Journal of Chromatography A, 1343 (2014) 109–118

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Hydrophilic modification gigaporous resins with poly(ethylenimine) for high-throughput proteins ion-exchange chromatography

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ARTICLE INFO

Article history:
Received 25 December 2013
Received in revised form 11 March 2014
Accepted 22 March 2014
Available online 1 April 2014

Keywords:
Hydrophilicity
Gigaporous microspheres
Anion-exchange medium
Branched polyethyleneimine
High-throughput

ABSTRACT

High hydrophilicity of gigaporous microspheres based on a copolymer of poly(glycidyl methacrylate)-*co*-divinyl benzene (PGMA-DVB) was successfully realized through coating the branched polyethyleneimine (PEI) in PGMA-DVB microspheres. PEI with various molecules weights and different branching agents were identified in terms of protein recovery as evaluation approach. For this evaluation, PEI_{600} (M_w = 600) and poly (ethylene glycol) diglycidyl ether (PEGDE, M_w = 400) were used as modification agent and branching agent, respectively. The modified microspheres showed good permeability and revealed a certain mechanical strength. After modification, the protein recovery increased from 40% to >90%. The protein recovery increased with the branched generations and the first and second generations could give the protein recovery of 93% and 96%, respectively. Meanwhile, the ionic capacity also showed a rising trend in the range of 0.11–0.32 mmol/mL with the branched generations. But the dynamic binding capacity of protein (bovine serum albumin, BSA as the model protein) increased at first and then decreased. Analysis of the dry microspheres structure by mercury intrusion method as well as observation of the branched PEI on PGMA-DVB membrane in aqueous solution indicated that excess PEI chains with the extended state in the second generation would block the small pores and decrease the accessible surface area. Therefore, the protein capacity on the second generation, on the contrary, was lower than that on the first generation. Meanwhile, it was found that the PEI chains in the modified microspheres changed their construction from the extended to the collapsed state with increase of NaCl concentration. And the corresponding pore size of the modified microspheres increased with salt concentration through low-field nuclear magnetic resonance. Dynamic binding capacity of proteins on the modified supports did not significantly change with increase of the flow rate. The media showed good performance for separation three model proteins at high flow rate of 1084 cm/h. This modified gigaporous microspheres had a large potential in application for rapid separation of biomolecules.

1. Introduction

High capacity at high flow rate has being in pursuit of the goal for high throughput chromatographic separation of proteins. Generally, the chromatographic media used for separation of proteins are microspheres based on polysaccharide, such as agarose (Sepharose^{GE}) and dextran (Sephadex). These media with small pores (5–30 nm) [1] were not favor to the mass transfer of proteins, especially macromolecular size proteins. Therefore, the adsorption equilibrium and mass transport of proteins cost more time in order to obtain high protein capacity in the media with small pores [2]. This means that low flow rate must be used in purification resulting in prolonging contact time between proteins and chromatographic support. Thus, the denaturalization or loss of biological activation would easily come out. Ideally, the chromatography media should provide a high selectivity and efficiency even if it is operated at high flow rate [3], which is usually obtained on the monolith [4,5] and membrane chromatography [6]. Therefore, there has being a growing interest in alternative support, such as the rigid gigaporous microspheres [7].

Our research group has developed an easy and novel method to prepare gigaporous poly(styrene-divinyl benzene) (PS-DVB) [8] and polyglycidyl methacrylate-divinyl benzene (PGMA-DVB) [9] microspheres with 300–500 nm pore size, which can be used as a perfusion chromatographic support [10–15]. These media enable better access of macromolecules to the inner of the particle through

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http://dx.doi.org/10.1016/j.chroma.2014.03.064
0021-9673/

the combination of convective and diffusive flow [16]. As a consequence, the time required for a chromatographic separation is reduced, and meanwhile, the loading capacity is also independent of the superficial velocity.

However, these gigaporous resins containing styrene or divinylbenzene showed relative hydrophobicity as a result of their specific nature, which would lead to proteins non-specific adsorption and denaturalization [17]. Therefore, surface hydrophilicity modification would be very necessary for these resins to separate proteins. Several methods had been previously reported for hydrophicity of gigaporous microspheres, including physically coating of the modified agarose [18], covalently binding polyvinyl alcohol (PVA) [19] and dextran [20]. In these mentioned approaches, all of the hydrophilic macromolecules, such as agarose, PVA, and dextran, used for modification was neutral and with hydroxyl groups. Therefore, the modified microspheres must be derived furtherly through reaction of hydroxyl groups when they were used for a variety of chromatography model including ion-exchanged [21], hydrophobic interaction chromatography [22], and affinity chromatography [23,24], etc. Usually, these derivations need more modification procedures and increased the final economic cost.

A kind of hydrophilic molecule, polyethyleneimine (PEI) with abundant amino groups, has been widely used in modification of supports with micro-grad pores, such as monolith, membrane, and gelation, for chromatography [25–29]. Tan' groups used PEI in coating the monolithic column based on a copolymer of glycidyl methacrylate and ethylene dimethacrylate (PGMA-EDMA) and finally obtained anion-exchanged monolith [30]. Liu et al. modified membrane using PEI and obtained ion-exchanged membrane [31]. To the best of our knowledge, there is little report in hydrophilicity of the polymer microspheres with PEI for chromatographic media. It seemed to be that PEI as macromolecules was not easy to diffuse into the inner of the usual microspheres due to their small pore size of less than 50 nm. However, the gigaporous microspheres used in this study had the through-pore with 150–200 nm that could permit big size molecules freely moving in the microspheres. This item had been proved by our previous modification using hydrophilic macromolecules [18,20]. In this method, PEI was expected to not only cover the hydrophobic sites [32] in the microspheres, but also provide ion-exchanged sites for capture of proteins.

In this study, PEI was used for modification of gigaporous microspheres based on a copolymer of glycidyl methacrylate (GMA) and divinyl benzene (DVB) for the first time. Meanwhile, important parameters, including different molecule weight of PEI, various branching agents and the generation of branch, were investigated in terms of their influence on proteins recovery and dynamic binding capacity of proteins. Otherwise, the construction of PEI chains in the microspheres was observed by atom force microscope, and it was found that PEI chains changed from the extended state to collapsed state with the increasing of salt concentration. This should make it possible to rapidly capture the proteins and mass transfer.

2. Experimental

2.1. Materials and equipments

PGMA-DVB gigaporous microspheres were prepared by our group according to our previous report [9]. PEI with different molecular weight (300, 600 and 1800) was obtained from XIYA company (Shandong, China). Diepoxyl ether with different molecular weights, including diethylene glycol diglycidyl ether (DGDE, M_w = 218), poly(ethylene glycol) diglycidyl ether (PEGDE, M_w = 400) and poly (ethylene glycol) diglycidyl ether (PEGDE, M_w = 600), were purchased from XIYA company (Shandong, China). Bovine serum albumin (BSA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Other reagents used in experiments were of analytical grade and bought from Beijing Chemical Factory (Beijing, China).

2.2. Modification of PGMA-DVB microsphere with PEI

Modification of PGMA-DVB microspheres with PEI was shown in Fig. 1. The scheme began with attaching PEI with required molecules to PGMA-DVB microspheres in dimethyl sulfoxide (DMSO) for 24 h at 60 °C, followed by washing with 100 mL DMSO. In the next step, the branching agent with two epoxy groups was added in excess amount and reacted with amines of the PEI for 24 h at 60 °C, subsequently, the same procedure was repeated for two cycles and the branched PEI was obtained on PGMA-DVB microspheres. PEI with different molecular weights (M_w = 300, 600, 1800) and different branching agents, including diethylene glycol diglycidyl ether (M_w = 218), poly(ethylene glycol) diglycidyl ether (M_w = 400), and poly (ethylene glycol) diglycidyl ether (M_w = 600), were used in each of these modification reactions. In the following descriptions, the modified PGMA-DVB obtained by different steps will be named by PGMA-DVB-PEI$_m$ n, (i.e. PGMA-DVB-PEI$_{600}$ 1 represents PGMA-DVB modified by PEI with molecular weight of 600 after the first step; when PGMA-DVB-PEI$_{600}$ 1 subsequently reacts with the branching agents, the resulting microspheres will be called as PGMA-DVB-PEI$_{600}$ 2; in the same way, the modified microspheres in the next steps are named by PGMA-DVB-PEI$_{600}$ 3 (It was defined as the first branched generation), PGMA-DVB-PEI$_{600}$ 4, and PGMA-DVB-PEI$_{600}$ 5 (It was defined as the second branched generation), etc. The detailed descriptions were shown in Table 1.

2.3. FT-IR spectroscopy of PGMA-DVB and PGMA-DVB-PEI

The final microspheres were dried under vacuum at 60 °C until constant in weight prior to be characterized by JASCO FT/IR-400/600 spectrometer (JASCO, Inc., USA). The dried microspheres (0.5%) were mixed with possium bromide (KBr) powder and grinded to homogeneous fine powder. Then the mixture was added into the mold for preparation of pellet. The pellet was inserted into the IR sample holder and the fine powder spectrum was obtained. In the same method, the control experiment was performed with PGMA-DVB microspheres.

2.4. Measurement of microspheres pore diameter and morphology in dry state

Pore diameter of PGMA-DVB before and after modification with PEI, in their dry state, were conducted by an AutoPore IV 9500 mercury porosimetry (Micromeritics, USA). The specific surface area and porosity of the samples was measured by N_2 adsorption at 70 °C on a Micrometritics ASAP2020 analyzer (Micromeritics, USA) and calculated by the Brunauer–Emmett–Teller (BET) method. The differences between PGMA-DVB microspheres and PGMA-DVB-PEI were studied. Experiments were performed in accordance with the protocol given in the AutoPore IV 9500 operator's manual. The morphologies of the microsphere before and after modification were observed by scanning electron microscope (SEM) (JSM-6400, JEOL Lim. Co., Japan). The detailed method of samples preparations for SEM observation was described as following. Microspheres were re-suspended in distilled water. Then, the dispersion was dropped on a piece of aluminum foil and dried at an ambient atmosphere. The sample was placed on a metal stub with double-sided conductive adhesive tape, and was coated with a thin gold film under reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan).