UNOsphere™ Q Support Technical Data

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Introduction

UNOsphere Q is a new high-capacity, high-throughput anion exchange capture support, based on acrylamide and vinylic monomers, designed for process chromatography. Unlike conventional beads, this new support is produced in a single reaction in which monomer, ligands, and cross-linker together produce the final derivatized species, enhancing manufacturing reproducibility. This support 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 supports.

Capacity, Recovery, and Productivity

The new support was designed with large-diameter pores and a high surface area to maximize capture speed and macromolecule capacity. UNOsphere Q was found to be highly competitive with other process supports having similar functional groups when compared at a constant operating pressure of 14.7 psi (Table 1). Studies used a 1.1 x 20 cm (20 ml) column equilibrated with 10 mM Tris buffer, pH 8.5 (buffer A). BSA (5.0 mg/ml in buffer A) was loaded until 10% breakthrough occurred. Elution was performed with buffer A containing 0.5 M NaCl (buffer B). Chromatography was performed on a BioLogic DuoFlow™ system.

The high productivity exhibited by UNOsphere Q is probably due, in part, to its open architecture and low backpressure at high flow rates (Figure 1).

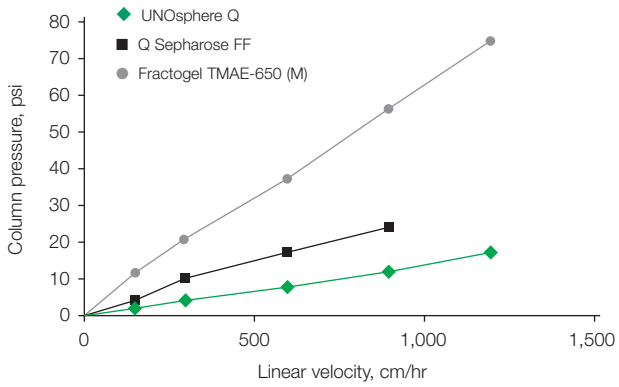

Fig. 1. Pressure/flow comparison for anion exchange supports. Conditions were as in Table 1. The Q Sepharose FF was run below 600 cm/hr, as recommended in manufacturer's literature.

Table 1. Comparison of properties of UNOsphere Q, Q Sepharose, and Fractogel TMAE supports.

Support	Linear Velocity (cm/hr)	Recovery (%)	BSA Binding Capacity (g/L)	Process Time (hr)	Productivity (g/L/hr)
UNOsphere Q	615	100.0	120.0	1.58	75.0
Q Sepharose FF	300	99.0	23.0	1.19	19.0
Fractogel EMD TMAE (M)	105	99.0	82.0	5.04	16.0

Properties were evaluated on a 1.1 x 20 cm (20 ml) column equilibrated with 10 mM Tris buffer, pH 8.5 (buffer A). BSA (5.0 mg/ml in buffer A) was loaded until 10% breakthrough occurred. Elution was performed with buffer A containing 0.5 M NaCl. Chromatography was performed on a BioLogic DuoFlow system.

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The capacity of UNOsphere Q, like other supports, is related to linear velocity. Figure 2 shows that for a 20 cm bed height column, dynamic binding capacity ranges from about 180 down to about 125 mg/ml over the velocity range of 150–1,200 cm/hr.

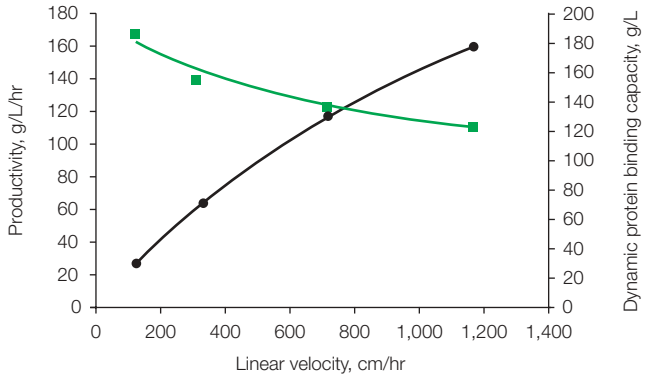

Fig. 2. Protein binding capacity (—) and productivity (—) of UNOsphere Q. Conditions were as in Table 1.

An inverse relationship exists between dynamic binding capacity and productivity, and the latter continues to increase even at 1,200 cm/hr. Productivity for UNOsphere Q compares favorably, at 14.7 psi constant pressure, with that of other process media (Figure 3).

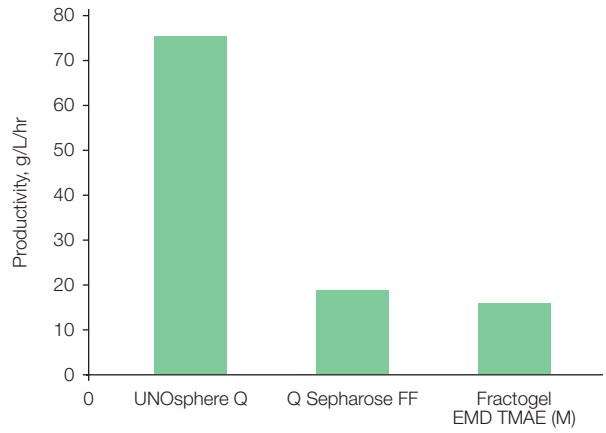

Fig. 3. UNOsphere Q productivity comparison. Conditions were as in Table 1.

Efficiency

Van Deemter analysis of an UNOsphere Q column showed that efficiency was, as expected, higher at very low flow rates but remained good at rates up to 1,200 cm/hr (Figure 4). Asymmetry was found not to vary over the entire experiment, indicating that the resin packed very uniformly and that no channeling or interaction of the sample with the support occurred.

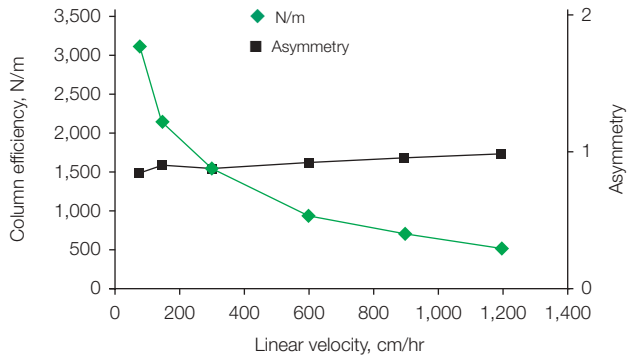

Fig. 4. Efficiency analysis of UNOsphere Q packing. The column was 1.1 x 20 cm, and was injected with 50 µl of 10% acetone in water. N is the number of theoretical plates, and L is the column length.

Base Stability

Resistance to NaOH during sanitization or storage is of considerable importance for a process chromatography support. We have collected data at up to 10,000 hr of storage in 0.1 and 1.0 N NaOH. Figure 5 shows that little effect on the dynamic binding capacity of UNOsphere Q is seen with either concentration of NaOH.

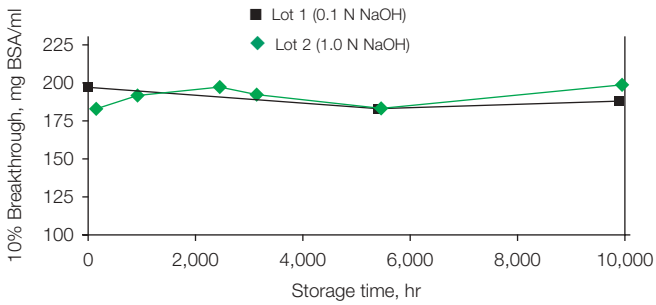

Fig. 5. Dynamic binding capacity of UNOsphere Q after extended storage in NaOH. BSA solution, 5.0 mg/ml in 20 mM Tris buffer, pH 8.3 (binding buffer), was loaded on a 2 ml column at 300 cm/hr. At 10% breakthrough, loading was stopped and the column was washed with binding buffer until the absorbance at 280 nm was 2% of the test solution. The bound protein was eluted with 20 mM Tris buffer, pH 8.3, containing 1.0 M NaCl, and recovery was determined by absorbance at 280 nm.

Virtually identical retention times for several test proteins were seen at up to 10,000 hr of storage in 1.0 N NaOH (Figure 6).

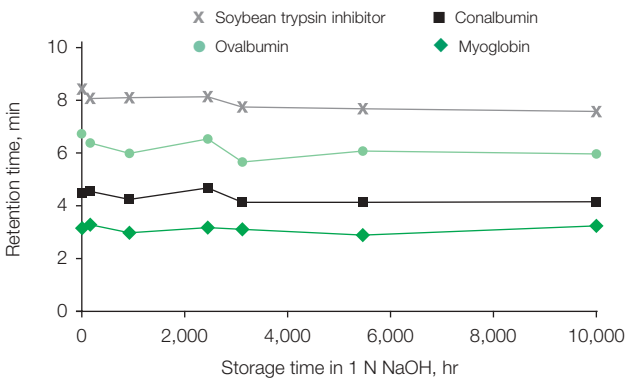

Fig. 6. Effect of storage on UNOsphere Q retention times. Support media were stored in 2 volumes of 1.0 N NaOH, which was replaced weekly throughout the test cycle. At the predetermined test interval, a small aliquot (~10 ml) was removed, washed, and packed in a Bio-Scale™ chromatography column. Retention times were determined with Bio-Rad anion exchange standards (catalog #125-0561).

Solvent Effects

High salt or chaotropic agent concentrations are often required for the chromatography of inclusion body proteins, column regeneration, and so on. Table 2 shows that addition of these chemicals does not increase column pressures much above atmospheric at most flow rates.

Table 2. Column pressure in various test solvents over a range of linear velocities.*

	Pressure (psi) at Given Linear Velocity (cm/hr)				
Solvent	**150**	**300**	**600**	**900**	**1,200**
20 mM Tris, pH 8.5	2	2	4	8	9
1 M NaCl	2	3	6	9	11
1 N NaOH	3	3	6	9	10
6 M guanidine HCl	7	9	15	22	ND**
8 M urea	6	14	28	43	52

* Support 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 Tris buffer, pH 8.5, then with the test solvent, and run at the velocities indicated. Between tests, the column was reequilibrated with Tris buffer.

** ND, not determined

Conclusions

UNOsphere Q has been shown to have high capacity and recovery at high linear velocity. Dynamic binding capacity, pressure and flow properties, and productivity compared favorably with other process chromatography media.The support was found to retain these favorable pressure and 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 was found to have essentially no effect on dynamic binding capacity or retention times for model proteins.

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