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RESEARCH PAPER

Effect of Porogen Solubility Parameter on Structure of Chromatographic Supports with Large Pores

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Abstract: The macroporous microspheres were prepared through suspension polymerization based on a copolymer of glycidyl methacrylate and ethylene glycol dimethacrylate. The effect of porogen on the microspheres structure was evaluated in terms of pore size and surface area. Porogen contained dichloromethane (δ = 9.7 (cal cm^{-3})$^{1/2}$) and *N*-octanol (δ = 10.3 (cal cm^{-3})$^{1/2}$) which were correspond to a good and poor solvent, respectively. The solubility parameter of porogen was controlled in the range of 9.89–10.09 (cal cm^{-3})$^{1/2}$. The pore size of microspheres increased with the increase of difference value of solubility parameter between the polymer and the porogen. On the contrary, the surface area of microspheres decreased. The anion exchange media was prepared through coupling poly(ethylene imine) in the microspheres and the proteins transport was determined by frontal analysis method. The macroporous microspheres with 257 nm pore size could still afford a high proteins capacity (45.1 mg mL^{-1}). These macroporous supports showed great potential in rapid separation of proteins.

Key Words: Porogen; Solubility parameter; Macroporous microspheres; Protein; Separation

1 Introduction

The high through-put separation of biomolecular has been a hot topic with the development of pharmaceuticals. Chromatography, as a main technology, plays an important role in separation and purification of proteins, in which the chromatographic support is the key of the technique. The supports based on the agarose are widely used for separation of proteins due to its good biocompatibility and chemical stability. However, this kind of matrix has poor mechanical strength, resulting in enduring the low operation pressure. The small pores of this support, which are in the range from 3 to 50 nm[1], bring a low binding capacity and throughput of proteins, especially for large biomolecules or particles, such as virus and virus-like particles. The macroporous supports based on the polymers show some advantages in terms of high mechanical strength and large pore size (more than 100 nm). This kind of supports can tear more than ten megapascals and afford a rapid transport of large molecules such as proteins[2].

The agarose-based macroporous polymer supports are mainly prepared by suspension radical polymerization. In this method, it is not easily to fabricate the large through-put pore in the polymer microspheres. Hahn *et al*[3] reported a perfusion support that could allow protein transfer in convective way. POROS is the typical representatives of the perfusion chromatography that is composed of two kinds of pores, including throughpores (500–800 nm) and diffusion pores (20–100 nm). The pore structure was not easy to accurately control due to its complicated preparation process. The fabrication of pores needs two steps, production of nanoparticles and formation of aggregation[4]. Sun's group[5,6]

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prepared gigaporous microsphere based on polymethacrylate, through compound porogen containing calcium carbonate particles and organic solvents. In this method, the throughpores were not well-distributed in the microspheres because of the poor compatibility between inorganic particles and organic solvents. Zhou *et al*[7,8] used reverse micelles swelling method to prepare a gigaporous supports. Design of porogen was proved to be an efficiency method for fabrication of the throughpores in the microspheres. These large pores in supports could improve the transport of proteins. Meanwhile, it was found that the supports with the large pores afforded low surface area. As a result, the binding capacity of proteins was low on these supports[9, 10]. It was concerned by many researchers that how to increase the capacity of proteins on the macroporous media. Our group ever used a method of atom transfer radical polymerization to prepare the polymethacrylate microspheres with the throughpores that were in the range from 200 to 300 nm[11,12]. In addition, the microspheres could contain higher surface area than ones from another ordinary polymerization method. Meanwhile, it was found that porogen had drastic influence on structure of the microspheres. But the detailed influences were not explored in previous studies.

In present study, the effect of porogen on the microspheres structure was evaluated in terms of pore size, surface area, morphology, and binding capacity. The solubility parameter of porogen affecting the microspheres structure was also investigated.

2 Experimental

2.1 Instrumentation and materials

Glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EDMA), poly(vinylalcohol) (PVA, M_w = 1700 ± 50, 87% alcoholysis degree), and poly(ethylene imine) (PEI, M_w = 600) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Azobisisobutyronitrile (AIBN), dichloromethane (DCM), *n*-octanol (OA), and sodium lauryl sulfate (SDS) were produced by Shanghai Chemical Plant (Shanghai, China). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the above chemicals were used as received.

Pore diameter and surface are of the microspheres, in their dry state, were measured on an AutoPore IV 9500 mercury intrusion porosimetry (Micromeritics, USA) and specific surface area measuring instrument (V-Sorb 2800, Nanjing Gold APP Instruments, China). The morphologies of these microspheres were observed by scanning electron microscope (SEM) (FEI Quanta400F, FEI Lim. Co., Japan). The dynamic binding capacity of proteins was measured by ÄKTA Purifier 10 (GE, USA).

2.2 Methods

2.2.1 Preparation of macroporous microspheres by suspension polymerization

In a typical suspension polymerization, as shown in Fig.1, AIBN (0.040 g), GMA (1.0 mL), EDMA (1.0 mL), DCM (1.5 mL) and OA (1.5 mL) were added to an ampule. Then the reaction mixture was added into 50 mL of aqueous solution that contained 0.5 g of PVA and 0.05 g of SDS under stirring at 200 rpm. The reactor was heated to 60 ºC and the polymerization proceeded at 60 ºC for 8 h. The reaction solution was filtered and the resulted microspheres were then soxhlet extracted by acetone for 24 h. Finally, the microspheres were vacuum dried at 50 ºC for 24 h. After that, the dried microspheres were stored at room temperature.

2.2.2 Modification of PGMA-EDMA microspheres with PEI and determination of ion exchange capacity

To measure protein capacity of PGMA-EDMA microspheres, PEI was used to modify the supports to obtain anion exchange media. This derivatization was carried out according to previous study[13]. The mixture containing PGMA-EDMA (5.0 g), dimethyl sulfoxide (50 mL) and PEI (5.0 g) was heated to 60 ºC for 24 h. After the reaction finished, the microspheres (PGMA-EDMA-PEI) were filtered and washed with water. The ion exchange capacity of these microspheres was measured according to the previous method[14]. The detailed procedure was as the following steps: (1) 30 mL of NaOH (1.0 M) was passed through the column filled with the supports; (2) the column was cleaned with water to remove –OH adsorbing on the surface; (3) 50 mL of HCl (0.10 M) was passed through the column and collected in a flask; (4) 30 mL of NaCl (1.0 M) was passed through the column and collected in the same flask as described in step 3; (5) all of the collection was titrated by NaOH solution. The ion exchange capacity (Q) was calculated as follows:

$$Q = \frac{C_1V_1 - C_2V_2}{V_0} \quad (1)$$

where, C_1 and C_2 are the concentration of HCl and NaOH, V_0, V_1 and V_3 are the volume of supports, HCl and NaOH, solutions respectively.

2.2.3 Determination of binding capacity of proteins by frontal analysis

Frontal breakthrough curves were measured for feeds

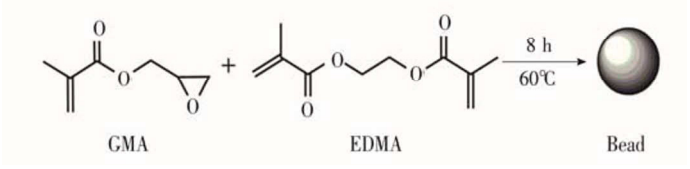

Fig.1 Scheme for preparation of PGMA-EDMA microspheres