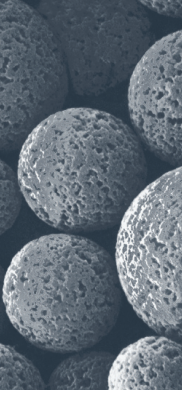

CHROMATOGRAPHY

UNOsphere™ S Cation Exchange Support

- Efficient capture of biopharmaceutical molecules from crude feedstreams
- Ultrahigh binding capacities at fast linear velocities
- Hydrophilic polymeric beads engineered for high mechanical stability and low backpressures
- Robust polymer designed to withstand repetitive clean-in-place cycles
- Biopharmaceutical manufacturing quantities available
- Fully supported for regulatory submission

Achieve High Productivity Using UNOsphere S Cation Exchange Support

Be Productive

In the bioprocess industry, the isolation of biomolecules from crude feedstock is one of the most demanding chromatographic steps in the downstream process.

Biopharmaceutical manufacturers are under increasing economic pressure to reduce drug production costs.

These factors require the media used in the capture step to have very high binding capacities at fast linear velocities while maintaining low column backpressure.

UNOsphere is a patented* new-generation polymeric support, based on a single-step polymerization process that delivers high productivity in the capture step.

UNOsphere Polymer Technology

The genesis of UNOsphere support is based on the single-step polymerization process used to prepare UNO® continuous-bed columns. Incorporation of the sulfonic acid ligand into the matrix during polymerization leads to consistent batch-to-batch reproducibility. UNOsphere beads are macroporous (>2,000 Å), leading to fast binding kinetics and high binding capacities (Table 1). Careful selection of monomers and crosslinkers provides unrivaled base stability and bead rigidity.

* US patent 6,423,666 B1.

Properties of UNOsphere S Support

Most production chromatography systems have maximum pressure limits of 3 bar. The median particle size of UNOsphere S support is 80 µm, which generates a backpressure less than 1.5 bar at 1,200 cm/hr (Figure 1). The highly macroporous nature of UNOsphere S support provides high binding capacities that range from 30–60 mg IgG/ml support in the linear velocity range of 150–1,200 cm/hr (Figure 1).

Harsh conditions, such as clean-in-place and corrosive buffer systems, may affect the long-term stability of chromatographic media. The robustness of UNOsphere S support allows it to survive these conditions with minimal loss of performance (Table 1).

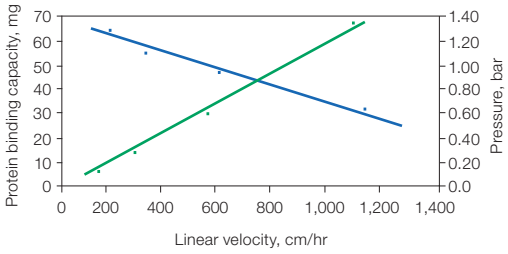

Fig. 1. Binding and backpressure properties of UNOsphere S support. Column size, 1.1 x 20 cm; sample, 2 mg/ml human IgG; buffer, 50 mM sodium acetate, pH 5.0. (—), backpressure; (—), 10% breakthrough capacity.

BIO-RAD

Table 1. Properties of UNOsphere S support.

Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Total ionic capacity	280 ± 30 µeq/ml
Dynamic binding capacity*	
150 cm/hr	60 mg IgG/ml
1,200 cm/hr	30 mg IgG/ml
Median particle size	80 µm
Recommended linear flow rate range**	50–1,200 cm/hr
Chemical stability	
1.0 M NaOH (20°C)	Up to 2,000 hr
1.0 M HCl (20°C)	Up to 200 hr
pH stability range	pH 1–14
Regeneration conditions	1–2 M NaCl
Storage conditions	20% Ethanol 0.1 N NaOH for 30 days

* 10% breakthrough capacity determined with 4.5 mg/ml human IgG in a 1.1 x 10 cm column.

** UNOsphere S support packed into a 20 cm bed height and run at 1,200 cm/hr generates less than 2 bar backpressure.

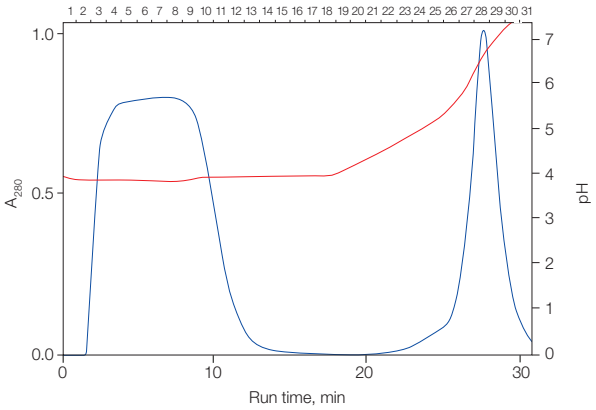

Fig. 2. Purification of murine IgG_1 on UNOsphere S column. Column size, 0.5 x 10 cm (2 ml); sample, 15 ml (6.6 mg) of murine IgG_1-conditioned medium. The sample was loaded onto the column in 20 mM citrate-phosphate buffer, pH 4.0, washed, and eluted in a linear gradient of 0–100% 20 mM citrate-phosphate, pH 8.0 in 10 column volumes at a flow rate of 2.0 ml/min (600 cm/hr). Each fraction was 2.0 ml. (—), A_{280}; (—), buffer pH.

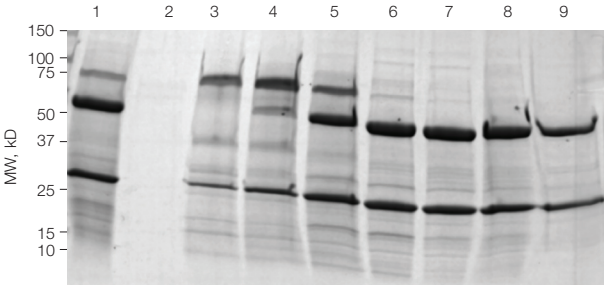

Fig. 3. SDS-PAGE gel of UNOsphere-purified murine IgG_1. Fractions from the chromatography run shown in Figure 2 were separated on a 4–20% precast gel. On left, reference standards; lane 1, conditioned medium; lane 2, flowthrough; lanes 3–8, fractions 25, 26, 27, 28, 29, and 30; lane 9, protein A-purified murine IgG_1 from culture medium.

Capture Performance

UNOsphere S support is designed for high-efficiency capture of monoclonal antibodies from crude feedstreams. Murine IgG_1 (6.6 mg) was captured and eluted from a 2 ml UNOsphere S column (Figures 2 and 3); assays of the load and eluate demonstrated a recovery of 97%. No antibody was detected in the flowthrough (Figure 3, lane 2). The 10% breakthrough capacity for this murine IgG_1 antibody is 12.8 mg/ml (at 600 cm/hr) from conditioned medium.

Technical Assistance

Regulatory Support Files are available upon request. Bio-Rad Laboratories is an ISO 9001 registered corporation. For additional information and technical assistance, contact your local Bio-Rad office. In the USA and Canada, call 1-800-4-BIORAD.

Visit us on the Web at **www.bio-rad.com/unosphere/** for more information on Bio-Rad's complete line of process chromatography media.

For more information about UNOsphere S support, request bulletins 2678, 2774, 2780, and 2849. For more information about the chromatographic performance of UNOsphere support, refer to the Bibliography. (Note that the authors refer to UNOsphere as BRX).

Bibliography

Hunter AK and Carta G, Protein adsorption on novel acrylamido-based polymeric ion exchangers. I. Morphology and equilibrium adsorption, J Chromatogr A 897, 65–80 (2000)

Hunter AK and Carta G, Protein adsorption on novel acrylamido-based polymeric ion exchangers. II. Adsorption rates and column behavior, J Chromatogr A 897, 81–97 (2000)

Ordering Information

Catalog #	Description
156-0111	UNOsphere S Support, 25 ml
156-0113	UNOsphere S Support, 100 ml
156-0115	UNOsphere S Support, 500 ml
156-0117	UNOsphere S Support, 10 L

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*