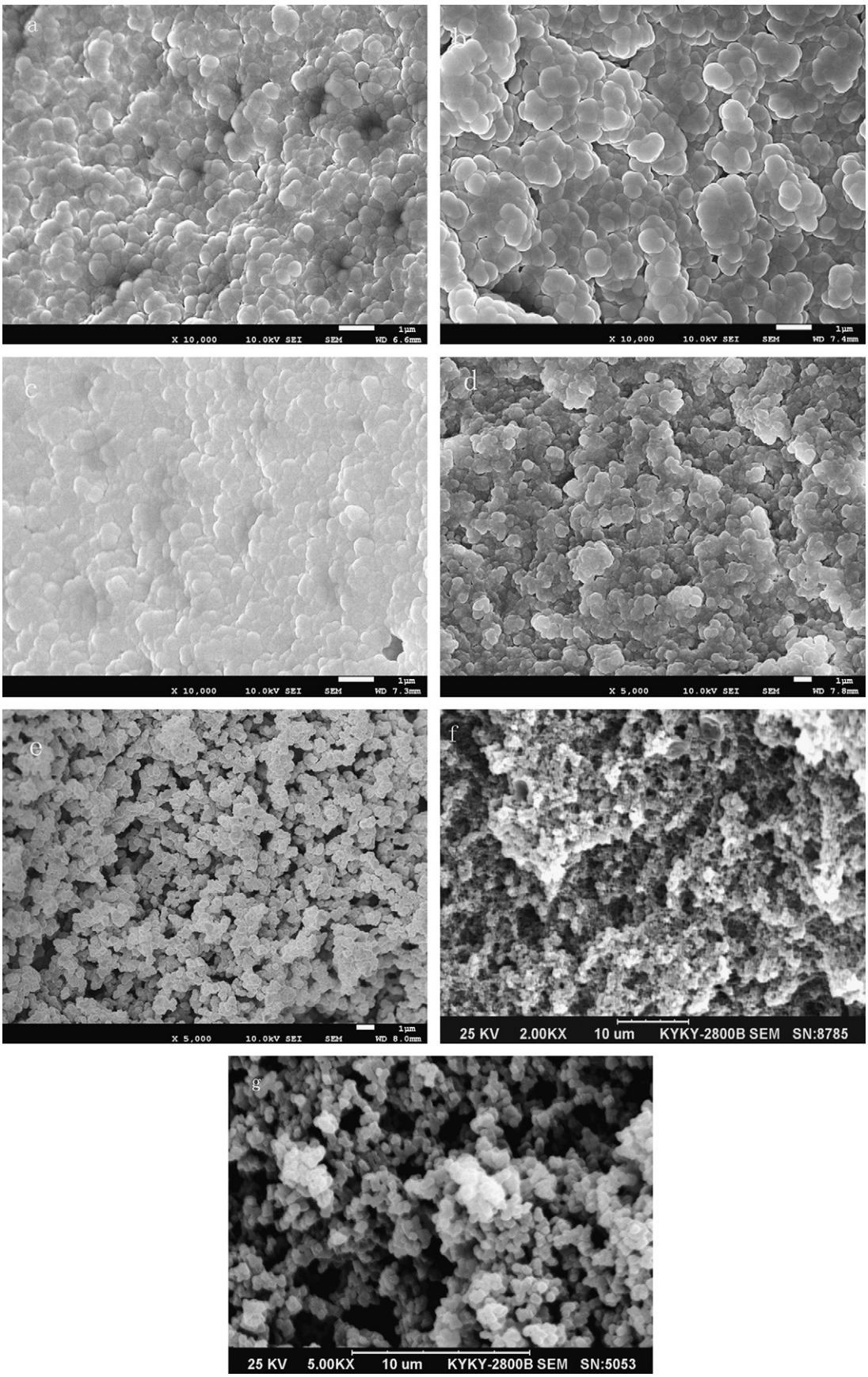

Fig. 3. Scanning electron microscopy of samples.

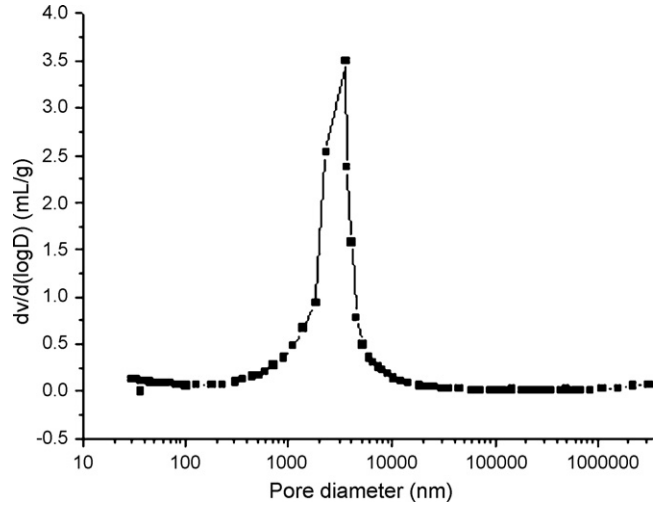

Fig. 4. Pore size distribution profiles for the monolith by mercury intrusion porosimetry.

2.3. Determination of the content of hydroxyl group on the polymeric monolith

The average loading of hydroxyl groups on the hybrid monolithic column was determined by titration of the excess of acetic acid after acetylation of the resin with acetic anhydride [32]. 0.15 g of monolith was placed in a tube, along with 1.0 mL of acetic anhydride and 5.0 mL of pyridina. Then the tube was put into a thermostat-controlled water-bath at 60 °C for 12 h.After that, 10 mL of water was added into the tube to make the excess acetic anhydride change to acetic acid. The solution was then titrated with 1.0 mol L^{-1} NaOH solution at the temperature of 25 °C. Dihydroxyphthalophenone was used as the indicator. Blank assay was operated at the same way. The loading of hydroxyl groups on the hybrid monolithic column was taken as the average of three parallel experiments.

2.4. Chromatographic characters of the monolith

2.4.1. HPLC instrument and conditions

A 1100 system from Agilent Technologies (Shanghai, China) was applied to chromatographic studies. The HPLC system consisted of a quaternary pump with an online vacuum degasser, an autosampler with variable injection capacity from 0.1 to 100 μL and UV detector. Chromatographic separation of Lys was achieved on the polymeric monolithic column (50 mm × 4.6 mm i.d.). All sample solutions injected in the chromatographic system were filtered through a millipore membrane (0.45 μm) to remove particles and large aggregates. Agilent liquid chromatography system chemical software was used and operated under Windows xp for data acquisition and integration. The UV detector was set at 280 nm and the temperature was 25 °C. The sample injection volume of the autosampler was 5.0 μL.

2.4.2. Selection of mobile phase

In order to investigate the effects of pH and concentration of mobile phase on the elution of Lys, a series buffer of different concentration and pH were investigated which were 0.002, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mol mL^{-1} and pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, respectively.

2.4.3. Separation of lysozyme

Chicken egg white was separated from fresh eggs and diluted to 50% (V/V) with phosphate buffer (50 mmol, pH 7.0). The diluted egg white was homogenized in an ice-bath and centrifuged at 4 °C and 12,000 rpm for 15 min. The supernatant fluid was used as a lysozyme source. The chromatographic separation was performed using a gradient which was: water for the first 3 min, 80% of water and 20% of 0.05 mol L^{-1} Na_2HPO_4 aqueous solution for the next 3 min, and 0.05 mol L^{-1} of Na_2HPO_4 aqueous solution for the last 3 min. The chromatography of separation was showed in Fig. 5.

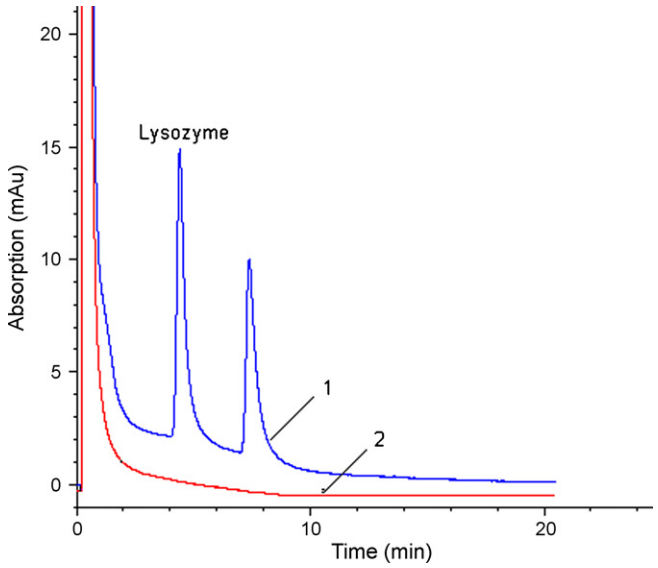

Fig. 5. Chromatogram of the separation of Lys from egg white. HPLC conditions: the prepared hybrid monolith, 50 mm × 4.6 mm i.d.; Chromatographic conditions: the gradient: water for the first 3 min, 86% water and 14% 0.05 mol L^{-1} Na_2HPO_4 aqueous solution for the next 3 min, and 0.05 mol L^{-1} Na_2HPO_4 aqueous solution for the last 3 min. 1: Chromatogram by the hybrid monolith Mf; 2: Chromatogram by the organic monolith Mg.

2.5. Determination of binding capacity of the hybrid monolith for Lys

To determine the dynamic binding capacity of hybrid monolithic column for Lys, frontal analysis of the column was carried out with 2 mg mL^{-1} Lys in the mobile phase of 0.05 mol L^{-1} Na_2HPO_4 aqueous solution (pH = 9.2). The binding capacity (Q) was caculated by the following equation:

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

where V_{HB} (mL) is the half breakthrough volume of Lys, V_0 (mL) the dead volume of the column, c (mg mL^{-1}) the concentration of Lys in the mobile phase and m (g) is the dry weight of the hybrid monolith.

3. Results and discussions

3.1. Characterizations of the hybrid skeleton structure monolith

3.1.1. IR spectrum of monolith

The vinyl ester resin and the prepared hybrid monolith were taken for Fourier-transfer IR characterization and the spectrums were shown in Fig. 2. Comparing to Fig. 2a, Fig. 2b showed that the absorption spectrum of the hybrid monolith displays readily identifiable peaks at 2852 cm^{-1} and 2930 cm^{-1} were characteristic of C–H symmetric and anti-symmetric stretching vibration. The strong absorption observed at 1510 cm^{-1} was caused by C–O–C. The absorptions observed at 1200 cm^{-1} and 1050 cm^{-1} were caused by $-SO_3$. The stretching band at 3500 cm^{-1} indicated the presence of O–H multimer.

Table 1
The conditions of polymerization.

No.	Vinyl ester resin (mL)	AIBN(g)	$NaHSO_3$ (g)	CCl_4 (mL)	Dodecyl alcohol (mL)	$FeCl_2$ (g)	Cetanol (g)	Methanol (mL)	Water (mL)
Ma	1.0	0	0	0.05	1.0	0.005	0	0	0
Mb	1.0	0	0.01	0	1.0	0.005	0	0	0.1
Mc	1.0	0	0.01	0.05	1.0	0	0	0	0.1
Md	1.0	0	0.05	0.05	1.0	0	0	0	0.1
Me	1.0	0	0.01	0	1.0	0	0	0	0.1
Mf	1.0	0	0.01	0.05	1.0	0.005	0	0	0.1
Mg	1.0	0.009	0	0	0	0	0.8	0.1	0

The polymerization was realized by ATRP. Vinyl ester resin was used as the monomer, CCl_4 was the initiator, $NaHSO_3$ was both as the coadunate initiator and adjunct, $FeCl_2$ was the catalytic agent, dodecyl alcohol was the porogen agent and methanol was the solvent. The results showed that the hybrid material had been polymerized.

3.1.2. SEM figures of monoliths

There are many factors including initiator, catalyst and $NaHSO_3$, influence the morphology of monoliths. These factors have been studied in preparation of monoliths. In the usual process of ATRP, $FeCl_2$ and CCl_4 (which are being used as catalyst and initiator, respectively) are indispensable, but we have found that when their amounts are changed there is no obvious change from SEM and IR spectrum. As we know, some inorganic salt can alter the activity of the free radical through controlling the concentration of it in the process of ATRP, and then to control the molecular mass to get a more relatively structure. So, $NaHSO_3$ is firstly used both as inorganic adjunct and coadunate initiator in conjunction with $FeCl_2$ and CCl_4 in the polymerization. In Table 1, the representative conditions were listed, and the corresponding morphologies were shown in Fig. 3. Fig. 3 showed that there was no porous in a, b, c, and d which indicated that there was no porous structure in the polymeric monolith without one of CCl_4, $NaHSO_3$ and $FeCl_2$ being added in the process. Fig. 3e (Table 1 Me) showed that $NaHSO_3$ could take the place of $FeCl_2$ and CCl_4 to lead the polymerization, and from which, a particle accumulation structure was obtained. Fig. 3f showed that the monolith Mf had a skeleton structure by the ratio of 1:0.4:5.4 ($NaHSO_3$:CCl_4:$FeCl_2$, mol ratio). All these results indicated that the conjunction of vinyl ester resin, $NaHSO_3$, CCl_4 and $FeCl_2$ could lead a skeleton structure in the process of ATRP. So, Mf in Table 1 was selected as the optimized condition.

3.1.3. The pore size distribution

The pore diameter distribution of Mf in Table 1 was characterized by mercury intrusion porosimetry which was shown in Fig. 4. From the figure it was obtained that the general pore volume, average pore diameter and interval porosity were 1.489 mL g^{-1}, 0.89 μm and 69.76%, respectively.

3.2. Mechanism of skeleton structure monolith

The monolith was polymerized by normal in situ free radical polymerization in our lab previously [25]. The initiator was 2,2-azobisisobutyronitrile (AIBN), and without $FeCl_2$, $NaHSO_3$ and CCl_4 being added. Fig. 3g was its morphology. The SEM showed that the pores were obtained by the aggregated particles obviously. $FeCl_2$, CCl_4 and $NaHSO_3$ instead of AIBN were added into the polymeric process to polymerize by ATRP in the present work which led a skeleton structure polymeric monolith. The results suggested that the mechanism of ATRP seemed to play an important role in forming skeleton structure. In the process, $NaHSO_3$ was partly used as adjunct to adjust the activity of free radical by combine with it. Besides, $NaHSO_3$ in conjunction with CCl_4 was used as allied initiator in the process. The results which have been claimed in Section 3.1.2 showed that the skeleton structure is due to the conjunction of addition of $FeCl_2$, CCl_4 and $NaHSO_3$.

3.3. Separate Lys from egg white

Fig. 5 (1) showed the chromatogram, and in which three distinct peaks could be observed. Lys was separated from egg white in a short time with high resolution, and the second peak was due to Lys. Fig. 5 also showed that two different results were obtained by the two different monoliths. In Fig. 5, chromatogram 1 was obtained by the monolith Mf, and chromatogram 2 was obtained by the monolith Mg. In addition, the chromatograms obtained by Ma, Mb, Mc, Md are the same to Fig. 5 (2). The two different chromatograms showed that the hybrid monolith Mf can be directly used in separating Lys from egg white, but the others cannot. The reasons are as follow: in this experiment, the hybrid monolith Mf has proper pore size to separate Lys; moreover, there are many negatively charged sulfonic groups in the hybrid monolith Mf, and the action of cation-exchange between the hybrid monolith and Lys lead the present result. But there is no proper pore (such as Ma, Mb, Mc, Md) or sulfonic group (such as Fig. 3g) on the organic monolith, and so they cannot be directly used in separating Lys from egg white.

Furthermore, the column revealed a good stability in the experiments. A hybrid monolith has been used under the given mobile phase to separate Lys from egg white and benzene and its homologs from the mixture, respectively. The RSD of retention time is 0.15% (n = 11) and 0.12% (n = 11), respectively. The pressure drop and column efficiency did not change with the accumulation of the number of injections.

3.4. Effect of concentration and pH of mobile phase on the elution of Lys

The concentration and pH value of the mobile phase were investigated in Section 2.4.2. The results were shown in Figs. 6 and 7, respectively. Fig. 6 showed that there was little effect on the elution of Lys when the concentration of Na_2HPO_4 aqueous solution was changed from 0.002 mol L^{-1} to 0.5 mol L^{-1}. But when water was used as the mobile phase, Lys was strong retained to the monolith. So, a much lower concentration which was 0.05 mol L^{-1} Na_2HPO_4 aqueous solution was selected as the elution buffer. A gradient which was: water for the first 3 min, 0.05 mol L^{-1} Na_2HPO_4 aqueous solution for the next 3 min, and 0.1 mol L^{-1} Na_2HPO_4 aqueous solution for the last 3 min.

Fig. 7 showed the effect of pH value on the elution of Lys. When pH value was lower than 9, Lys was strongly retained on the monolith. When pH value was 9 or higher than 9, Lys was eluted quickly. This is because that there are negatively charged sulfonic groups on the monolith surface. In addition, when pH value of mobile phase is higher than 3, the hydroxyl group on the monolith will be hydrolyzed, which lead negatively charged stationary phase, too. Under the actions of the two kind of negatively charged groups, when the pH value is lower than 9, it is much lower than the isoelectric point of Lys (11.0), and the Lys is positively charged. So, there will be ion exchange between the monolith and Lys, and the

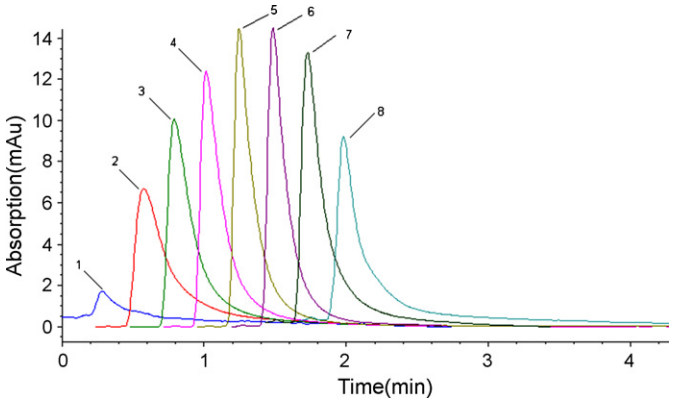

Fig. 6. Effect of buffer salt concentration on the elution of lysozyme with the monolith being used as the HPLC stationary phase HPLC conditions: the prepared hybrid monolith, 50 mm × 4.6 mm i.d.; sample: Lys, 0.1 mg mL^{-1}; volume: 5.0 μL. Mobile phases: 1. water; 2. 0.002 mol mL^{-1} of Na_2HPO_4 aqueous solution; 3. 0.01 mol mL^{-1} of Na_2HPO_4 aqueous solution; 4. 0.02 mol mL^{-1} of Na_2HPO_4 aqueous solution; 5. 0.05 mol mL^{-1} of Na_2HPO_4 aqueous solution; 6. 0.1 mol mL^{-1} of Na_2HPO_4 aqueous solution; 7. 0.2 mol mL^{-1} of Na_2HPO_4 aqueous solution; 8. 0.5 mol mL^{-1} of Na_2HPO_4 aqueous solution.

Lys will not be eluted. When pH value is 9 or higher than 9, it is nearly to the isoelectric point of Lys, and there is little charge in the Lys. So, there is seldom exchange between Lys and monolith which lead to no retention. But if pH of the mobile phase was 10 or more, it will be too high to fit for the HPLC system and the monolith. So, 0.05 mol L^{-1} Na_2HPO_4 (pH = 9.2) was selected as the elution mobile phase.

3.5. Separation of benzene and its homologs from the mixture

Moreover, the monolith was also used to separate benzene and its homolog from the mixture with the mobile phase methanol/water (70/30, v/v). The chromatogram was shown in Fig. 8. The three peaks were benzene, biphenyl and alcohol phenyl, respectively in order. According to the order of the peaks, it could be explained by the theory of hydrophobic interaction chromatography successfully.

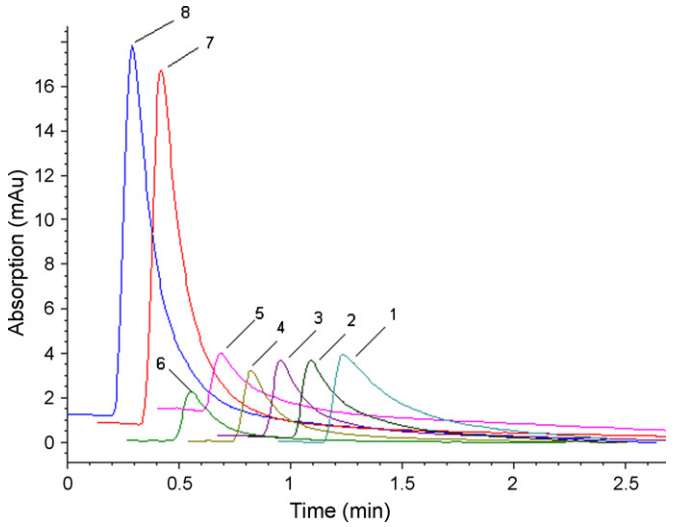

Fig. 7. The pH effect on the elution of lysozyme HPLC conditions: the prepared hybrid monolith, 50 mm × 4.6 mm i.d.; sample: Lys, 0.1 mg mL^{-1}; volume: 5.0 μL. Mobile phase: buffer phosphate with the pH (with orthophosphoric acid or caustic soda aqueous solution to adjust pH) as follows: 1. pH = 3.0; 2. pH = 4.0; 3. pH = 5.0; 4. pH = 6.0; 5. pH = 7.0; 6. pH = 8.0; 7. pH = 9.0; 8. pH = 10.0.

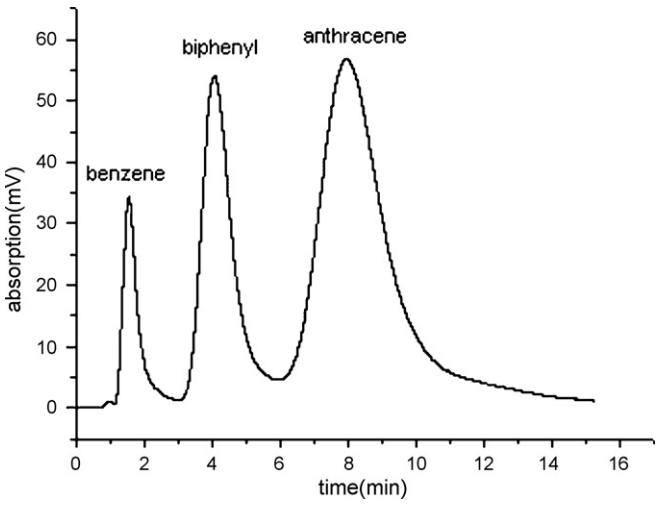

Fig. 8. Chromatogram of benzene and its homolog. HPLC conditions: the prepared hybrid monolith, 50 mm × 4.6 mm i.d.; samples: benzene, biphenyl and alcohol phenyl, 0.1 mg mL^{-1}, respectively; volume: 5.0 μL. Mobile phase: methanol/water (70/30, v/v). The peaks are benzene, biphenyl and alcohol phenyl, respectively.

3.6. Content of hydroxyl group of the monolith

It has been obtained that the content of hydroxyl group was 17.8% according to the titration in Section 2.3. The result was calculated by the following formula:

$$X\% = \frac{(V_0 - V_1)CM}{1000W} \times 100\%$$

V_0: the volume of NaOH that was consumed in the blank assay (mL)
V_1: the volume of NaOH that was consumed in the titration of nominal sample (mL)
C: concentration of standard solution (mol L^{-1})
M: the molar mass of OH
W: the quality of sample (g)

3.7. Dynamic binding capacity of the hybrid monolith for Lys

According to the process being described in Section 2.5, the binding capacity of the hybrid monolith for Lys is 1.10 mg g^{-1}.

4. Conclusion

A novel hybrid organic–inorganic monolithic column which was polymerized by ATRP has been prepared. Natrium bisulfurosum was firstly used both as added substance and initiator in the polymeric monolithic column. A skeleton structure with low back pressure was obtained in this work. The results showed that the monolith can be used as HPLC stationary phase successfully without those time-consuming and numerous chemical modifications. Moreover, high resolution was obtained in a short time in the separation of Lys from chicken egg white. The results suggested that such kind of monolithic column could be used as a simple, cheap, effective solid phase to HPLC.

Acknowledgements

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References

[1] S. Hjertén, J.L. Liao, R. Zhang, J. Chromatogr. 473 (1989) 273.
[2] F. Svec, E.C. Peters, D. Sykora, J. Chromatogr. A 3 (2000) 887.
[3] F. Svec, J. Sep. Sci. 2 (2004) 1419.
[4] B.G. Belinkii, Russ. J. Bioorg. Chem. 32 (2006) 323.
[5] G.S. Chirica, V.T. Remcho, J. Chromatogr. A 924 (2001) 223.
[6] A.M. Siouffi, J. Chromatogr. A 1000 (2003) 801.
[7] F. Svec, J. Chromatogr. B 841 (2006) 52.
[8] L. Xu, H.K. Lee, J. Chromatogr. A 1216 (2009) 5483.
[9] T. Nema, E.C.Y. Chan, P.C. Ho, Talanta 82 (2010) 488.
[10] X.C. Wang, X.C. Lin, Z.H. Xie, J. Chromatogr. A 1216 (2009) 4611.
[11] E.F. Hilder, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 1044 (2004) 3.
[12] M.M. Zheng, G.D. Ruan, Y.Q. Feng, J. Chromatogr. A 1216 (2009) 7739.
[13] M.M. Zheng, B. Lin, Y.Q. Feng, J. Chromatogr. A 1164 (2007) 48.
[14] L.J. Yan, Q.H. Zhang, J. Zhang, J. Chromatogr. A 1046 (2004) 255.
[15] M.L. Chen, M.M. Zheng, Y.Q. Feng, J. Chromatogr. A 1217 (2010) 3547.
[16] L.T. Arenas, T.A.S. Aguirre, A. Langaro, Polymers 44 (2003) 5521.
[17] G. Schottne, Chem. Mater. 13 (2001) 3422.
[18] C.K. Chan, S.L. Peng, I. Chu, Polymers 42 (2001) 4186.
[19] M. Malik, V. Choudhary, I.K. Varma, Rev. Macromol. Chem. Phys. 40 (2000) 139.
[20] B.A. Milleville, W. Bladergroen, New organic peroxide catalyst for curing vinyl ester resins, in: 45th Annual Conference, Composites Institute, Society of the Plastic Industry, Session 17A, February 12–15, 1990.
[21] J.M. Sands, B.K. Fink, S.H. McKnight, C.H. Newton, J.W. Gillespie, G.R. Palmese, Clean Prod. Process. 2 (2001) 228.
[22] G.L. Yang, H.Y. Liu, L.G. Bai, Micropor. Mesopor. Mater. 112 (2008) 351.
[23] G.L. Yang, H.Y. Liu, Y.H. Zhang, J. Chromatogr. A 1129 (2006) 231.
[24] L.G. Bai, H.Y. Liu, G.L. Yang, Chin. J. Chem. 26 (2008) 2063.
[25] K. Branovic, D. Forcic, J. Ivancicetal, J. Chromatogr. B 801 (2004) 331.
[26] Y.L. Huo, P.J. Schoenmakers, W.T. Kok, J. Chromatogr. A 1175 (2007) 81.
[27] M. Bedair, Z.E. Rassi, J. Chromatogr. A 1079 (2005) 236.
[28] M. Kato, M. Kamigaito, M. Higashimura, Macromolecules 28 (1995) 1721.
[29] V. Percec, B. Barbooiu, Macromolecules 28 (1995) 7970.
[30] J.G. Gao, H.L. Du, H.N. Sun, Chin. J. Catal. 10 (1989) 422.
[31] R.Y. Zhang, L. Qi, P.Y. Xin, Polymers 51 (2010) 1703.
[32] S. Xie, F. Svec, J.M.J. Fréchet, J. Polym. Sci. Polym. Chem. 35 (1997) 597.