ABSTRACT

Use of novel, synthetic aza-arenophilic gels for immunoglobulin purification and reversible enzyme immobilization

Sherryline Jogie-Brahim

In 1990, Ngo and Khatter first reported on a new synthetic affinity gel, called the aza-arenophilic gel, for the purification of immunoglobulins. This gel was synthesized using Sepharose CL-4B as the support, a pentahalogenated pyridine moiety, a tertiary amine and a capping nucleophile.

The present study is a further extension of this pioneering work. It focuses on the synthesis of a wide variety of aza-arenophilic gels in which the various synthons were varied. These included the nature of the support (Sepharose CL-4B and Fractogel), the pentahalogenated pyridine (2,4,6-trifluoro-3,5-dichloro pyridine and pentafluoropyridine), the tertiary amine [4-(dimethylamino)pyridine, 1-methylimidazole, isoquinoline, 1,2,4-triazole and 1-H-tetrazole] and the capping nucleophiles (2-mercaptoethanol, 2-mercaptopyridine, phenethylamine, 2,5-dimercapto-1,3,4-thiadiazole, 2-mercaptoethanol-divinylsulphone-2-mercapto ethanol and divinylsulphone-2-mercaptoethanol).

The optimization of conditions for the synthesis of these aza-arenophilic gels was investigated. The results obtained show that the reaction of Sepharose CL-4B with the pentahalopyridine and the tertiary amine is very rapid with activation being achieved in as little as 0.5 hour.

Albumin, the major serum protein, is often a contaminant in the purification of immunoglobulins using synthetic affinity matrixes. Studies indicate that using a phosphate binding buffer (0.01M containing 0.1 – 2.0M NaCl) in the pH range of 7.0-8.0, a working temperature range of 10 – 30^{0}C and appropriate eluting buffers such as 0.1M NaOAc, pH 2.9 or 0.01M NaH_2PO_4 (containing 0.4M triethylamine and 1.5M NaCl), pH 7.4 could essentially minimize and possibly eliminate the contamination of albumin during IgG purification.

Studies conducted on the optimization of the IgG binding conditions reveal that when the pH of the binding buffer is varied > 95% IgG purity is obtained over the entire pH range investigated (6.0-8.0) for all the gels studied except Gels IV, VII and XI which was found to bind between 25-28% of albumin and 72-75% IgG at pH 6.5 (Gels IV and VII) and pH 7.4 (Gel VII).

The selective binding of IgG on to these gels is found to be favoured in the presence of NaCl. In the absence of NaCl, significant albumin contamination was observed. The actual concentration of NaCl required to effect a pure IgG fraction depended upon the pentahalopyridine used and the nature of the capping nucleophile and tertiary amine employed. Essentially pure IgG can be obtained from these gels if a high salt binding buffer (0.01M NaH_2PO_4 containing 2.0M NaCl) is employed followed by elution with either an acidic buffer (0.1M NaOAc, pH 2.9), a gradient buffer (0.01M NaH_2PO_4 containing 1.5M – 0.0M NaCl) or by elution with the binding buffer containing acetonitrile (0.96M) or triethylamine (0.4M). Pure IgG can also be obtained under low salt binding conditions (0.01M

NaH_2PO_4 containing 0.15M NaCl) provided the column is washed with a high salt buffer (0.01M NaH_2PO_4 containing 1.5M NaCl) after binding followed by elution with an acidic buffer.

This study also demonstrates that the use of a Cibracon Blue affinity column (Blue Avidgel P) "in-tandem" with an aza-arenophilic column could be used to remove further contaminating albumin from IgG samples. This could be especially useful with gels such as Gel VII which showed significant albumin binding.

These aza-arenophilic gels, in addition to binding IgG, were also shown to reversibly bind enzymes such as urease, β-galactosidase and peroxidase. The bound enzyme can be effectively desorbed (>80%) using a 0.1M NaOAc buffer (pH 2.9) within 30 minutes. Hence these supports can be used many times and spent bioreactors can be readily regenerated in situ. These gels retained up to 60-80% of their initial activity after 217 days when stored at 4^0C in a phosphate buffer (0.05M, pH 7.0)

This present study also demonstrates that aza-arenophilic bioreactors containing non-covalently immobilized enzymes can be incorporated into flow injection manifolds for the quantitation of analytes. With respect to a urease bioreactor, calibration curves were found to be linear over the urea concentration range of 0.05 – 30 mM with a detection limit of 41 x 10^{-6} M. Up to 20 samples can be manually analysed per hour, with the bioreactor exhibiting good within-day (8h) (0.93% for a 4mM and 1.75% for a 20mM) and day-to-day (2.06% for a

4mM and 2.25% for a 20mM) precisions. Excellent recovery yields were also achieved (98 – 102.8%).

Keywords: aza-arenophilic gels, affinity chromatography, immunoglobulin purification, affinity ligands, capping nucleophiles, heterocyclic base, immobilized enzymes, flow injection systems.