

Process engineering approach to large-volume methacrylate monolith synthesis for plasmid purification

The purification of plasmid DNA is hampered by the low performance of conventional chromatographic adsorbents which possess small particle pore diameters. Most of these adsorbents are tailored for the high adsorption capacity of proteins with size less < 10 nm. In columns packed with such adsorbents, large molecules such as pDNA with sizes > 100 nm adsorb predominantly at the particle outer surface, thus resulting in a low binding capacity. A monolith is a continuous phase consisting of a single piece of a highly porous organic or inorganic solid material. The most important feature of this material is that all the mobile phase is forced to flow through its large pores. As a consequence, mass transport is enhanced by convection; dramatically reducing the long diffusion time required by particle based chromatographic supports. Therefore, chromatographic separation process on monoliths is practically not diffusion-limited. The large pores of these monoliths allow penetration of large pDNA molecules to the internal surface area at high flow rate with low pressure drop. Different types of monolithic supports are currently available based on preparation and chemistry. These are silica-based, polyacrylamide-based and gel-based monolithic resins. Polymethacrylate monolithic support is an optimal adsorbent for pDNA separation. These adsorbents have large pore diameters and thus no significant hindrance to convective mass transport. They are resistant to pH, non-toxic, economically favorable to synthesize and can be easily modified by functionalizing with anion-exchange, hydrophobic interaction or affinity ligand. The flexibility and the ease to tailor their pore and surface characteristics to the target pDNA molecule through alteration in synthesis conditions make them more attractive.

The construction of large-volume methacrylate monolithic columns for preparative-scale plasmid purification is obstructed by the enormous release of exotherms, thus introducing structural heterogeneity in the monolith pore system. A remarkable radial temperature gradient develops along the monolith thickness, reaching a terminal temperature that supersedes the maximum temperature required for the preparation of a structurally homogenous monolith. A novel heat expulsion technique is employed to overcome the heat build-up during the synthesis process. The enormous heat build-up is perceived to encompass the heat associated with initiator decomposition and the heat released from free radical-monomer and monomer-monomer interactions. The heat resulting from the initiator decomposition was expelled along with some gaseous fumes before commencing polymerisation in a gradual addition fashion. Characteristics of a 50 mL monolith synthesised using this technique showed an improved uniformity in the pore structure radially along the length on the monolith. Chromatographic characterisation of this adsorbent displayed a persistent binding capacity of 14.5 mg pDNA/mL of the adsorbent. The adsorbent was able to fractionate a clarified bacteria lysate in only 3 min (after loading) into RNA, protein and pDNA respectively. The pDNA fraction obtained was analysed to be a homogeneous supercoiled pDNA.