ABSTRACT

Second generation aza-arenophilic gels for antibody purification and enzyme immobilization.

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The present study is a further extension of the pioneering work of Ngo et al and Jogie-Brahim on the synthesis and use of aza-arenophilic gels as synthetic affinity matrices for the purification of immunoglobulins, mainly IgG. Here a different procedure is adopted for the synthesis of the second generation of aza-arenophilic gels. The heterocyclic base (4-aminopyridine or 4-amino(methyl)pyridine) was first covalently linked to the support (an aldehyde functionalized polyacrylate) and then reacted with the halopyridine (3,5-dichloro-2,4,6-trifluoropyridine or pentafluoropyridine), followed by the capping nucleophile (2-mercaptoethanol, 2-mercaptopyridine, ethylene glycol, phenethylamine, 2,5-dimercapto-1,3,4-thiadiazole).

The optimization of the synthesis conditions was investigated. The results showed that the affinity gel which showed the best compromise between binding capacity and ease of regeneration was synthesized using 0.25 mmol heterocyclic base, 6.0 mmol halopyridine and 60.0 mmol capping nucleophile/mL gel respectively.

The results also showed that the IgG binding selectivity of the affinity gel increased as the content of NaCl in the binding buffer was increased (up to 4.0 M NaCl). Using 4.0 M NaCl and eluting with NaOAc

(0.1 M, pH 2.9), followed by 30% propan-2-ol/ NaOAc (0.1 M, pH 2.9) IgG is obtained in > 76% in the first step and > 95% in the second step. On the other hand, binding buffers containing salts such as $(NH_4)_2SO_4$, K_2SO_4, Na_2SO_4 and $MgCl_2$ increased albumin contamination. Also, varying the eluting conditions to a low salt concentration, alcohols or neutral systems resulted in significant albumin contamination.

In addition to the binding of immunoglobulins, these second generation aza-arenophilic gels were also shown to be suitable supports for the reversible immobilization of enzymes such as β-galactosidase, peroxidase and urease. Enzyme desorption is achieved in 20 minutes using Glycine-HCl (0.1 M, pH 2.9). Furthermore, storage stability studies indicated that the immobilized enzyme is capable of retaining up to 75% of its initial activity after a period of 400 days when stored in a phosphate buffer (0.01 M, pH 7.4) at 4 ^{o}C.

Keywords: aza-arenophilic gels, affinity chromatography, immunoglobulin purification, affinity ligands, immobilized enzymes.