Biochemical Engineering Journal 37 (2007) 332–337

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Short communication

# Fabrication of superporous cellulose beads with grafted anion-exchange polymer chains for protein chromatography

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Received 10 April 2007; received in revised form 29 May 2007; accepted 4 June 2007

**Abstract**

Glycidyl methacrylate was grafted onto the pore surface in superporous cellulose beads using ceric ammonium nitrate as initiator. By modifying the grafted and ungrafted matrices with diethylaminoethyl (DEAE) groups, anion exchangers were produced. Under a proper grafting reaction condition, the adsorption capacity of bovine serum albumin (BSA) to the DEAE-G-SC was over two times increased as compared to the ungrafted DEAE-SC and no nonspecific adsorption of BSA was observed. Moreover, the intraparticle diffusive mass transfer was not affected by this grafted polymer chains. However, the flow hydrodynamic experiments revealed that the grafted polymer chains led to the decrease of flowthrough pores. So the convective mass transport in the particles was significantly reduced, leading to the decrease of column efficiency with flow velocity. Despite this unfavorable effect, however, due to the high adsorption capacity of the grafted beads, the DEAE-G-SC column could still be operated at a DBC of 20–35% higher than the normal DEAE-SC column in the range of 150–600 cm/h.


*Keywords:* Grafting polymerization; Superporous cellulose bead; Anion exchanger; Protein adsorption; High capacity; Chromatography

## 1. Introduction

Perfusion matrices represent a class of chromatographic materials introduced as an improvement to traditional matrices [1]. The perfusion packing materials are characterized by presenting two sets of pores: the diffusion pores of 80–150 nm and the flowthrough pores of 600–800 nm enabling finite intraparticle convection into the bead. The favorable features of the intraparticle convection and low mass transfer resistance make perfusion materials useful in high-speed chromatography. However, it should be noted that the introduction of large pores would result in the decrease of specific surface area, therefore leading to the decrease of protein binding capacity [2]. To overcome this disadvantage, it is necessary to modify perfusion stationary phases to acquire high binding capacity at high speed.

Grafting polymer chains or polymer layers onto the backbone of polymer materials has been adopted as a simple and convenient method for modifying the chemical and physical properties of polymer materials [3], of which surface-initiated polymerization using an initiator is widely used. During the polymerization reaction, the polymer chains "grow" from the active site on the surface induced by initiator; the graft density and length could be controlled by adjusting the monomer and initiator concentration as well as reaction time [4]. The most advantage of grafting polymerization is the introduction of a large amount of functional groups. Now, a few studies have demonstrated that cerium(IV) ion initiated grafting of polymer chains onto porous supports could effectively increase the binding capacity of proteins [5,6].

We have recently fabricated superporous cellulose (SC) microspheres [7] by using submicron-sized $CaCO_3$ particles as a solid porogenic agent to form perfusive pores. Introduction of superpores is essential to overcome the restriction of pore diffusion towards macromolecules, but the static adsorption capacity (33 mg BSA/ml) was not as high as expected. Hence, in order to enhance the adsorption capacity of the superporous cellulose bead, we have herein adopted the method of grafting polymerization. Namely, glycidyl methacrylate was grafted onto the bead pores to fabricate a grafted superporous cellulose matrix (G-SC). After modifying the matrices with diethylaminoethyl groups, anion exchangers were prepared. The static and dynamic binding properties of the two kinds of anion exchangers were compared to examine the improvements in adsorption ability by the grafting polymerization. Some parameters related to the

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doi:10.1016/j.bej.2007.06.006

**Nomenclature**

| | |
|---|---|
| $c_0$ | initial or inlet protein concentration (mg/ml) |
| $d_p$ | mean particle diameter (m) |
| $D_p$ | effective pore diffusivity ($m^2$/s) |
| $K$ | bed (Darcy) permeability ($m^2$) |
| $K_d$ | dissociation constant in the Langmuir equation (mg/ml) |
| $L$ | column length (m) |
| $\Delta p$ | pressure drop across column (Pa) |
| $q_m$ | adsorption capacity described by the Langmuir equation (mg/ml wet bead) |
| $q_{10}$ | dynamic binding capacity at 10% breakthrough (mg/ml wet bed) |
| $Q$ | mobile phase flow rate (ml/min) |
| $t_0$ | retention time under non-retained condition (min) |
| $t_{10}$ | time at 10% breakthrough (min) |
| $u$ | superficial velocity of mobile phase (m/s or cm/h) |
| $V_t$ | total volume of chromatographic column (ml) |

*Greek letters*

| | |
|---|---|
| $\varepsilon$ | bed voidage |
| $\varepsilon_p$ | particle porosity |
| $\mu$ | viscosity (Pa S) |

pore structure were also measured to more extensively discuss the effects of the grafting polymerization.

## 2. Materials and methods

### 2.1. Materials

Glycidyl methacrylate (GMA) was purchased from Yuanji (Shanghai, China) and purified by distillation under reduced pressure. Ceric ammonium nitrate (CAN) was received from Guangfu (Tianjin, China) and used without further purification. Tris-(hydroxymethyl) aminomethane (Tris), diethylaminoethyl chloride (DEAE-Cl) and bovine serum albumin (BSA) with a purity of 96% were obtained from Sigma (St. Louis, MO, USA). All the other reagents were of analytical grade from local sources.

### 2.2. Preparation of grafted superporous cellulose beads

The fabrication procedure of the superporous cellulose beads (SC) has been described in detail in the previous work [7]. After double cross-linking and reducing, the SC beads were grafted with GMA monomer by a modified method described previously [6]. Briefly, 1 g SC beads were added to 40 ml of 0.125 mol/l $HNO_3$ solution in a three-necked round bottom flask kept in a water bath maintained at 60 °C. The suspension was purged with nitrogen gas for 10 min, and CAN at a final concentration of 5–15 mmol/l was added under stirring and nitrogen purging. After 30 min, 10 ml GMA in 50 ml dioxane was added, and the stirring was continued under nitrogen for 4–10 h. The reaction occurs as given in Fig. 1. The grafted SC beads (G-SC) were separated on a sintered glass filter and washed with 10% (v/v) acetone solution. The beads were dehydrated by stepwise transfer into aqueous solutions of acetone (10% increment, from 10 to 90% acetone) and finally anhydrous acetone. Finally, the beads were extracted with acetone in a Soxhlet apparatus for 6 h to remove any homopolymers. After replacing acetone in the beads with aqueous buffer, the G-SC beads were stored in 20% (v/v) ethanol aqueous solution at 4 °C for further use.

To investigate the effect of initiator concentration at 5–15 mmol/l, other reaction conditions were kept constant (50 mmol/l nitric acid, 10% (v/v) GMA, 60 °C, 6 h reaction). Then, the reaction time was also changed from 4 to 10 h by maintaining other conditions at fixed values (8 mmol/l CAN, other conditions the same as described above).

### 2.3. Determination of epoxy groups

By the grafting polymerization, polymer chains with epoxy groups were introduced into the beads, so we can check the reaction degree by determining the amount of epoxy groups. The epoxyl groups were determined by a reaction between the oxirane ring and sodium thiosulfate, following by titration with 10 mmol/l hydrochloride acid to neutralize any released $OH^-$ in the reaction [8]. In a typical procedure, 0.2 g drained G-SC

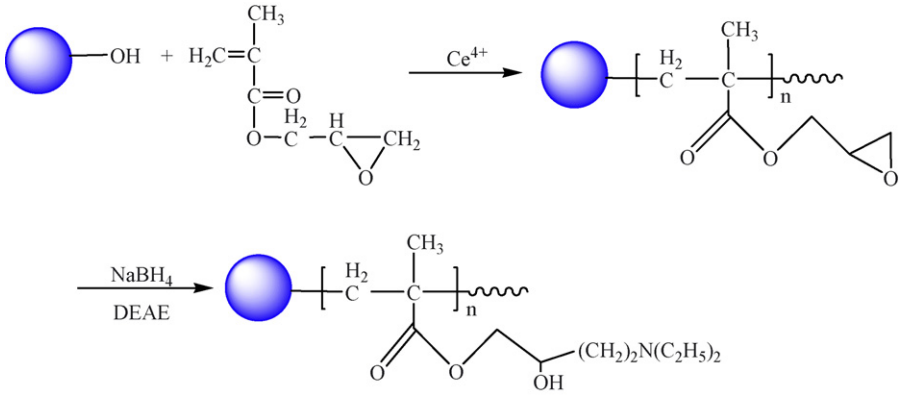


Fig. 1. Cerium initiated graft polymerization of superpores cellulose beads and the following modification with DEAE-Cl.