Analytica Chimica Acta 853 (2015) 617–624

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Preparation and characterization of a thermoresponsive gigaporous medium for high-speed protein chromatography

Jian-Bo Qu [a,*], Yan-Li Chen [a], Guan-Sheng Huan [a], Wei-Qing Zhou [b], Jian-Guo Liu [a], Hu Zhu [a], Xiao-Yun Zhang [a,*]

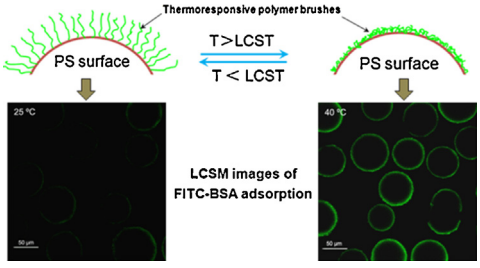
[a] State Key Laboratory of Heavy Oil Processing, Center for Bioengineering and Biotechnology, China University of Petroleum (East China), Qingdao 266580, PR China

[b] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, PR China

HIGHLIGHTS

- A high-speed thermoresponsive bio-separation medium was prepared in two steps.
- Non-specific adsorption of proteins on thermoresponsive medium was greatly reduced.
- Separation of proteins was achieved by only adjusting column temperature.
- It was able to separate proteins at the mobile phase velocity up to 2167 cm h^{-1}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 19 September 2014
Accepted 22 September 2014
Available online 26 September 2014

Keywords:
Gigaporous polystyrene microspheres
Thermoresponsive polymer brushes
Protein chromatography
N-isopropylacrylamide
Atom transfer radical polymerization

ABSTRACT

A high-speed thermoresponsive medium was developed by grafting poly(*N*-isopropylacrylamide-*co*-butyl methacrylate) (P(NIPAM-*co*-BMA)) brushes onto gigaporous polystyrene (PS) microspheres via surface-initiated atom transfer radical polymerization (ATRP) technique, which has strong mechanical strength, good chemical stability and high mass transfer rate for biomacromolecules. The gigaporous structure, surface chemical composition, static protein adsorption, and thermoresponsive chromatographic properties of prepared medium (PS–P(NIPAM-*co*-BMA)) were characterized in detail. Results showed that the PS microspheres were successfully grafted with P(NIPAM-*co*-BMA) brushes and that the gigaporous structure was robustly maintained. After grafting, the nonspecific adsorption of proteins on PS microspheres was greatly reduced. A column packed with PS–P(NIPAM-*co*-BMA) exhibited low backpressure and significant thermo-responsibility. By simply changing the column temperature, it was able to separate three model proteins at the mobile phase velocity up to 2167 cm h^{-1}. In conclusion, the thermoresponsive polymer brushes grafted gigaporous PS microspheres prepared by ATRP are very promising in 'green' high-speed preparative protein chromatography.

1. Introduction

The rapid development of biotechnology makes the separation and purification of biomacromolecules increasingly important. Liquid chromatography has been the most important technique at all scales of protein purification owing to its high resolution and mild

* Corresponding authors. Tel.: +86 532 86981566; fax: +86 532 86981566.
E-mail addresses: jbqu@upc.edu.cn (J.-B. Qu), zhangxy@upc.edu.cn (X.-Y. Zhang)

http://dx.doi.org/10.1016/j.aca.2014.09.034
0003-2670/

separation conditions [1]. Conventional chromatography techniques, such as reversed phase liquid chromatography (RPLC), ion-exchange chromatography (IEC), hydrophobic interaction chromatography (HIC), have some limitations in protein separation [2]. The use of organic solvents (RPLC) or high salt concentration (IEC and HIC) during the separation process may damage proteins, resulting in the loss of bioactivity. This has prompted the development of a new mode of chromatography based on an aqueous mobile phase. Recently, thermoresponsive chromatographic systems have been reported as an interesting option for controlling the separation of biomolecules [3]. Temperature-responsive materials, particularly PNIPAM, have the potential to be used in a variety of chromatographic modes, including size exclusion chromatography (SEC) [4,5], HIC [6,7], IEC [8,9], and affinity chromatography [10], in which elution of the bound target biomolecules can be induced by a small change in temperature. Thermoresponsive stationary phases are typically prepared by grafting temperature-responsive polymers onto chromatographic matrices with porosity, such as silica beads [6,11], monolithic silica columns [12], polysaccharide gels [8,13], polystyrene particles [4,14]. The main advantage of these smart chromatographic media is that they can separate mixtures of biomolecules in a pure aqueous environment under isocratic conditions, which offers potential for cost-effective purification in a more environmentally friendly manner.

Although, previous investigations on thermoresponsive stationary phases show their potential applications in the purification of commercially valuable components from agricultural, food, pharmaceutical and other complex streams, there still exist some limitations in the thermoresponsive media so far reported. First, the supports have been dominated by silica beads (or monoliths) and polysaccharide gels. The former have poor chemical stability at low (pH < 2) or high pH (pH > 7.5), which makes them unsuitable for the conditions often employed for the industrial cleaning of equipment [8,15]. The latter have poor mechanical strength and can only be operated under low pressure [16]. Secondly, most conventional porous microspheres with normal diameters of 10–30 nm have stagnant mass transfer problems during the separation of biomacromolecules, and there has been little published literature examining the separation of large proteins. There is a need to develop and evaluate thermoresponsive media on new types of supports. Gigaporous polystyrene microspheres with strong mechanical strength, good chemical stability and high mass transfer rate offer potential for the purification of biomacromolecules, either in preparative or analytical modes. Zhou et al. reported a novel method to prepare gigaporous PS microspheres with pore diameter of about 300–500 nm [17]. After further hydrophilization and derivatization, these particles can be used as perfusion protein chromatographic supports and media [18–21].

Atom transfer radical polymerization (ATRP) is an attractive polymer grafting method allowing surface to obtain well-defined polymer brushes by a surface-immobilized ATRP initiator [12,22]. In this study, taking gigaporous PS microspheres as a base support, we prepared a novel thermoresponsive high-speed protein chromatographic medium using surface-initiated ATRP. The gigaporous structure of the medium was characterized by scanning electron microscope and mercury porosimetry. Then, flow hydrodynamics and chromatographic performance of a column packed with the medium were investigated in detail to testify the feasibility and potential of the gigaporous PS microspheres grafted with thermoresponsive brushes for high-speed protein chromatography.

2. Experimental

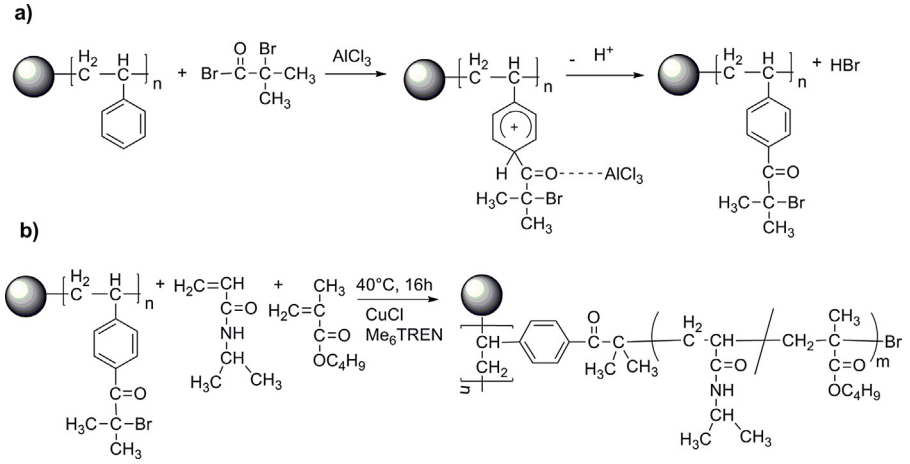
2.1. Materials and reagents

The gigaporous PS microspheres used in this work were synthesized by suspension polymerization as described in previous paper [17]. The product gives out the specific surface area as 22.69 $m^2 g^{-1}$, the average diameter as 65 μm (25–100 μm range), and the average pore size as 280 nm (100–600 nm range).

N-isopropylacrylamide (NIPAM, >98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Bovine serum albumin (BSA), acid phosphatase (ACP) and trypsin were obtained from Amresco (USA). Butyl methacrylate (BMA, 99%), ethylenediamine tetraacetic acid (EDTA, AR) and 2-bromoisobutyryl bromide (CP) were obtained from Chengdu Xiya Chemical Reagent Co., Ltd. (China). Tris (2-aminoethyl) amine (TREN), fluorescein isothiocyanate (FITC, 95%) and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) were from Alfa Aesar (UK). Anhydrous aluminum chloride ($AlCl_3$, AR), carbon disulfide (CS_2, AR), copper(II) chloride ($CuCl_2$, 98%) and copper(I) chloride (CuCl, 98%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). High purity argon and liquid nitrogen were ordered from Qingdao Tianyuan Gas Manufacturing Co., Ltd. (China). Tris[2-(dimethylamino)ethyl] amine (Me6TREN) was synthesized according to the literature procedure [23]. Other reagents were of analytical grade from local sources.

2.2. Apparatus

The gigaporous structure of PS microspheres and PS–P(NIPAM-*co*-BMA) was observed by scanning electron microscopy (SEM)



Scheme 1. (a) Bromoacetylation of PS microspheres and (b) reacting route of PS–Br grafted with poly(NIPAM-*co*-BMA) brushes.