UNOsphere™ S Resin Technical Data

Tech Note

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Chromatography

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Abstract

UNOsphere S is a cation exchange resin. It can be used for efficient separation of proteins, nucleic acids, viruses, plasmids, and other biomolecules. It delivers high productivity in the capture step, owing to its high binding capacity at fast linear velocities, while maintaining low column backpressure. In this tech note, we highlight the different properties of UNOsphere S Resin. We also show a comparative analysis of this resin with two other commercially available cation exchange resins and demonstrate that UNOsphere S Resin is relatively superior in terms of its productivity/g/L/hr, making it an ideal capture resin in biopharmaceutical manufacturing.

Introduction

UNOsphere S is a high-capacity, high-throughput cation exchange capture resin for process chromatography based on acrylamido and vinylic monomers. Unlike conventional resins, this one is produced in a single reaction in which monomer, ligands, and crosslinker together produce the final derivatized species, enhancing manufacturing reproducibility. This resin was characterized with respect to dynamic protein binding capacity, protein recovery, pressure and flow properties, packing efficiency, and base stability. Comparative studies were done with commercially available resins.

Capacity, Recovery, and Productivity

The resin is designed with large-diameter pores and a high surface area to maximize capture speed and macromolecule capacity. UNOsphere S is highly competitive with other process resins that have similar functional groups when compared at a constant operating pressure of 14.7 psi (Table 1).

The high productivity exhibited for UNOsphere S Resin is due in part to its open architecture and low backpressure at high flow rates (Figure 1).

The capacity of UNOsphere S, like other resins, is inversely related to linear velocity. Figure 2 shows that for a 20 cm bed height column, dynamic protein binding capacity ranges from about 60 to about 30 g/L over the velocity range of 150 to 1,200 cm/hr.

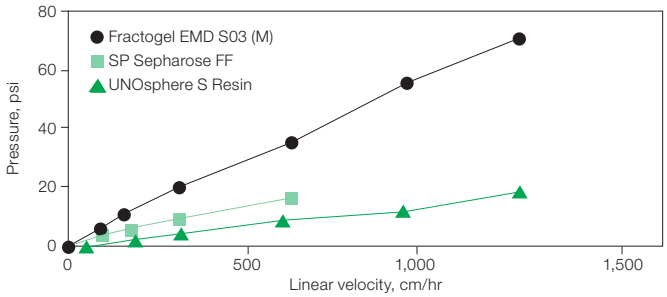

Fig. 1. Pressure/flow comparison for cation exchange resins. Conditions were as in Table 1. The SP Sepharose FF was not run faster than 600 cm/hr, as recommended by manufacturer's literature.

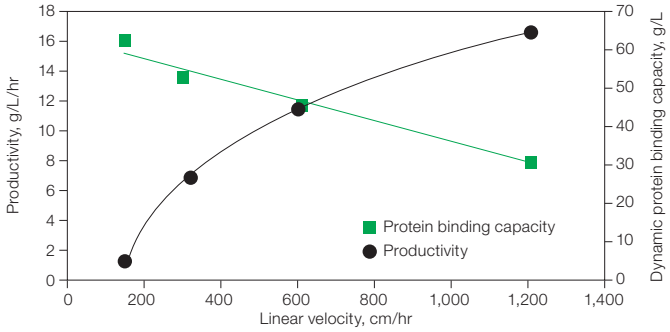

Fig. 2. Protein binding capacity and productivity of UNOsphere S Resin. Conditions were as in Table 1.

Table 1. Comparison of three commercial cation exchange resins. Studies used a 1.1 x 20 cm (20 ml) column equilibrated with 20 mM sodium acetate buffer, pH 5.0 (buffer A). Human IgG (polyclonal, 1.0 mg/ml) was in buffer A. Elution was with buffer A containing 0.5 M NaCl (buffer B). Chromatography was performed on a BioLogic DuoFlow™ System (Bio-Rad Laboratories, Inc.) at 14.7 psi.

Resin	Linear Velocity, cm/hr	Recovery, %	Human IgG Binding Capacity, g/L	Process Time, hr	Productivity, g/L/hr
UNOsphere S Resin	1,100	98.6	32.0	0.60	53.3
SP Sepharose FF	500	97.8	14.3	0.77	18.5
Fractogel EMD SO3 (M)	231	97.0	66.4	6.50	10.2

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Productivity continues to increase even at 1,200 cm/hr. The productivity of UNOsphere S compares favorably at 14.7 psi constant pressure with that of other process resins (Figure 3).

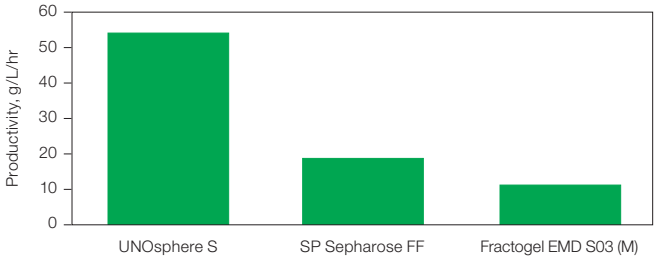

Fig. 3. UNOsphere S Resin productivity comparison. Conditions were as in Table 1.

Pressure/Flow Performance

UNOsphere S Resin was designed to achieve the highest productivity (grams of drug per operational hour per liter of resin) possible. UNOsphere Resins can be run at the highest linear velocities and loading capacities allowed by the column and chromatography system. Figure 4 illustrates the pressure/flow performance for UNOsphere S Resin.

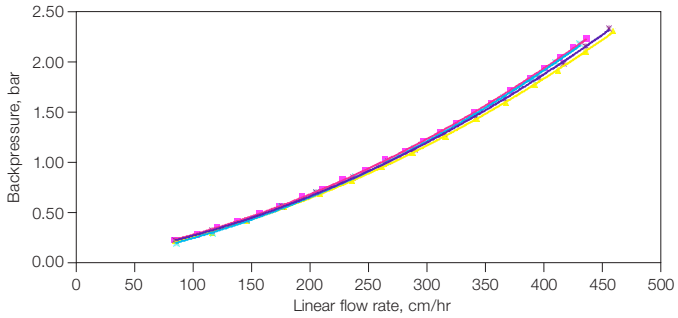

Fig. 4. Normalized pressure/flow curves for comparison of running buffers on UNOsphere S. 10 mM sodium acetate, 150 mM NaCl, pH 5.0 (—); 0.1 M NaCl (—); 1.0 M NaCl (—); PBS, pH 7.0 (—).

Efficiency

Van Deemter analysis of a column packed with UNOsphere S Resin showed that efficiency was, as expected, higher at very low flow rates but quite good at rates up to 1,200 cm/hr (Figure 5). Asymmetry did not vary over the entire experiment, indicating that the resin packed very uniformly and no channeling or interaction of sample with the resin occurred.

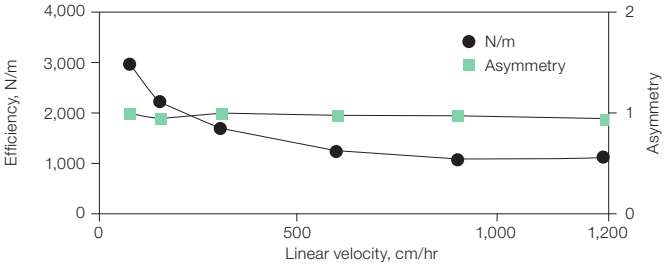

Fig. 5. Efficiency analyses of UNOsphere S Resin packing. Column was 1.1 x 20 cm; 50 µl of 10% acetone in water was injected.

Base Stability

Resistance to sanitization or storage in NaOH solution is of considerable importance for a process chromatography resin. Thus far we have collected data at up to 10,000 hr of storage in 0.1 and 1.0 N NaOH. Figure 6 shows little effect on the dynamic binding capacity of UNOsphere S Resin with either concentration of NaOH.

Retention times for several test proteins were virtually identical at up to 10,000 hr of storage in 1.0 N NaOH (Figure 7).

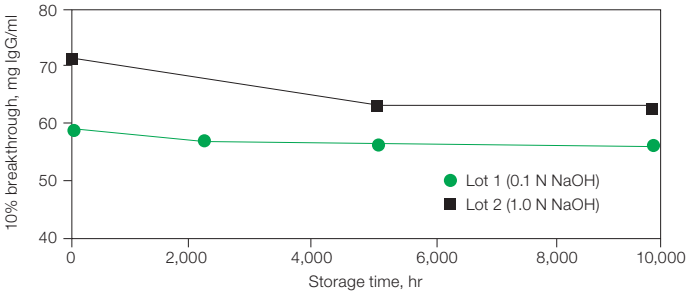

Fig. 6. Resistance to storage in NaOH. IgG solution, 4.4 mg/ml in 50 mM sodium acetate buffer, pH 5.0 (binding buffer), was loaded on a 2 ml column at 300 cm/hr. At 10% breakthrough (BT), loading was stopped and the column was washed with binding buffer until the absorbance at 280 nm was 2% of the test solution. The bound protein was eluted with 20 mM Tris-HCl buffer, pH 9.0, containing 0.5 M NaCl, and recovery was determined by extinction at 280 nm.

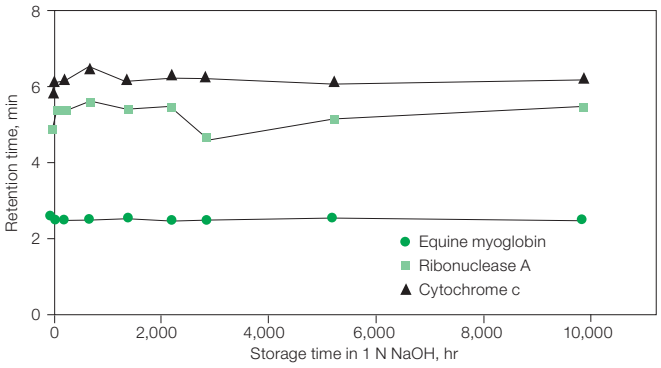

Fig. 7. Effect of NaOH on retention time. Resin was incubated in 2 volumes of 1.0 N NaOH, which was replaced weekly throughout the test cycle. At the predetermined test interval, a small aliquot (~10 ml) was removed, washed, and packed in a Bio-Scale™ Chromatography Column. Retention times were determined with Bio-Rad anion exchange standards.

Solvent Effects

High salt or chaotropic agent concentrations are often required for chromatography of inclusion body proteins, column regeneration, and other purposes. Table 2 shows that addition of such chemicals does not raise column pressure much above atmospheric pressure at most flow rates. For other properties of UNOsphere S Resin, see Table 3.

Table 2. Column pressure in various test solvents over a range of linear velocities. UNOsphere S Resin was suspended in 1 M NaCl and packed into a 1.1 x 20 cm column at 1,200 cm/hr. The column was equilibrated with 20 mM sodium acetate, pH 5.0, and then with test solvent and run at the velocities indicated. Between tests, the column was reequilibrated with sodium acetate.

	Pressure (psi) at Given Linear Velocity, cm/hr				
Solvent	**150**	**300**	**600**	**900**	**1,200**
20 mM sodium acetate, pH 5.0	2	5	7	10	13
1 M NaCl	2	5	8	12	15
1 M NaOH	4	8	12	17	24
6 M guanidine-HCl	5	12	21	32	44
8 M urea	9	16	22	ND*	ND*

* ND, not determined.

Table 3. Properties of UNOsphere S Resin.

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	80 µm
Total ionic capacity	269 ± 50 meq/ml
Dynamic binding capacity	60 mg IgG/ml at 150 cm/hr 10% BT capacity determined with 4.5 mg/ml hIgG in 1.1 x 10 cm column
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2.0 bar at flow rate of 1,200 cm/hr (20 x 20 cm packed bed, 1.17 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15–1.20
pH stability	1–14
Shipping solution	20% ethanol or 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	
1.0 M NaOH (20°C)	Up to 2,000 hr
1.0 M HCl (20°C)	Up to 200 hr*
Shelf life	5 years

* Data derived under accelerated conditions at 60°C.

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 1-800-424-6723.

Visit **bio-rad.com/web/UNOsphereSData** for more information about Bio-Rad's UNOsphere S Resin.

For more technical information, request bulletins 2774, 2780, 2849, and 6713. For more information about the chromatographic performance of UNOsphere Resin, refer to the bibliography (note that the authors refer to UNOsphere Resin as BRX).

Conclusions

UNOsphere S Resin has high capacity and recovery at high linear velocity. Dynamic binding capacity, pressure/flow properties, and productivity compared favorably with other process chromatography resins. The resin retained these favorable pressure/flow properties in the presence of common chaotropic agents and at various pH values and salt concentrations. Long-term storage in 0.1–1.0 N NaOH caused minimal reduction in dynamic binding capacity and had essentially no effect on retention times for marker proteins.

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