

## Organic Co-Polymer Monolithic Columns for Liquid Chromatography Mixed Mode Protein Separations

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**Abstract :** Organic mixed mode monolithic columns were fabricated from; glycidyl methacrylate-co-ethylene dimethacrylate-co-stearyl methacrylate, using glycidyl methacrylate and stearyl methacrylate as co monomers representing 30% and 70% respectively of the liquid volume with ethylene dimethacrylate crosslinker and 2,2-dimethoxy-2-phenylacetophenone as the free radical initiator. The monomers were mixed with a binary porogenic solvent, comprising propan-1-ol, and methanol (0.825 mL each). The monolith was formed by photo polymerization (365 nm) inside a borosilicate glass tube (1.5 mm ID and 3 mm OD x 50 mm length). The monolith was observed to have formed correctly by optical examination and generated reasonable backpressure, approximately 650 psi at a flow rate of 0.2 mL min<sup>-1</sup> 50:50 acetonitrile: water. The morphological properties of the monolithic columns were investigated using scanning electron microscopy images, and Brunauer-Emmett-Teller analysis, the results showed that the monolith was formed properly with  $19.98 \pm 0.01$  mm<sup>2</sup> surface area,  $0.0205 \pm 0.01$  cm<sup>3</sup> g<sup>-1</sup> pore volume and  $6.93 \pm 0.01$  nm average pore size. The polymer monolith formed was further investigated using proton nuclear magnetic resonance, and Fourier transform infrared spectroscopy. The monolithic columns were investigated using high-performance liquid chromatography to test their ability to separate different samples with a range of properties. The columns displayed both hydrophobic/hydrophilic and hydrophobic/ion exchange interactions with the compounds tested indicating that true mixed mode separations. The mixed mode monolithic columns exhibited significant separation of proteins.

**Keywords :** LC separation, proteins separation, monolithic column, mixed mode

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