

ABSTRACT

PREPARATION AND CHARACTERIZATION OF MOLECULARLY IMPRINTED CRYOGELS FOR CARBONIC ANHYDRASE PURIFICATION

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In this study, molecularly imprinted poly(HEMA-MAH) cryogels were synthesized and used for purification of carbonic anhydrase from bovine blood. Cryogels were characterized by its swelling degree, macroporosity, flow rate and FTIR, SEM, surface area and elemental analysis. Maximum swelling degree of the cryogel was found to be 8.01 g H₂O/g while their macroporosity was about 70 %. Flow rate increased with increasing macroporosity. Incorporation of MAH monomer into cryogel structure was shown with elemental analysis and FTIR. With SEM photographs it was shown that cryogels were highly porous and had sponge like morphology. Their surface area was found to be 29.3 m²/g with BET studies. Maximum carbonic anhydrase adsorption was found to be 3.16 mg/g with MES buffer (pH 6.0) while temperature had no significant effect over the adsorption. Adsorption capacity was decreased with increased chromatographic flow rate to 4 mL/min. Tree-dimensional structure of the enzyme was studied with fluorometric analysis. Cryogels were re-used for 10 cycle and their adsorption capacity decreased only 10 %. Cryogel selectivity were investigated with albumin, hemoglobin, IgG, γ -globulin and lysozyme and found to be 15.26, 60.05, 21.88, 17.61 and 17.42, respectively. These cryogel used for purification of carbonic anhydrase from bovine blood and purified with 84 fold and 80 % recovery. Purity of the enzyme was shown with SDS-PAGE and zymogram.

Key words: Carbonic anhydrase, cryogel, molecularly imprinted polymer.