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Preparation and evaluation of a large-volume radial flow monolithic column

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Abstract

In this study, a 38 mL monolith with homogeneous porous structure was produced by a single polymerization from glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) in the presence of porogens and an initiator. The uniform temperature distribution within the reaction system was achieved by adding reactant mixture continuously and enhancing the heat transfer ability of the polymerization system. Homogeneous porous structure in the monolith was proved by SEM and the pore size distribution profiles measured by mercury intrusion porosimetry. Experimental results from proteins separation indicated that the dynamic capacity and resolution of radial flow monolithic column were independent of flow rates. Furthermore, the pressure drop on the column was linearly dependent on the flow rate and did not exceed 1.7 MPa even at a flow rate of 50 mL/min, which proved that the prepared monolith could be used in the quick separation and preparation of biopolymers.

Keywords: Monolithic column; GMA–EDMA; Temperature controlling; Porous structure; Radial flow

1. Introduction

Monolithic supports represent one of the newest developments of chromatographic stationary phases for biomolecular separation [1]. Their internal structure with highly interconnected pores consists of macropores (pore diameters > 50 nm), mesopores (pore diameters between 50 and 2 nm) and micropores (pore diameters < 2 nm). The macropores can ensure excellent convective mass transfer between the mobile and the stationary phases, which increase the mobility of the molecules to be separated by several orders of magnitude compared to traditional bead counterparts [2,3]. While mesopores and micropores can afford enough active sites for grafting function groups that endow the monolithic support with some chromatographic separation capabilities. The high resolution and capacity, which are independent of flow rate, can be achieved in monoliths, and even under the high flow rate, the pressure drop is moderate [4–11]. Due to such characteristics, they are suitable for the quick separation and preparation of biopolymers, such as proteins [5–8], polypeptides [8,9] and oligonucleotides [5,10]. Consequently, they are considered as the fourth generation of sorbent materials for protein separations [11].

Glycidyl methacrylate-based monoliths were introduced in the 1990s [12,13], which were polymerized from GMA and EDMA in the presence of porogens and initiators. Despite many papers have described their applications in the analytical scale, only a very few in the scale-up separation of biopolymers [14,15]. The main problem lies in that the accurate control of the polymerization temperature for large monoliths need to be solved. Generally, “unstirred” mode

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is applied in the preparation of monoliths [12,16–19], i.e., the monolith is moulded in a cartridge directly. The preparation process is very simple, while the polymerization is highly exothermic, so the occurrence of substantial temperature gradients in large moulds is inevitable, and the volume of prepared monoliths with homogenous porous structure is limited [20–22]. Temperature is one of key variables in controlling the porous structure. Thus, adjusting the polymerization temperature allows the tuning of average pore size within a broad range spanning two orders of magnitude [17]. If in situ unstirred mode is applied in the polymerization of large-volume monoliths, several mixing effects caused by stirring will not appear in the system, but temperature gradient will be produced by the large amount of heat evolved in the polymerization, which would distort the uniformity of the porous structure of monoliths. Peters et al. [16] proposed that during the preparation of large-volume monoliths, continuous and slow addition of the reactant mixture could control the polymerization rates so as to decrease the temperature gradient in the system. The method can only minimize the extent of the reaction exotherm, but it does not improve the heat transfer in the reaction system resulting in the limited volume of prepared monoliths. Podgornik et al. [20] prepared several annular monoliths with different outer diameters by use of "tube in a tube" technique, and then embed one into another. In such a way, the reactant mixture in a single preparation can be reduced, and consequently the temperature distribution can be effectively controlled. Nevertheless, it is complicated to prepare large volume monoliths, which may cause the damage of monoliths and the occurrence of large nesting dead volume.

To ensure the desired performance of monoliths, it is crucial to remove the heat so that the temperature distributes uniformty in the preparation of monoliths. In this paper, according to the characteristics of annular monolithic structure, we designed a thermostated annular reactor to enhance the heat transfer ability between the polymerization system and the circumference, and adopted an approach for continuous and slow addition of the mixture to reduce the heat evolved in unit time. By the method, a 38 mL porous polymer monolith with homogenous pore size distribution was obtained. In addition, a special housing was designed to provide a uniform flow distribution in the radial direction over the entire monolith bed.

2. Experimental

2.1. Instruments and reagents

2.1.1. Instruments

Annular reactor and radial flow column were designed and produced by our laboratory. A 501-type thermostat (Shanghai, China), KYKY-1000B scanning electron microscope (SEM, Scientific Instrument Factory Chinese Academy of Sciences, Beijing, China), 9310-type mercury intrusion porosimeter (MIC, USA), K-type thermocouple (Shenyang, China), AL-708 temperature-controlling apparatus (Xiamen Yudian Automation Engineering Co. Ltd., China) and As3120 Ultrasonic cleaners (Automatic Science Instrument Co. Ltd.) were purchased from the respective companies. P200 II pump, HPLC system comprising semi-preparative pumps P260 allowing flow rates up to 50 mL/min, UV200 II detector and HPLC hardware/software (data workstation), were produced by Dalian Elite Analytical Instruments Co. Ltd.

2.1.2. Reagents

GMA, EDMA, benzoyl peroxide (BPO), cyclohexanol, 1-dodecanol, diethylamine, were purchased from Acros Organics (NJ, USA). Bovine serum albumin (BSA), goat serum and Tris were bought from Sino-American Biotechnology company, Beijing, China, other reagents used in this study were of analytical grade. The water used in the experiments was deionized.

2.2. Preparation and modification of monoliths

BPO (1 wt.% with respect to monomers) was dissolved in four parts of a mixture consisting of 60% GMA and 40% EDMA. The porogenic solvents (six parts in total) consisting of 90% cyclohexanol and 10% 1-dodecanol were admixed slowly to the monomers. The polymerization mixture was purged with nitrogen for 30 min in order to remove oxygen, and enable the initiator to dissolve completely and monomers to mix homogenously. Fig. 1 shows the flow chart of polymerization. In the presence of nitrogen, the polymerization mixture was slowly pumped into the thermostated annular reactor at a flow rate of 0.32 mL/min through a stainless tube using a P200 II pump. The diameters of the outer and inner annuluses were 50 and 8 mm, respectively. After addition, the reactor was sealed at both ends, and then the polymerization was proceeded for 24 h at 55 °C.

The temperature profile was measured by placing three T-type thermocouples directly into the polymerization mixture as shown in Fig. 1. For the mold, two thermocouples

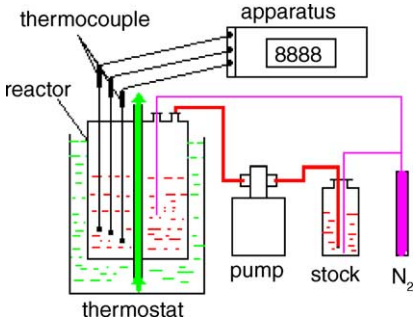

Fig. 1. Flow chart for the preparation of annual monolithic stationary phases.

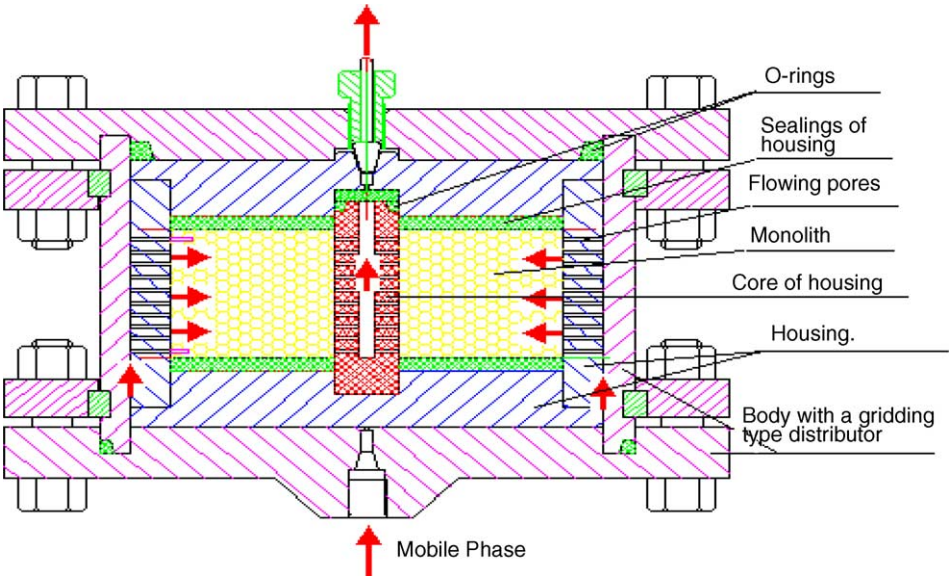

Fig. 2. Schematic presentation of the housing and column body for an annual monolith.

were placed at distances 9 and 19 mm from the center of the reactor ("center" and "outer" positions, respectively), and the third one was halfway between the two ("middle" position). All the thermocouples were put vertically and could be drawn in axial direction. AL-708 temperature-controlling apparatus was used to measure the different temporal and spatial temperature distribution by drawing the thermocouples.

After polymerization, the monolith was removed from the reactor, and the height of the monolith was adapted to 20 mm. The entire monolith was then placed in a radial column cartridge (as shown in Fig. 2). Consequently, the column was provided with fittings, attached to the HPLC system. Ethanol was pumped through the column at a flow rate depending on the flow resistance of the monolith to remove the porogens and other residual soluble compounds.

The column was washed with 10 column volumes of deionized water. To introduce weak anion-exchange diethylaminoethyl (DEAE) groups on the monolith, 50 mL of pure diethylamine was pumped and recycled through the unit at a flow rate of 5 mL/min for about 3 h using P200 II pump. In order to obtain the corresponding polymer with l-(*N*,*N*-diethylamino)-2-hydroxypropyl groups, the column was then removed from the chromatographic system, sealed at both ends with plugs and heated in a thermostated bath at 70 °C for 8 h. The reaction scheme is presented in Fig. 3. The modified column was then reattached to the HPLC system and washed with 10 column volumes of deionized water to remove the residual diethylamine.

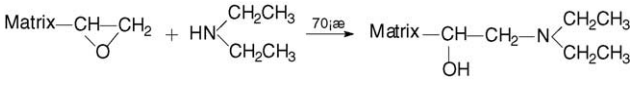

Fig. 3. Reaction of epoxide groups with diethylamine.

2.3. Dynamic capacity measurement

The frontal analysis was employed to determine dynamic capacity of the monolith with BSA as the model protein. At first, BSA was dissolved in a binding buffer (buffer A, 20 mM Tris–HCl, pH 7.4) at a concentration of 2.5 mg/mL. Then, the feed solution was pumped into the column at a defined flow rate, and the absorbance of the effluent was measured at 280 nm. When the signal of the detector was stable, the pump was switched off. After that, the column was regenerated by buffer B (2 M NaCl in buffer A). The capacity of the monolithic column was calculated on 50% of the final absorbance of the breakthrough curve.

2.4. Determination of porosity and SEM of the monoliths

After all of the chromatographic experiments were completed, the matrix was removed from the stainless steel housing. The monolith was divided into inner, middle and outer portions which located at positions 9, 14 and 19 mm from the center of monolith, respectively. The samples were further cut into smaller pieces, and vacuum-dried at 50 °C for 12 h. To compare the uniformity of internal porous structure of the monoliths, the pore size distribution of samples from different portions was measured by porosimeter, and microstructure was determined by SEM.

3. Results and discussion

3.1. Effect of polymerization temperature distribution on porous structure of monoliths

As mentioned above, the temperature distribution is important in controlling the porous structure of monoliths. Gen-

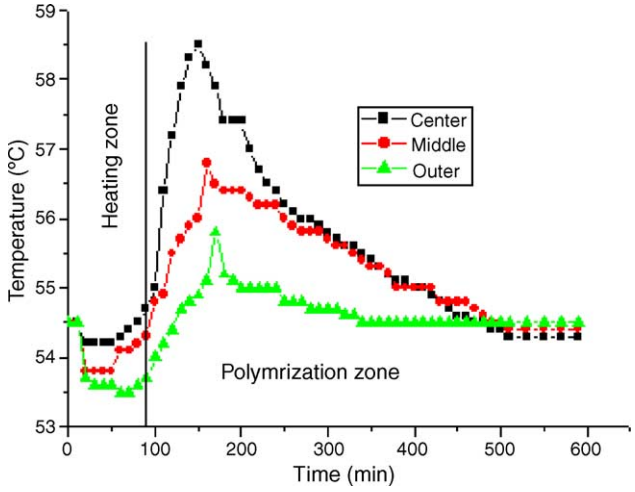

Fig. 4. Temperature profile curves obtained at 9 mm (center), 14 mm (middle) and 19 mm (outer) from the center of the reactor during the polymerization of GMA–EDMA at 55 °C. For conditions see Section 2.

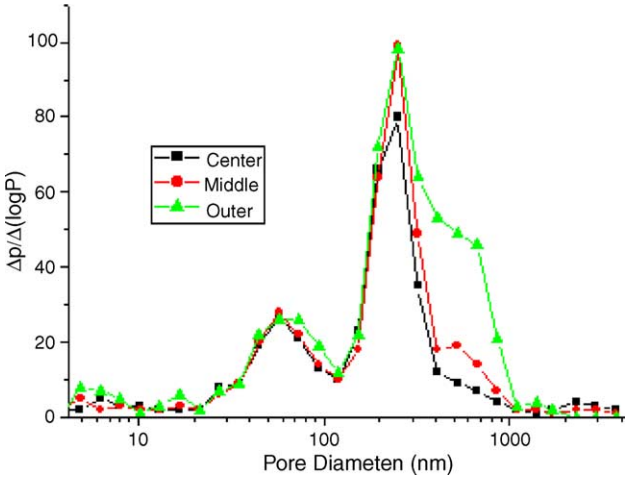

Fig. 5. Differential pore size distribution curves at different portions of the poly(GMA–EDMA) monolith. For conditions see Section 2.

erally, the higher the temperature, the smaller the pores, and vice versa [16,17]. During the reaction, the occurrence of temperature gradient was inevitable for the application of unstirred mode and poor heat transfer ability in the system. Thus, inhomogenous porous structure may occur in the preparation of large-scale monoliths, which might affect the chromatographic performance. To obtain uniform temperature distribution, continuous and slow addition of the mixture was employed in place of the batch reaction to reduce the heat evolved. Meanwhile, the reactor was immerged in the thermostated bath, and hot water flew through the inner channel of the reactor (as shown in Fig. 1) to remove the heat in the center of the reactor and enhance the heat exchange of the system. Thus, the temperature distribution becomes uniform (as shown in Fig. 4).

The reactant mixture at room temperature was continuously added into the reactor at 55 °C. The temperature of the reactant mixture first decreases, and then rises as presented in the heated zone of Fig. 4. As the temperature rises in the system, BPO decomposes and the resulting radicals initiate polymerization in solution. Meanwhile, the heat evolved further triggers the decomposition of BPO, so that the temperature of the system rises to a maximum rapidly as shown in the frontal zone of Fig. 4. At this moment, temperature is the critical factor for controlling polymerization rates. As the reaction goes on, the concentration of monomers decreases, and the reaction rate slows down simultaneously. Hence, the system temperature lowers gradually to get an equilibrium. Now, the concentration becomes the critical factor of controlling reaction rates at this stage. From the three temperature distribution profiles shown in Fig. 4, it appears that the temperature at the center is higher than those at the outer, and the maximum recorded temperature is 58.5 °C, only 3.5 °C higher than the bath temperature (55 °C). The radial temperature gradient is only 3 °C. So it may be noted that the temperature-controlling approach goes well, and the polymerization proceeds under uniform temperature. According to the analysis above, it seems that the polymerization could be divided into temperature-controlling zone (located before the maximum) and concentration-controlling zone (after the maximum). In the temperature-controlling zone, the initiator decomposes more rapidly than before as the rise of temperature, which destroys the uniformity of the temperature distribution. Therefore, it is beneficial to prepare large-scale monoliths at lower temperatures. Obviously, the so-called lower temperatures must be higher than the decomposition temperature of initiators. By contrast, in the concentration-controlling zone, the concentration of the mixture imposes a major impact on the reaction, while the temperature is a minor factor. As summarized above, in the preparation of large-scale monoliths, the gradual temperature rise is favorable to the uniformity of temperature distribution, which results in the preparation of monoliths with homogenous porous structure.

Fig. 5 shows differential porosity distribution curves at different portions of the poly(GMA–EDMA) monolith prepared at 55 °C. We can see that the portions of curves overlap each other before 250 nm of Fig. 5. That is, the temperature of the system is uniform in most time of the reaction, and only slight differences exist around the maximum point. Such results also testify the uniformity of temperature distribution in the measured system. While the distribution in the large pores zone (pore size bigger than 250 nm) is different slightly. The proportion of large pores in the center is smaller than that of the outer part, which conforms to the temperature distribution of polymerization.

The SEM with magnification of 7500 at center, middle and outer portions of the monolith is presented in Fig. 6. It can be seen that the micro morphology of different portions is similar. The pores in the matrix are highly interconnected, forming a porous network of channels. These prove that the experimental methods are efficient to control the homogeneity of porous structure in the prepared large-scale monoliths again.

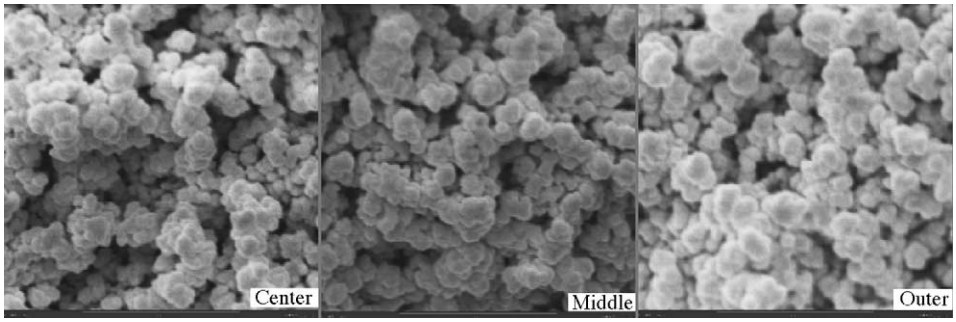

Fig. 6. SEM of monolith produced by copolymerization of GMA and EDMA. For conditions see Section 2.

3.2. Correlation of flow rate and back pressure

Since the constructed monolithic columns are mainly used for semipreparative or, in some cases, also for preparative purification and separations, high flow rates are expected to be used. To examine the suitability of the 38 mL unit for high flow rates, the pressure drop was measured. The results are presented in Fig. 7. It can be seen that even at the flow rate of 50 mL/min the pressure drop is below 2.0 MPa. The relation is linear, demonstrating no deviation from the Hagen–Poiseuille equation occurred even at the highest flow rate applied. The possibility of running a monolithic column at high flow rates means that separation and purification can be completed in a very short time.

3.3. Relation between the dynamic capacity and flow rates

As stationary phases, the dynamic capacity of monoliths is one of the most critical affecting factors for chromatographic analysis. It is important to study the effects of flow rate on dynamic capacity for the rapid separation of biopolymers. Fig. 8 presents four breakthrough curves at different flow rates. From the overlapping of the normalized breakthrough curves, it can be concluded that the dynamic capacity of the prepared monoliths is independent of flow rates. The total column capacity reached 1.6 g of BSA, corresponding to 42 mg/mL of the support, much higher than that of CIM (BIA Separation, 30 mg/mL) and same as that of UNO-Q (42 mg/mL) [11]. Moreover, the backpressure was quite low at the examined flow rates, and even when the flow reached 40 mL/min, the pressure was lower than 2 MPa. Conditions: flow rate: 10, 20, 30 and 40 mL/min; sample: 2.5 mg/mL BSA in a 20 mM Tris–HCl buffer, pH 7.4; detection: UV at 280 nm.

3.4. Correlation between resolution and flow rates

To examine the relation between resolution and flow rate, a gradient separation of goat serum at five flow rates were employed. As can be seen from Fig. 9, with volume of the eluent as abscissa, all chromatograms at different flow rates overlap each other, which proves the independence of flow rates. When the flow rate reaches 40 mL/min, the linear velocities on the inner and outer surface of the monoliths will be 480 and 76.2 cm/h, respectively. That is, the linear velocity of inner surface is 6.3 times higher than that of the outer. The

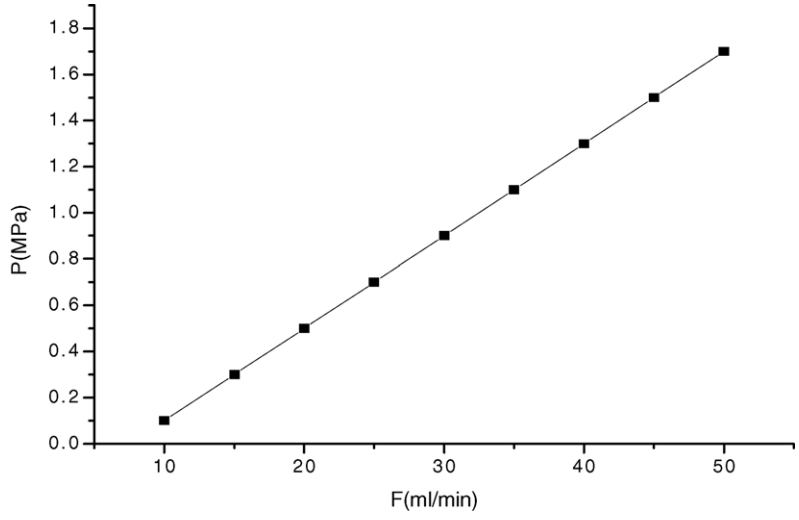

Fig. 7. Effect of flow velocity on back pressure in the monolith of GMA–EDMA. For conditions see Section 2.

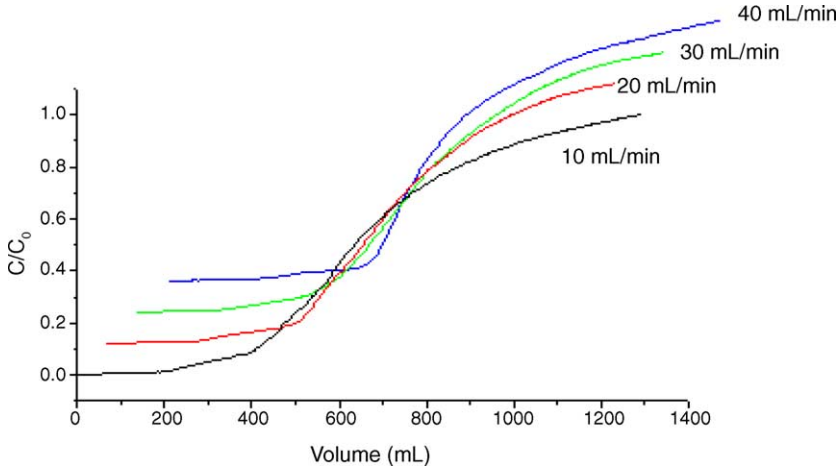

Fig. 8. Effect of the flow rate on the dynamic binding capacity.

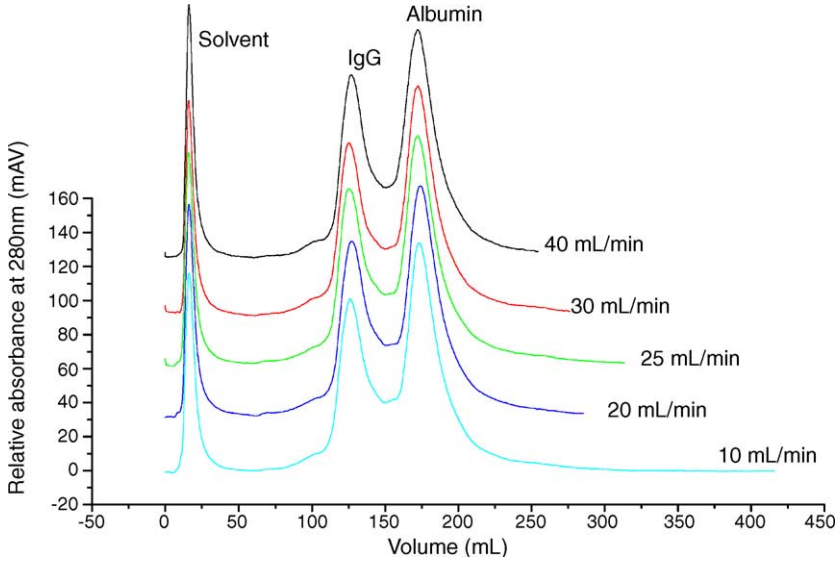

Fig. 9. Effect of the flow rate on the separation efficiency.

efficiency of chromatographic separation remains the same even in such high flow rates. It means that the monolithic columns are superior to packed columns in the separation of biopolymers, and the radial chromatography can be directly linearly enlarged.

Separation of goat serum at five flow rates (10, 20, 25, 30 and 40 mL/min) normalized to the elution volume. Conditions: mobile phase (buffer A) 20 mM Tris–HCl buffer, pH 7.4; (buffer B) 20 mM Tris–HCl + 2 M NaCl, pH 7.4; gradient, 0–30% buffer B in 200 mL; detection: UV at 280 nm.

4. Conclusions

In the experiments, the uniformity of polymerization temperature distribution and homogenous porous structure of prepared monoliths were achieved by the methods designed for large-scale monoliths preparation, such as, continuous addition of reactant mixture and improved heat exchange. The experiments have proved that the dynamic capacity and resolution are independent of flow rate, while the backpressure is moderate even at higher flow rates. So it is especially suitable for the separation and preparation of bio-samples. As the rapid development of bio-science, the preparation, separation and purification of bio-products have been the key roles in realizing the industrialization of high-techs. Nowadays, the throughputs of samples, and the time consumed for separation and purification have attracted more and more attentions. The unity of high throughputs, high resolution and short consuming time is the goal of researchers. So we can predict that the modified large-volume monoliths will play important roles in many fields, such as industrial catalysis, solid phase extraction, chromatographic separation and cir-

culate reactors. The applications of monoliths in these fields will not only greatly reduce the operating pressure of industrial reactions, preparation and separation, but also increase the throughputs in unit time and shorten the periods of producing new products.

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