

Application of a Synthetic Ligand Adsorbent for the Purification of Immunoglobulins

Introduction

Affinity adsorbents based on synthetic affinity ligands as opposed to compounds derived from natural sources offer many advantages, particularly robustness, low cost, ease of sanitisation and lack of biological contamination (e.g. viral vectors). Recently developed techniques such as rational design and combinatorial chemistry allow generation of vast array of new synthetic ligand structures which may be utilised in downstream processing. These techniques have been used to identify novel affinity ligands for the purification of immunoglobulins, particularly IgG (1,2). In this presentation, applications and properties of Mimetic A™ A6XL, a newly available optimized affinity adsorbent for large scale purification of antibodies are described.

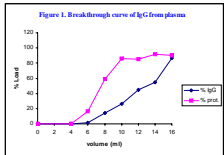
Mimetic A affinity adsorbent provides an effective means for purification of polyclonal antibodies from diverse sources (Table 1).

Table 1. Selectivity of Mimetic A for polyclonal IgG's

Source	efficiency	purity
Bovine (serum)	+++	+++
Chicken (serum)	+	+
Goat (serum)	+++	+++
Goat (whey)	+	++
Human (plasma)	+++	+++
Mouse (ascites)	+++	+++
Porcine (serum)	+++	+++
Rabbit (serum)	+	++
Sheep (serum)	+++	+++

Key:
 +++ +80-100%
 ++ +60-80%
 + +40-60%
 + 20-40%

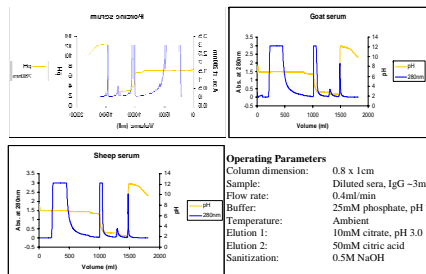
Note, that purity of the eluted IgG's can be considerably improved by chromatography under non-saturating conditions. Capture of IgG from human plasma is illustrated in Figure 1.



Column dimensions: 0.8 x 1cm.
 Sample: diluted plasma, IgG ~3mg/ml
 Flow rate: 0.4ml/min
 Buffer: 25mM phosphate, pH 7.0

The purification of polyclonal IgG from diverse sources is illustrated in Figure 2.

Figure 2. Purification of IgG from mammalian sera.

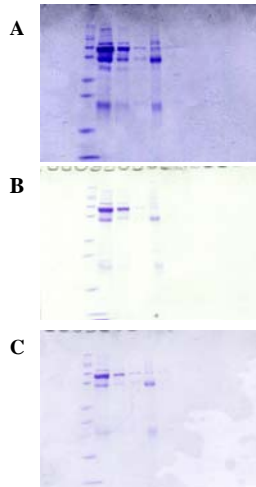


Operating Parameters
 Column dimension: 0.8 x 1cm
 Sample: Diluted sera, IgG ~3mg/ml
 Flow rate: 0.4ml/min
 Buffer: 25mM phosphate, pH 7.0
 Temperature: Ambient
 Elution 1: 10mM citrate, pH 3.0
 Elution 2: 50mM citric acid
 Sanitization: 0.5M NaOH

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The purity of the eluted IgG (Figure 3) is equivalent to that obtained when using Protein A/G based affinity adsorbents.

Figure 3: SDS-PAGE gel showing the purity of the eluted IgG. Lane 1: Molecular weight markers; Lane 2: Load; Lane 3: Non bound fraction; Lane 4: Wash; Lane 5: Eluted IgG.

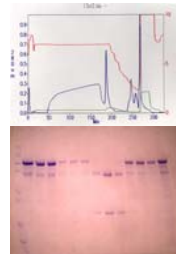


Lane 1: Molecular weight markers
 Lane 2: Load
 Lane 3: Non bound fraction
 Lane 4: Wash
 Lane 5: Eluted IgG

Purification of monoclonal antibodies using Mimetic A.

The application of Mimetic A affinity adsorbent in the purification of murine monoclonal antibody from cell culture medium containing 3 to 10% fetal calf serum is illustrated in Figure 4.

Figure 4: Purification of murine monoclonal antibody from cell culture media containing fetal calf serum.



A: Elution profile (pH gradient elution). Other conditions as in Figure 2
 B: SDS-Page of selected fractions

The recovery and purification of murine monoclonal IgG from a low titre conditioned cell culture medium, containing 5% fetal calf serum and humanised monoclonal antibody from protein free cell culture medium is summarised in Table 2.

Table 2. Purification of murine IgG₂ from low titre cell culture medium and humanised IgG from protein free cell culture medium.

Feed	Load	Recovery
monoclonal IgG, 20µg/ml, in 5% fetal calf serum	1mg/ml adsorbent	0.85 mg/ml adsorbent
Humanised IgG, 1.5mg/ml, in protein free medium	100% breakthrough 10mg	5mg IgG/ml adsorbent

The binding capacity of Mimetic A affinity adsorbent is strongly influenced by the concentration of antibody present in starting sample. In addition, the presence of additives in cell culture (pluronic F68, phenol red) can influence the efficiency of the adsorbent. Consequently, purification of IgG's from cell culture requires optimization. In most instances dialysis against a neutral buffer leads to improved binding of the antibody to the adsorbent.

Properties of synthetic ligand affinity adsorbent

Table 3. Properties of Mimetic A adsorbent

Property	Mimetic A
Ligand Type	Synthetic, defined structure
Operational pH range	2-14
NaOH resistant	Yes
Temperature range °C	4 - 121
Human IgG binding capacity	>25 mg/ml
Binding pH	6 - 9
Elution pH	2 - 4
Undetectable ligand leakage	Yes

Figure 5. Breakthrough curves for pure human IgG

Column dimension: 1.5x6.0 cm
 Buffer: 25mM phosphate, pH 7.0
 IgG: 10mg/ml

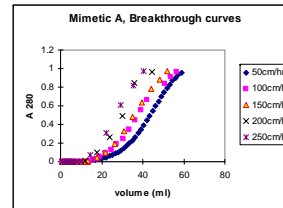


Figure 6. Flow properties of Mimetic A
 Column dimension: 3.2x 16 cm

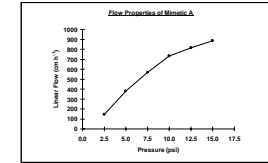


Figure 7. Dynamic binding capacity of Mimetic A for pure human IgG at 10% breakthrough
 Operating parameters as for Figure 5

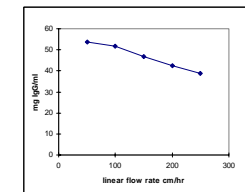
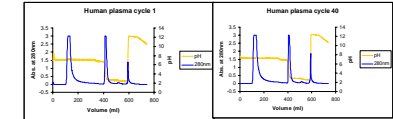


Figure 8. Re-use of Mimetic A
 Conditions as for Figure 2



SDS-Page gel of human IgG purification from plasma, cycle 40

Conclusions

Stable synthetic ligand affinity adsorbents such as Mimetic A offer alkali stable chromatography matrix which may be used for effective purification of IgG's from diverse sources.

References

- (1) Rongxiu, L., Dowd, V., Stewart, D.J., Burton, S.J. and Lowe, C.R. (1998), 'Design, Synthesis and application of Protein A mimetic', *Nature Biotechnology*, **16**, 190-201.
- (2) Teng, S.F., Sproule, K., Hussain, A. and Lowe, C.R. (1999), 'A Strategy for the Generation of Biomimetic Ligands for Affinity Chromatography. Combinatorial Synthesis and Biological Evaluation of an IgG Binding Ligand', *J.Mol.Rec.*, **12**, 67-75.

Acknowledgement:

We acknowledge members of ProMetic BioSciences for their contribution to this work.



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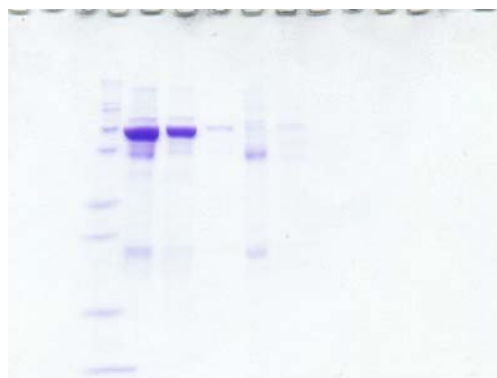
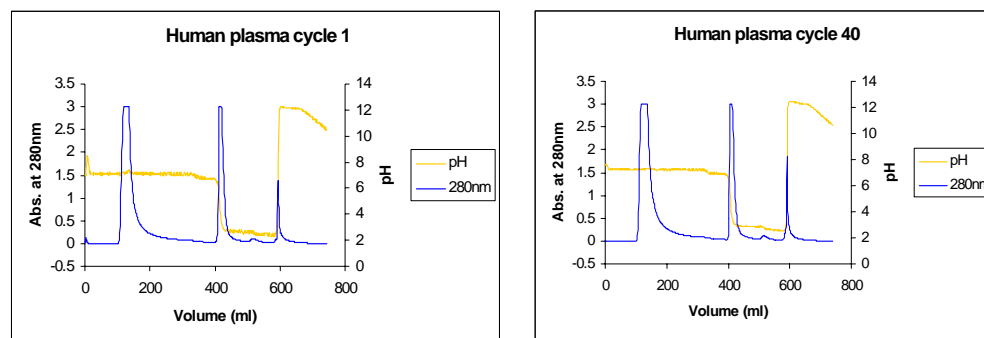


Figure 7. Dynamic binding capacity of Mimetic A for pure human IgG at 10% breakthrough

Operating parameters as for Figure 5

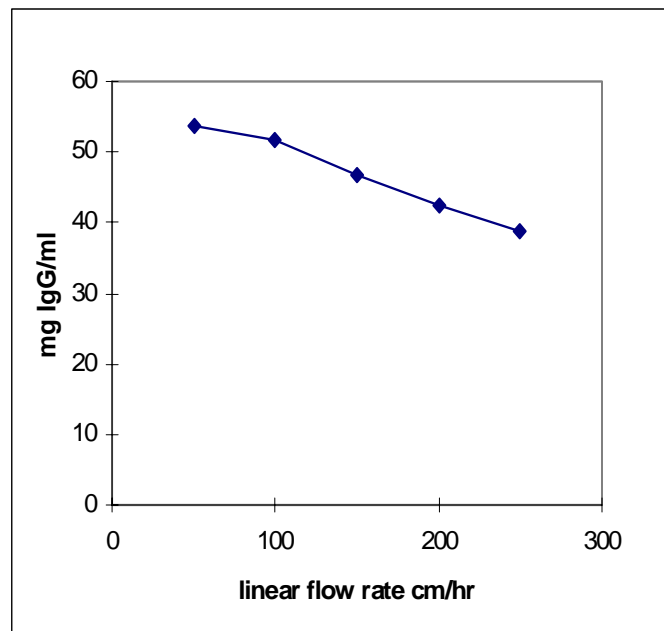


Figure 5. Breakthrough curves for pure human IgG

Column dimension: 1.5x6.0 cm

Buffer: 25mM phosphate, pH7.0

IgG: 10mg/ml

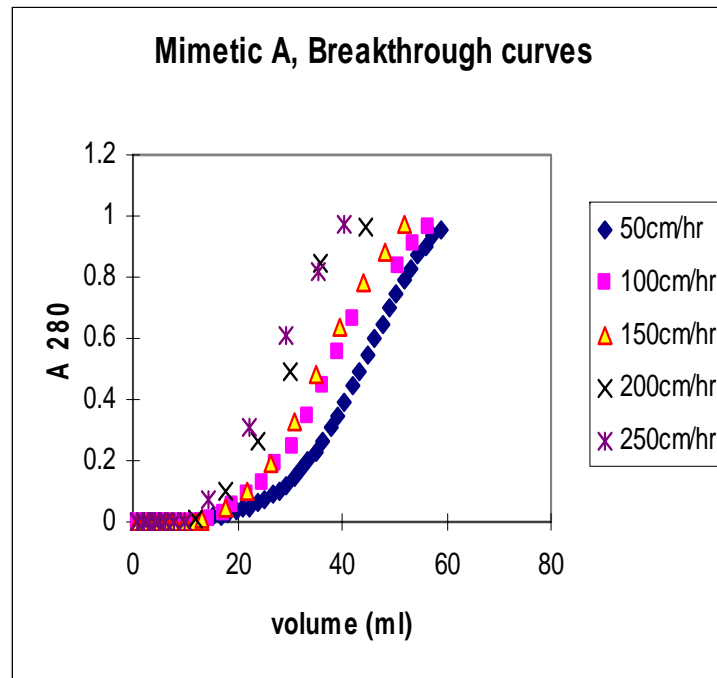


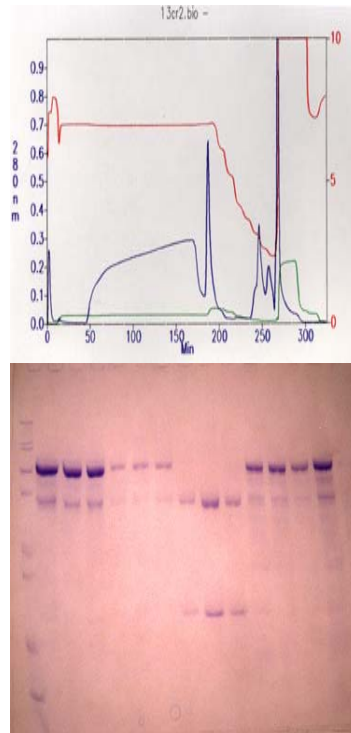
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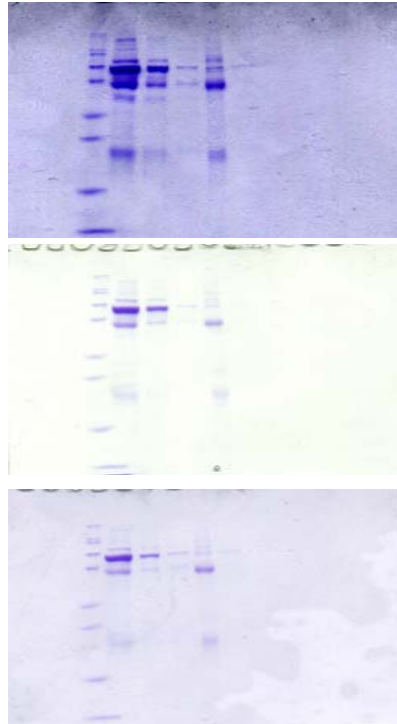
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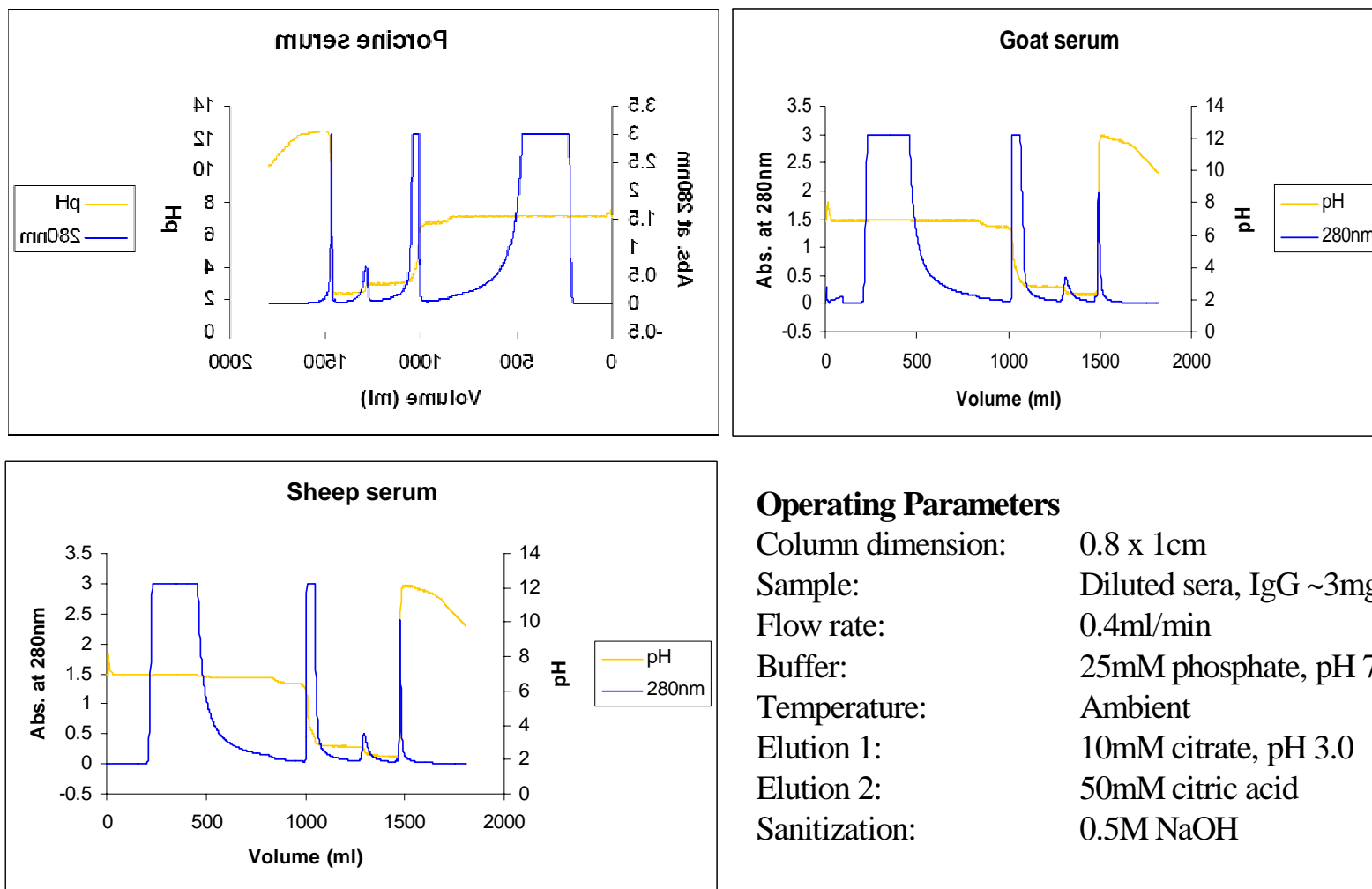
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Other conditions as in Figure 2)
B: SDS-Page of selected fractions

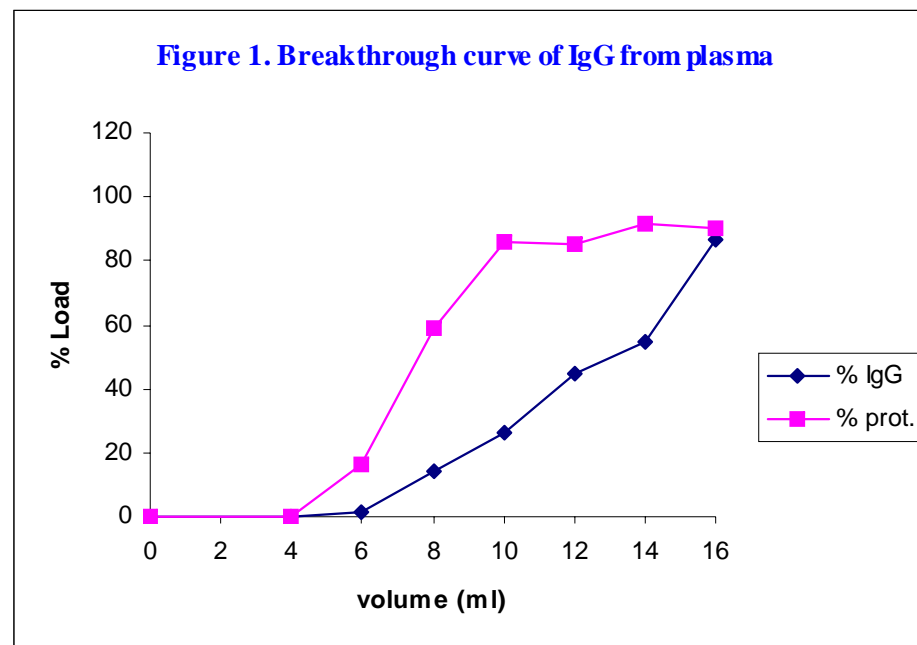
Western blot analysis



Lane 1: Molecular weight markers
Lane 2: Load
Lane 3: Non bound fraction
Lane 4: Wash
Lane 5: Eluted IgG

Figure 2: Purification of IgG from mammalian Serum





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Rabbit (serum)	+	++
Sheep (serum)	++++	++++

Key:

- ++++ 80-100%
- +++ 60-80%
- ++ 40-60%
- + 20-40%

Figure 6. Flow properties of Mimetic A

Column dimension: 3.2x 16 cm

