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Preparation of quaternary amine monolithic column for strong anion-exchange chromatography and its application to the separation of Enterovirus 71

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ABSTRACT

Large size virion is unable to diffuse into pores of conventional porous chromatography particles. Therefore, separation of virion by conventional column-packing materials is not quite efficient. To solve this problem, a monolithic column with large convective pores and quaternary amine groups was prepared and was applied to separate Enterovirus 71 (EV71, ≈5700–6000 kDa). Cross-section, pore structure, hydrodynamic performance, adsorption property and dynamic binding capacity of prepared monolithic column were determined. Double-pore structures, macropore at 2472 nm and mesopore at 5–60 nm, were formed. The porosity was up to 63.3%, which enable higher permeability and lower back pressure of the monolithic column than commercial UNO™ Q1 column. Based on the breakthrough curves, the loading capacity of bovine serum albumin was calculated to be 42.0 mg per column. In addition, prepared quaternary amine monolithic column was proved to be suitable for the separation of protein mixture by strong anion-exchange chromatography. As a practical application, prepared monolith column presents excellent performance to the separation of EV71 from virus-proteins mixture.

1. Introduction

A monolithic stationary phase is a continuous unitary porous structure prepared by in-situ polymerization or consolidation inside a column tubing and, if necessary, the surface is functionalized to convert it into a sorbent with the desired chromatographic binding properties [1]. Monoliths have advantages over conventional columns in respect of hydrodynamic properties, as they have a lower mass transfer resistance [2] and pressure drop [3]. These two properties play a critical role in performance, speed and scale up of chromatographic separation, especially in the purification and analysis of biomacromolecules.

In early 1990s, Svec et al. [4] prepared a novel continuous bed column with macroporosity and high capacity, and further aminated it with diethylamine. The amino functionalized porous polymeric column illustrated good separation to protein mixtures. Since then monolithic column attracted increasing attention both in scientific and engineering aspects. In recent years silica monolithic column [5] and polymer monolithic column [6] were extensively reported. Silica monolithic column is not suitable for application under strong acidic and basic condition. In contrast, polymer monolithic column has various advantages such as good stability, abundant synthesis materials and convenience in modification.

The structure of polymer monolithic column can be represented as an interconnected network of micrometer-sized flow-through channels. Owing to this structure, the solute transports to the surface of channel solely by convection instead by diffusion as observed in conventional media [3,7]. This provides good bed permeability and enables monoliths as an excellent stationary phase for fast separation to macromolecule [8]. Jungbauer et al. [9] established a chromatographic process based on monoliths for purification of infectious baculovirus and high efficiency was obtained even without prior concentration step. In the same study, the most efficient process consisted of the use of two different monoliths. An epoxy monolith was used as pre-column to eliminate lipids and quaternary amine (QA) monolith was used to bind and elute baculovirus. Virus recovery of 20–99% was achieved by this protocol.

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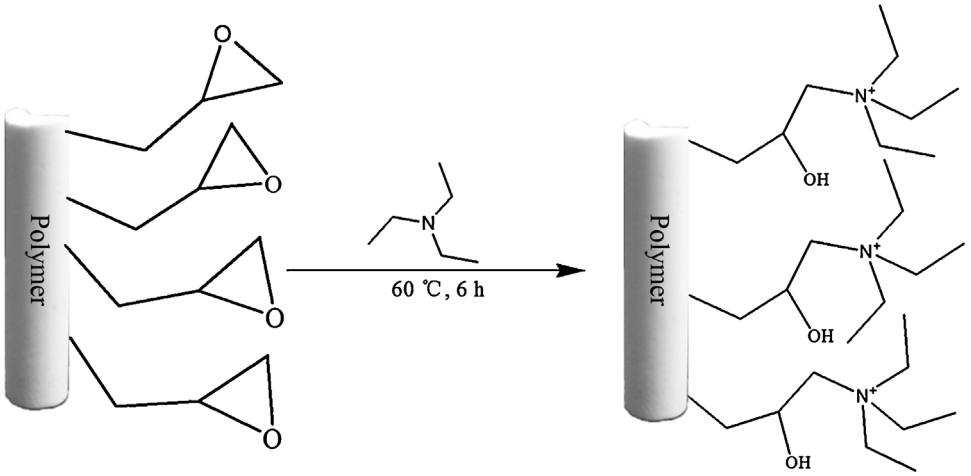

Fig 1. Amination reaction on the monolithic column.

CIM® Monolithic Column has particularly proven of value for purification and concentration of viruses, virus-like particles (VLPs), and large proteins [10]. In 2013, Rupar et al. [11] used CIM® QA column (BIA separations, Austria) for the fast purification of filamentous Potato Virus Y (PVY) (virus size, 740 nm × 11 nm) from plant tissue. Branovic et al. [12] successfully implement the enrichment of rubella virus and mumps virus by a CIM® DEAE column (BIA separations, Austria). Their work improves the sensitivity and selectivity of viral detection and makes it possible to isolate viral RNA from cell-free biological fluids.

Enterovirus 71 (EV71) is a major etiological agent causing the outbreaks of hand-foot-and-mouth disease (HFMD) in Asian countries [13,14]. It belongs to the enterovirus family in the picornaviridae and is similar to other enteroviruses (e.g. poliovirus and coxsackievirus) in structure and replication process [15]. EV71 infects mammalian cells (e.g. Vero) and produces empty particles (30 nm approximately) which consist of ≈60 copies of VP0 (≈36–38 kDa), VP1 (≈32–36 kDa) and VP3 (≈27 kDa) [16–18], thus the molecular weight of EV71 VLPs is estimated to be ≈5700–6000 kDa. The large molecular weight makes it difficult to be purified on conventional chromatographic supports. It was reported that EV71 is preferably purified by conventional techniques such as ultracentrifugation [17] or precipitation [19] followed by sucrose gradient/cesium chloride centrifugation or size exclusion chromatography. However these methods are laborious and time-consuming processes. It is urgent to explore an effective way for the purification of EV71 with high yield and faster processing time.

Monolithic chromatographic supports are an alternative to conventional supports due to their excellent mass transfer properties and their high binding capacity for virus. Ion-exchange chromatography is widely used as an initial chromatographic procedure and can be used for the purification of coxsackievirus B3 VLPs [20]. Venkatachalam ARK [21] reported a successful EV71 purification from serum-free culture medium by the CIM® DEAE-8f tube monolithic column. However, Serum-free culture medium is still immature and costly. In the conventional cell culture medium with serum, the serum proteins make the purification difficult. Therefore, we focus on the EV71 purification from cell culture medium with serum.

In this study, a poly (glycidyl methacrylate-co-ethylene dimethacrylate) monolithic column was synthesized, amination-functionalized and applied to the separation and purification of EV71. Functionalization was implemented by the reaction between epoxy groups and triethylamine. Resultant quaternary amine groups act as anion exchange groups for protein separation (Fig. 1).

2. Experimental

2.1. Chemicals

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), albumin egg, L-glutathione, bovine serum albumin (BSA), RNase-A and lysozyme C (Lyz) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Benzoyl peroxide (BPO), sodium chloride (NaCl) and dodecanol were purchased from Institute of Tianjin Chemical Reagent (Tianjin, China). Propanol and tetrahydrofuran were purchased from Tianjin FuYu chemical plant (Tianjin, China). $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$ were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Triethylamine was purchased from Guangdong guanghua science and technology co., Ltd (Guangdong, China). Inactivated crude extracts of Enterovirus 71 (EV71) were received from the Institute of Medical Biology, Chinese Academy of Medical Sciences (Yunnan, China). Commercial UNO™ Q1 monolith column (Serial No. Q1-429377, quaternary amine type) was purchased from Bio-Rad Pacific Ltd (Hong Kong, China). Ultrapure water was obtained from an Aquapro AJY-2002-U ultrapure water machine (Shanghai, China).

2.2. Preparation of the monolithic column

The procedure for the preparation of monolithic column by in situ polymerization was as following. Initiator BPO (1 wt.% to monomers) was dissolved in monomer mixture of GMA and EDMA (80:20, v/v). Propanol, dodecanol and water (48:44:8, v/v) were mixed as porogens. Reaction solution was prepared by mixing monomer and porogens (5:9, v/v) and purged with nitrogen for 20 min. A stainless-steel tube (10 × 100 mm ID) with one end sealed was filled with the above reaction solution and then another end was sealed. After polymerization at 60 °C for 24 h, the column was attached to a HPLC system and washed with methanol at a flow rate of 0.5 mL min^{-1} to remove the porogenic diluents and other impurities. The mixture of triethylamine and tetrahydrofuran (1:1, v/v) was then pumped circularly through the column for 6 h at 60 °C. The amination reaction is shown in Fig. 1. Finally, the monolithic column was washed in the order of methanol and phosphate buffer (50 mM, pH 7.0) to remove the remaining chemical reagents.

2.3. Chromatography

Chromatographic experiments were performed on a BioLogic DuoFlow™ Chromatography System (Bio-Rad Laboratories, Inc., USA). A step gradient elution was applied in the separation of EV71