Novel TOYOPEARL® Hydrophobic Interaction Chromatographic Resin for Antibody Purification

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Introduction

Hydrophobic interaction chromatography (HIC) is an effective tool for the purification of biomolecules at analytical and preparative scales, especially in the intermediate purification step for proteins. In this mode, chromatographic performance is affected by various properties of the stationary phase as well as mobile phase variables. The selection of a HIC resin is key to obtaining high recovery, lower pressure drop and good selectivity.

A high binding capacity HIC resin, TOYOPEARL® Phenyl-600M, was developed for preparative chromatography. This resin was prepared by optimizing the pore size of base resin and the surface modification for the binding/elution of human immunoglobulin G (IgG). TOYOPEARL® resins are macroporous, rigid polymethacrylate beads, which show good pressure/flow properties resulting in fasterprocess throughput.

Phenyl-600M showed an excellent IgG binding capacity compared to commercially available phenyl-agarose and conventional TOYOPEARL® HIC resins. We report here the fundamental features of the resin and its application to antibody purification.

Experimental

Stationary Phases

Name	Matrix	Ligand	Mean diameter [μm]	
TOYOPEARL Phenyl-600M	polymethacrylate	phenyl	65	Developed in this study
TOYOPEARL Phenyl-600M	polymethacrylate	phenyl	65	Existing product suitable for protein purification
TOYOPEARL Butyl-600M	polymethacrylate	butyl	65	Existing products suitable for antibody purification
TOYOPEARL PPG-600M	polymethacrylate	Poly (propylene glycol)	65	
Phenyl Sepharose 6 Fast Flow (high sub)	agarose	phenyl	90	Commercially available phenyl-agarose

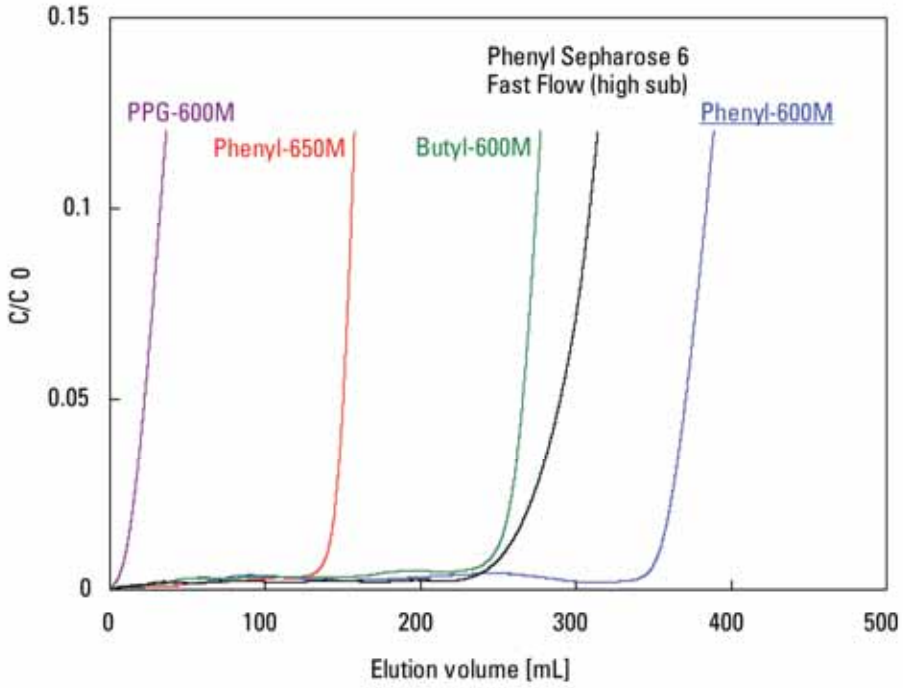
Chromatographic apparatus

- ☐ Pump: CCPM-II (Tosoh)
- ☐ UV detector: UV-8020 (Tosoh)
- ☐ Column oven: CO-8020 (Tosoh)

Sample (proteins)

- ☐ γ-globulin (human polyclonal IgG) (Kaketusken, Japan)
- ☐ Ribonuclease A from bovine pancreas (Sigma, USA)
- ☐ Lysozyme from chicken egg white (Sigma, USA)
- ☐ α-chymotrypsinogen A from bovine pancreas (Sigma, USA)

Figure 1. Breakthrough curves of γ-globulin



Column:	7.8mm ID X 20cm (9.6mL bed volume)
Feed:	1g/L g-globulin in 0.8mol/L $(NH_4)_2SO_4$, 0.1 mol/L sodium phosphate (pH 7.0)
Wash:	1.0mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Elution:	0.1mol/L sodium phosphate (pH 7.0)
Gradient:	stepwise
Flow rate:	2.39mL/min (300cm/hr)
Temperature:	25°C
Detection:	UV @ 280nm

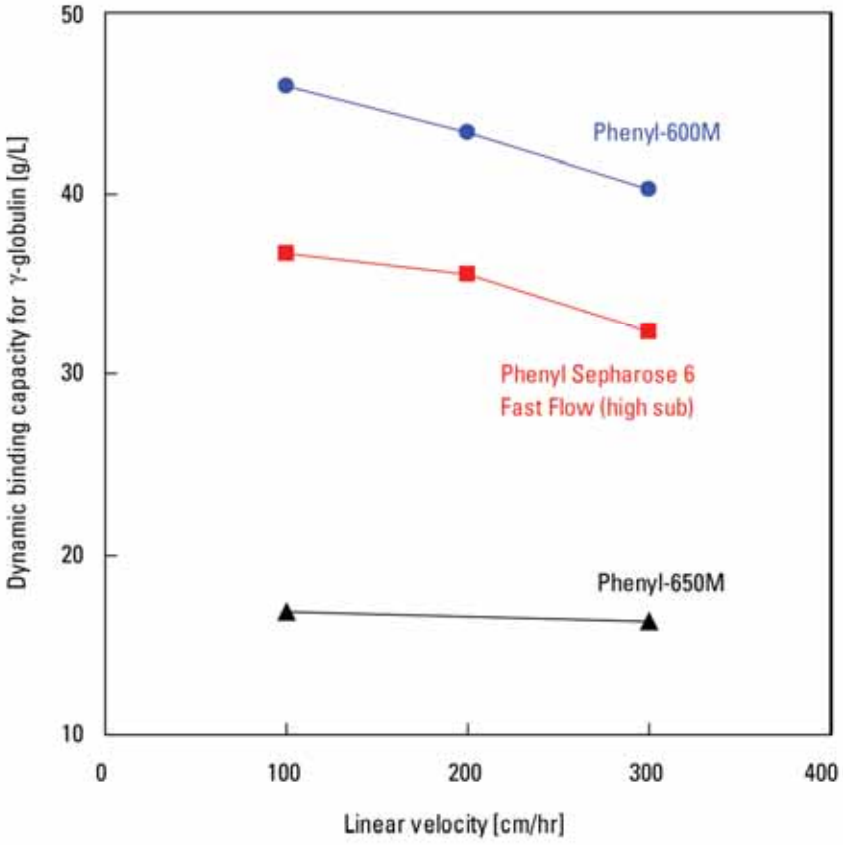
Table 1. Dynamic binding capacity, recovery and yield for γ-globulin

Resin	Dynamic binding capacity at 300 cm/hr [g/L-gel]	Recovery [%]	Yield [g/L-gel]
Phenyl -600M	40	91	36
Phenyl -650M	16	95	15
Butyl-600M	29	87	25
PPG -600M	3.4	-	-
Phenyl Sepharose 6 Fast Flow (high sub)	32	87	28

- Dynamic binding capacity was calculated at 10% breakthrough (Fig. 1).
- Yield [g/L-gel] = Dynamic binding capacity [g/Lgel] x Recovery [%] x 0.01
- Elution conditions are described in Fig. 1.

Phenyl-600M showed the highest binding capacity for γ-globulin compared to the other resins tested. In addition, the highest productivity was obtained due to high recovery.

Figure 2. Comparison of dynamic binding capacity at different linear velocities

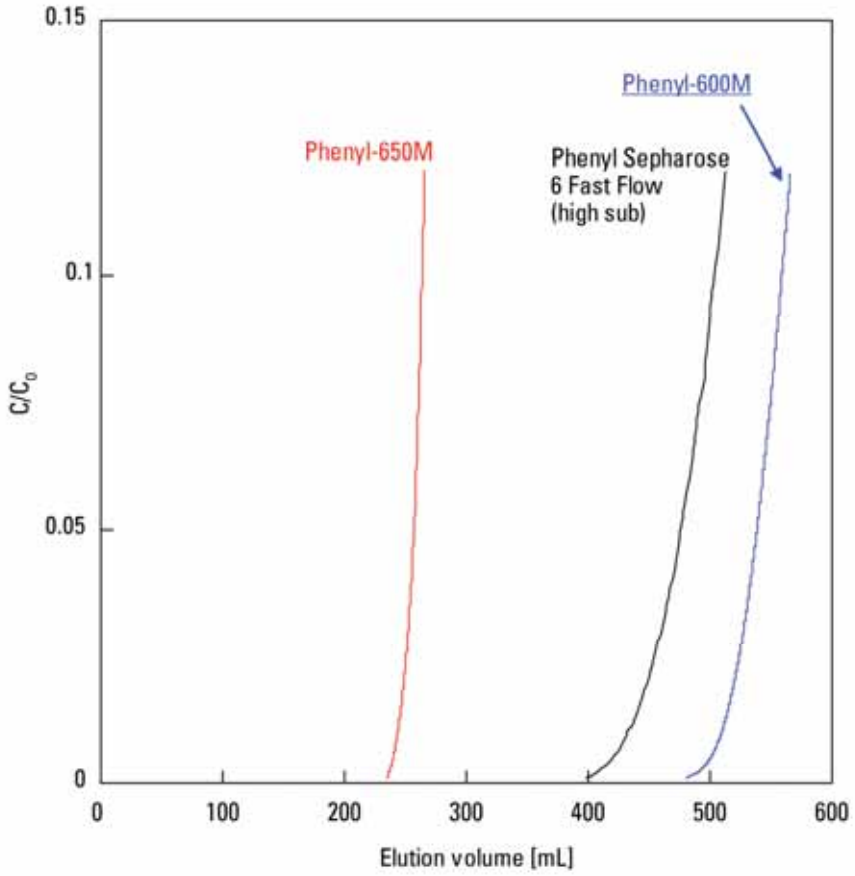


Column:	7.8mm ID X 20cm (9.6mL bed volume)
Feed:	1g/L g-globulin in 0.8mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Flow rate:	0.8mL/min (100cm/hr) 1.59mL/min (200cm/hr) 2.39mL/min (300cm/hr)
Temperature:	25°C
Detection:	UV @ 280nm

Dynamic binding capacity was calculated at 10% breakthrough.

Phenyl-600M showed higher γ-globulin binding capacity than the other phenyl resins at the linear velocities tested.

Figure 3. Breakthrough curves of lysozyme



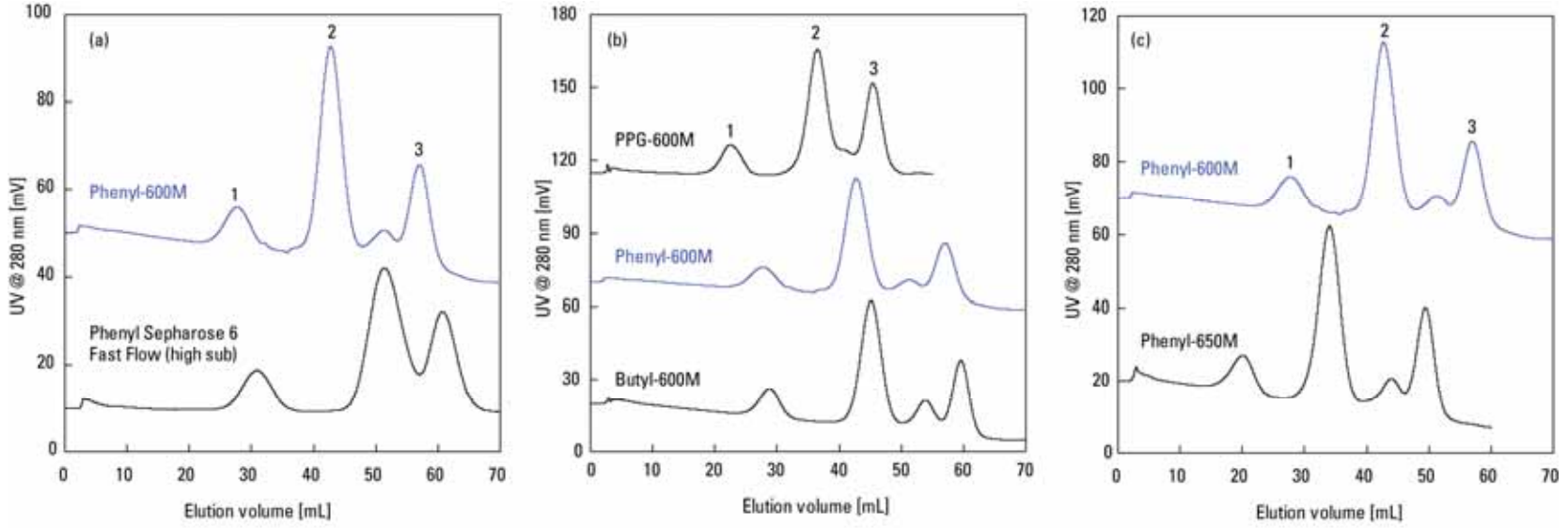
Resin	Dynamic binding capacity at 300 cm/hr [g/L-gel]
Phenyl-600M	58
Phenyl-650M	27
Phenyl Sepharose 6 Fast Flow (high sub)	52

Column: 7.8mm ID X 20cm (9.6mL bed volume)
Feed: 1g/L lysozyme in 1.8mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Flow rate: 2.39mL/min (300cm/hr)
Temperature: 25°C
Detection: UV @ 280nm

☐ Dynamic binding capacity was calculated at 10% breakthrough.

Phenyl-600M exhibited a high lysozyme binding capacity.

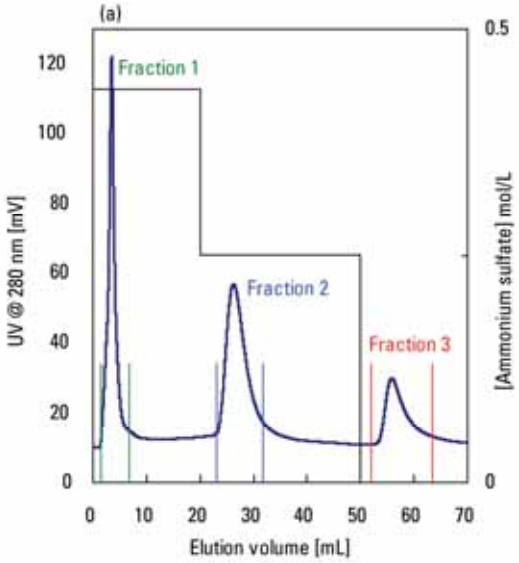
Figure 4. Protein separation on the HIC resins

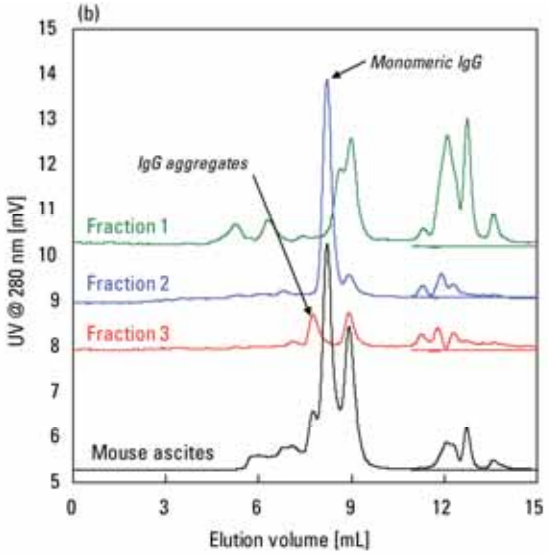


Column:	7.5mm ID X 7.5cm (3.3mL bed volume)
Sample:	1g/L of ribonuclease A (1), lysozyme (2) a-chymotrypsinogen A (3) in 0.72mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Sample load:	100µL
Gradient:	60min. linear gradient from buffer A to B
Buffer A:	1.8mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Buffer B:	0.1mol/L sodium phosphate (pH 7.0)
Flow rate:	1mL/min (136cm/hr)
Temperature:	Room temperature (ca. 25°C)
Detection:	UV @ 280nm

(a) Phenyl-600M showed different selectivity of the test proteins compared with Phenyl Sepharose 6 Fast Flow (high sub).
(b) The hydrophobicity of Phenyl-600M was between PPG-600M and Butyl-600M.
(c) While selectivity was similar to Phenyl-650M, the retention was stronger than Phenyl-650M.

Figure 5. Purification of monoclonal IgG in mouse ascites with TOYOPEARL Phenyl-600M





Column:	7.5mm ID X 7.5cm (3.3mL bed volume)
Sample:	Mouse ascites containing monoclonal IgG in 0.45mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Sample load:	100µL
Gradient:	Stepwise (0-20min. :buffer A, 20-50min. :buffer B 50-70min. :buffer C)
Buffer A:	0.43mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Buffer B:	0.25mol/L $(NH_4)_2SO_4$, 0.1mol/L sodium phosphate (pH 7.0)
Buffer C:	0.1mol/L sodium phosphate (pH 7.0)
Flow rate:	1mL/min (136cm/hr)
Temperature:	Room temperature (ca. 25°C)
Detection:	UV @ 280nm

Column:	TSKgel® G3000SW$_{XL}$ (7.8mm ID X 30cm)
Sample load:	100µL
Eluent:	0.3mol/L NaCl, 0.05mol/L sodium phosphate (pH 7.0)
Flow rate:	1mL/min
Temperature:	Room temperature (ca. 25°C)
Detection:	UV @ 280nm

(a) Separation of mouse ascites containing monoclonal IgG utilizing a decreasing salt concentration step gradient.
(b) HPLC (gel filtration chromatography) analysis of the mouse ascites feedstock and fractions 1-3 (Fig. 5a).
In fraction 2, monomeric IgG was efficiently separated from impurities, especially high molecular weight IgG aggregates.

Conclusions

We have demonstrated the following characteristics of TOYOPEARL® Phenyl-600M:

1. High dynamic binding capacity and recovery for proteins (Figure 1-3, Table 1)
2. Moderate hydrophobicity (Figure 4)
3. Different selectivity compared to commercial HIC resins (Figure 4)
4. Applicable to IgG purification (Figure 5)

These results indicate that this new phenyl resin can be a powerful tool for chromatographic purification of antibodies.