Purity Assessment Following Affinity Separation

Technical Overview

Introduction

PL-SAX 4000Å material has an open pore structure for the optimum separation of very large molecules such as immunoglobulins and at high flow rate delivers fast separations due to particle flow through.

This material was used to assess the purity of polyclonal antibodies (IgGs) after isolation from human serum using an affinity media. The bound polyclonal antibodies were eluted using 0.1 M glycine, pH 2.5, and to retain biological activity the pH was immediately adjusted to neutrality using sodium hydroxide (Figure 1).

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Conditions

Column:	Protein A affinity media, 5 x 50 mm
Binding Buffer:	0.01 M Tris HCl, pH 8
Flow Rate:	1.0 mL/min
Eluting Buffer:	0.1 M Glycine, pH 2.5
Flow Rate:	1.0 mL/min
Detection:	UV, 280 nm

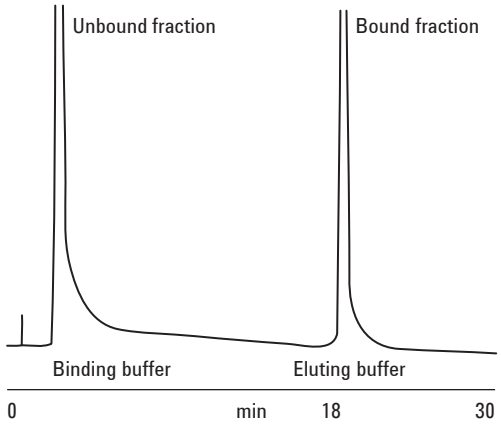

Figure 1. Polyclonal antibodies isolated from serum.

Using the PL-SAX 4000Å 8 µm high speed anion-exchange column the two fractions were chromatographed to determine both the purity of the IgG and also if any IgG was not retained by the affinity column. Each chromatographic run took less than 90 seconds (Figure 2)[1].

Conditions

Column:	PL-SAX 4000Å 8 µm, 4.6 x 50 mm (p/n PL1551-1803)
Eluent C:	0.01 M Tris HCl, pH 8
Eluent B:	C + 0.5 M NaCl, pH 8
Gradient:	Linear 0-100 % B in 2 min
Flow Rate:	4.0 mL/min
Detection:	UV, 280 nm

Peak Identification
1. IgG
2. Transferrin
3. Albumin

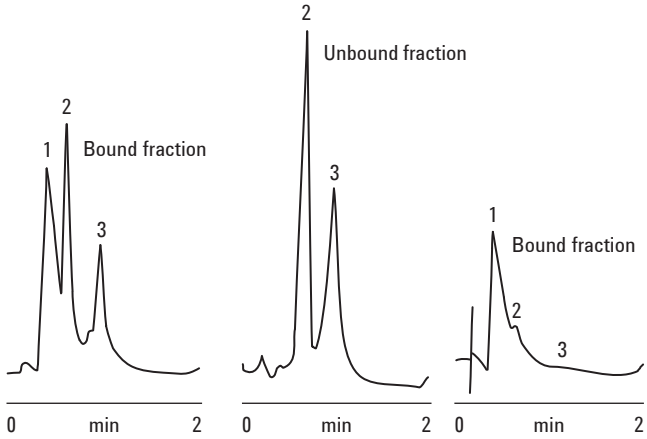

Figure 2. Chromatograms of serum and two polyclonal antibody fractions.

PL-SAX is a hydrophilic strong anion-exchange chromatographic packing material. It combines a rigid macroporous polystyrene/divinylbenzene (PS/DVB) polymer matrix with a chemically stable, quaternized polyethyleneimine coating to allow the analysis of biomolecules over a wide range of mobile phase conditions and pH. The physical stability of the media permits their use with high eluent flow rates and high speed gradients for very rapid separations. This excellent stability ensures both rapid equilibration between separations and the use of aggressive clean-up procedures employing high salt, NaOH, mineral and organic acids, and a wide range of organic solvents.

References

[1] Linda L. Lloyd and Frank P. Warner (1991) High speed analytical and preparative separation of biological macromolecules. In: *D L Pyle (Ed) Separations for Biotechnology. Elsevier Applied Science*.

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