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Large-volume methacrylate monolith for plasmid purification Process engineering approach to synthesis and application

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Abstract

The extent of exothermicity associated with the construction of large-volume methacrylate monolithic columns has somewhat obstructed the realisation of large-scale rapid biomolecule purification especially for plasmid-based products which have proven to herald future trends in biotechnology. A novel synthesis technique via a heat expulsion mechanism was employed to prepare a 40 mL methacrylate monolith with a homogeneous radial pore structure along its thickness. Radial temperature gradient was recorded to be only 1.8 °C. Maximum radial temperature recorded at the centre of the monolith was 62.3 °C, which was only 2.3 °C higher than the actual polymerisation temperature. Pore characterisation of the monolithic polymer showed unimodal pore size distributions at different radial positions with an identical modal pore size of 400 nm. Chromatographic characterisation of the polymer after functionalisation with amino groups displayed a persistent dynamic binding capacity of 15.5 mg of plasmid DNA/mL. The maximum pressure drop recorded was only 0.12 MPa at a flow rate of 10 mL/min. The polymer demonstrated rapid separation ability by fractionating *Escherichia coli* DH5α-pUC19 clarified lysate in only 3 min after loading. The plasmid sample collected after the fast purification process was tested to be a homogeneous supercoiled plasmid with DNA electrophoresis and restriction analysis.

Keywords: Methacrylate monolith; Large-volume; Plasmid DNA; Anion-exchange; Temperature

1. Introduction

The foreseeable increase in the demand for plasmid DNA (pDNA) molecules due to extensive developments in gene medicine has led to amplified efforts within both academia and industry to improve the effectiveness of plasmid production methodologies. A decade ago, production titers of plasmids were on the gram scale but presently amount in kilograms or even tonnes are now considered achievable [1–3]; hence it has been a major endeavour to revolutionalize existing plasmid purification methods as the majority of these schemes are not scalable enough to accommodate such large-scales. Electrostatic isolation of pDNA via anion-exchange liquid chromatography is the most frequently used technique for plasmid purification owing to its simplicity, widespread application, high resolving power and high capacity [2–6]. However, the performance of anion-exchange liquid chromatography for large-scale purification process hinges on the pore structure of the stationary phase [6,7].

Monolithic sorbents represent one of the newest developments of chromatographic stationary phases for biomolecule separation [7]. Their pore structure is made of highly interconnected pores consisting of macropores (greater than 50 nm), mesopores (between 50 nm and 2 nm) and micropores (less than 2 nm). The macropores ensure excellent convective mass transfer between the mobile and the stationary phase with molecular diffusion limited to the boundary layer. This increases the mobility of the molecules to be separated within the polymer. The mesopores and micropores present enough active sites for attaching functional groups that dictates the chromatographic separation capabilities of the monolithic polymer [8,9]. Molecules to be separated are transported to the active sites on the surface of the pores by bulk flow of the mobile phase. Consequently, there is virtually no transfer by diffusion. Since convective transfer is several orders of magnitude faster than diffusion, transport between mobile and stationary phases is faster as well. High resolution and capacity which are somewhat less dependent of flow rate can be achieved in monoliths,

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and even under the high flow rate, the pressure drop is moderate [10–14]. Due to such characteristics, they are suitable for rapid purification of large biomolecules such as proteins [11–14], oligonucleotides [5,11] and polypeptides [14,15].

Poly(glycidyl methacrylate-*co*-ethylene glycol dimethacrylate) monoliths have become very attractive stationary phases due to their advantageous hydrodynamic characteristics and the simplicity of their synthesis technique. They are basically prepared from the polymerisation of methacrylate monomers in the presence of a porogen and an initiator at a specific temperature [16,17]. Although various factors such as monomer content, porogen and initiator concentrations dictates the dynamics of the monolith pore structure formation, temperature is the most effective means of control as it maneuvers the production of monoliths with a wide range of pore size distributions from a single polymerisation feedstock [18]. Despite the fact that many papers have described the applications of methacrylate monolith on the analytical scale, only a few have explored the scale-up possibilities for the purification of biomolecules [19,20]. The pivotal hurdle here is the high exothermic level of the polymerisation reaction which becomes paramount in the case of large-volume monoliths and results in significant radial temperature gradients, thereby creating a non-uniform pore structure. Again, the unstirred nature of the polymerisation system leads to a limited capacity to efficiently distribute the heat of polymerisation. Podgornik et al. [21] prepared several annular monoliths with different outer diameters and embedded one into another. By this technique, the component concentration in the polymerisation feedstock is reduced and consequently the temperature distribution is controlled effectively. However, it can be quite difficult to prepare large-volume monoliths, because there is a high probability of damaging the pieces during the embedding process and this can lead to the introduction of dead volumes within the final monolith. Peters et al. [22] developed an approach to control the temperature increase during polymerisation by gradual addition of the feedstock. This lowers the polymerisation rate due to the limited amount of polymerisation feedstock per unit time. Although this method showed only moderate increase in temperature during the polymerisation and a more uniform pore size distribution was obtained, no additional chromatographic characterization was reported for the monolith. In this paper, we unveil a novel thermal expulsion technique for the synthesis of a large-volume methacrylate monolith and display its capability for rapid purification of pDNA from clarified bacteria lysate. The effect of radial temperature gradient on the pore properties of the polymer is also reported.

2. Experimental

2.1. Chemicals/reagents

Ethylene glycol dimethacrylate (EDMA) (MW 198.22, 98%), glycidyl methacrylate (GMA) (MW 142.15, 97%), cyclohexanol (MW 100.16, 99%), azobisisobutyronitrile (AIBN) (MW 164.21, 98%), methanol (HPLC grade, MW 32.04, 99.93%), ethidium bromide (MW 394.31, 10 mg/mL), 2-chloro-*N*,*N*-diethylethylamine hydrochloride (DEAE-Cl) (MW 172.10, 97%) were purchased from Sigma–Aldrich (Sydney, Australia). NaCl (MW 58.44, 99.5%), sodium dodecyl sulphate (SDS) (MW 288.38, 99.0%), Na_2CO_3 (MW 105.99, 99.5%), Tris (MW 121.14, 99.8%), EDTA (MW 292.3, 99.5%) were purchased from Amresco (Columbus, OH, USA). Agarose (Promega, Syndey, Australia) and 1 kbp (kilobase pair) DNA marker (Bio-Labs, New England, UK).

2.2. Chromatographic unit

The HPLC equipment (Biologic DuoFlow system, Bio-Rad, Melbourne, Australia) is designed with two F10 pumps for a maximum flow rate of 10 mL/min at 21 MPa, QuadTec UV/vis detector, SV5-4 select valve, BioLogic maximiser mixer, AVR9-8 switching valves, BioLogic Maximizer buffer blending system, pH monitor, F40 workstation, BioFrac fraction collector, AVR 7-3 sample inject valve, BioLogic rack, BioLogic DuoFlow software, USB bitbus communicator and Dell controller.Chromatographic column

The chromatographic column employed is a Bio-Rad glass column 10 cm × 2.5 cm (Econo-column chromatography columns, catalog no. 737-2512) connected with a movable flow adaptor (Flow Adaptor, 2.5 cm column I.D., 1–14 cm functional length, catalog no. 738-0017).

2.4. Plasmid sample

Plasmid DNA was isolated from *E. coli* DH5α-pUC19 bacteria after alkaline lysis using Wizard plus SV Maxipreps according to the manufacturer's instructions (Promega). The size of the pDNA was 2.7 kbp.

2.5. Procedures

2.5.1. Synthesis of methacrylate monolith

The methacrylate monolithic polymer was synthesised via free radical polymerisation of EDMA and GMA as monomers in cyclohexanol as a porogen. The polymerisation was initiated with AIBN. Fig. 1 shows schematically the technique adopted for the monolith synthesis. Cyclohexanol/AIBN mixture (P + I) and the monomer mixture (MM) were preheated to a temperature $T_0 = 50\,^{\circ}C$ and isothermally pumped (P1 and P2) simultaneously at flow rates of 0.05 and 0.03 mL/min, respectively, into the polymerisation mould (10 cm × 2.5 cm Bio-Rad, catalog no. 737-2512) which is immersed in a water bath at $T_p = 60\,^{\circ}C$ ($\Delta T = 10\,^{\circ}C$). The heat/fumes resulting from the AIBN decomposition was expelled prior to the isothermal pumping. The temperature profiles were traced by inserting thermocouples (Pyrosales, Sydney, Australia) directly into the polymerisation mixture at three radial points; centre, 6 mm and 12 mm positions. The polymerisation feedstock compositions were as follows; EDMA/GMA mixture (40/60, v/v) was combined with cyclohexanol/AIBN mixture in the proportion 40/60 (v/v) making a solution with total volume 40 mL. AIBN (1%, w/w, with respect to monomer) was used to initiate the polymerisation process. Both the monomer mixture and cyclohexanol/AIBN mixture were sonicated for 10 min and