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RESEARCH PAPER

Preparation of Mixed-mode Chromatography Supports Based on Gigaporous Polymer Microspheres

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Abstract: The mixed-mode chromatography supports were prepared, including anion exchange and hydrophobic interaction chromatography. The matrix was based on the gigaporous polymer microspheres from copolymer of glycidyl methacrylate and ethylene glycol dimethacrylate. The microspheres were modified by poly(ethylene imine) and butyl glycidyl ether for mix-mode chromatography. The effects of ion capacity and hydrophobic ligand density on protein capacity and recovery were evaluated. The results indicated that the protein capacity and recovery increased with ion capacity of the supports in the range of 0.2–0.5 M. The max capacity was 40 mg mL^{-1} and the protein recovery was more than 90%. The hydrophobic interaction chromatography occurred when hydrophobic ligand density was more than 0.03 M. The backpressure on these supports was less than 2 MPa at flow rate > 2000 cm h^{-1}. In addition, the high resolution could be retained even at high flow rate of 2880 cm h^{-1} for purification of Ig G from human serum. Therefore, this support showed a large potential in high through-put separation.

Key Words: Mixed-mode chromatography; Separation of protein; Gigaporous microspheres; High through-put

1 Introduction

High throughput separation and analytical methods become a hot topic with development of pharmaceuticals. Chromatography is a main method for separation of biomolecules, in which chromatographic supports is the key technology. The supports based on agarose were widely applied in separation due to their good biocompatibility and chemical stability, however, this support was not easy for realizing high throughput separation of biomolecules because of its nature of soft matrix and small pore size (3–50 nm)[1]. Polymer supports with large pores could realize rapid and high throughput separation due to their high mechanical strength (pressure resistance ≥ 10 MPa) and large pore (> 100 nm)[2].

Poros™ from PerSeptive Biosystem was one of typical polymer media with large pores[3], which was based on a copolymer of styrene and divinyl benzene (PSt-DVB). This media should be hydrophilically modified to decrease nonspecific adsorption of proteins on the support[4]. The modification process was complex and highly costly. Professor Sun's group ever prepared polyacrylate microsphere with gigapores using calcium carbonate and organic solvent as porogen, in which calcium carbonate could form large pores (more than 100 nm). However, these large pores did not uniformly distributed in the microspheres due to the poor compatibility between inorganic salt and organic system[5,6]. Zhou *et al*[7] develope the gigaporous microspheres of which the matrix was a copolymer of glycidyl methacrylate and divinylbenzene[2]. In our previous work, an anion exchange media through hydrophilic modification of these microspheres were prepared[7,8]. The dynamic capacity at high flow rate (8 mL min^{-1}) only decreased by 15% compared to that at low

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flow rate (1 mL min^{-1}). This result indicated that the media had good mass transfer for proteins. Recently, the microspheres based on a copolymer of glycidyl methacrylate and ethylene glycol dimethacrylate (PGMA-EDMA) were prepared through atom transfer radical polymerization (ATRP) in our group[9]. Compared with the matrix of PSt-DVB, the hydrophobicity of PGMA-EDMA decreased while the good mass transfer for proteins could be maintained. Otherwise, the epoxy groups in the microspheres could be further derived for the chromatographic media with various chromatography modes.

Mixed-mode chromatography incorporates multiple interaction modes between the stationary phase and the solutes in a feed stream. Compared to the single mode chromatography, it shows good selectivity for target solutes and high capacity. In some cases, one mixed-mode chromatography (MMC) column could afford higher efficiency than several corresponding columns[10–13]. Therefore, MMC could increase the separation efficiency through reducing the separation steps, especially in separation of complex samples, such as extraction of biomolecule fermentation broth[14], peptide, and extraction of medicine[15], etc.

In this study, a mixed-mode chromatography media, based on the matrix of PGMA-EDMA, was prepared through modification of poly(ethylene amine) and butyl glycidyl ether. This media incorporated anion exchange and hydrophobic interaction modes and could be applied to high throughput separation of proteins.

2 Experimental

2.1 Apparatus and materials

Separation of proteins was carried out on protein purifier (AKTA, Purifier 10, GE, USA) installing a Tricorn^{TM} 10/300 column (300 mm × 10 mm). Ultraviolet-visible light detector (L9, Shanghai INESA Scientific Instrument Co., Ltd) was used for measuring the concentration of proteins. The morphology of microspheres was observed by scanning electron microscopy (PEI Quanta400F, FEI, USA). The polymer microspheres (PGMA-EDMA) were prepared by our laboratory. Poly(ethylene amine) (M_w = 600, AR), butyl glycidyl ether (AR) and bovine serum albumin (BSA, > 98%) were purchased from Sigma-Aldrich (USA).Ultrapure water used for chromatography was made in our laboratory.

2.2 Experimental method

2.2.1 Preparation of MMC media

The MMC supports were prepared by two steps, and the process was shown in Fig.1. The first step was derivatization

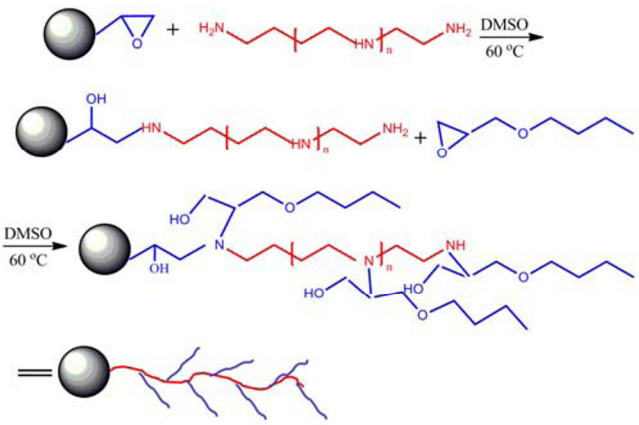

Fig.1 Schematic diagram of MMC supports preparation

of microspheres into anion exchange resin. PGMA-EDMA (5.0 g), poly(ethylene amine) (PEI, 15.0 g) were added into a flask which contained dimethyl sulfoxide (DMSO, 50 g), and then the flask was shaken in shaking table at 60 °C for 24 h. After that, the microspheres were filtered and washed with water to remove DMSO. The amines were linked on the microspheres surfaces to be used as ion exchange groups, and the ion capacity was detected by titration method. The second step was coupling hydrophobic ligands to the microspheres with amines. Butyl glycidyl ether (5.0 g) and DMSO (50 g) were added into the flask to react with the microspheres at 60 °C for 24 h. Then the microspheres were filtered and washed with DMSO and H_2O, respectively, also the ion capacity was determined again.

The ion capacity of MMC was determined according to the following process. 10 mL of MMC microspheres were packed into the column. Then these media were converted into the state containing OH^-. Then the microspheres were washed with water until the rinse water was neutral, and followed by successively addition of HCl solution (30 mL) and 1.0 M NaCl solution (30 mL), which were collected and titrated by 0.1 M NaOH solution with phenolphthalein as an indicator. The ion capacity (q) was calculated as follows:

$$q = (C_0V_0 - C_1V_1)/V_2 \quad (1)$$

where, C_0 and V_0 are concentration and volume of HCl, respectively; C_1 and V_1 are concentration and volume of NaOH, respectively; V_2 is the volume of MMC media in the column.

2.2.2 Estimation of static protein capacity

MMC supports (5.0 mL) and 5.0 mg mL^{-1} bovine serum albumin (BSA) in 50 mM pH 8.0 Tris-HCl solution were mixed in a 250-mL triangular flask, and shaken at room temperature for 24 h. The concentration of BSA was determined by measuring the absorbance at 280 nm and then calculating the concentration using a calibration curve. The protein capacity (Q) was calculated by the formula as follows:

$$Q = (C_0 - C_1)/V_2 \quad (2)$$