A Novel Desalting Column With High Mechanical Strength for Faster Desalting of Proteins

TSKgel
APPLICATION NOTE

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Introduction

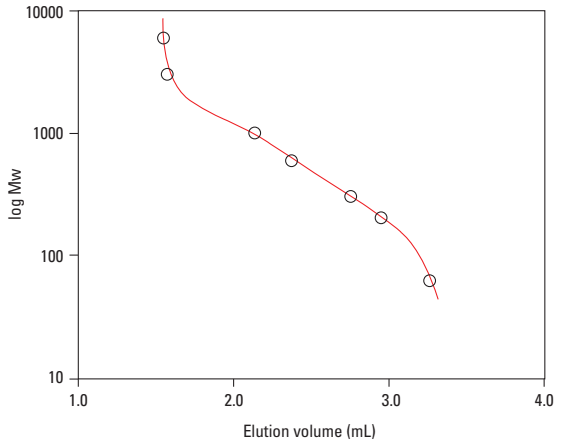
Desalting is a process that removes or reduces the salt concentration from a liquid stream or a collected fraction. In hydrophobic interaction (HIC), ion exchange (IEC), and size exclusion chromatography (SEC), proteins elute at high or elevated salt concentration. While various desalting techniques are available, desalting on the basis of size exclusion chromatography is widely used in biochemical laboratories.

A low MW exclusion limit, a large pore volume, and a high degree of hydrophilicity are the most desirable characteristics of desalting columns. Salts need full access to all pores, while proteins and other high MW species must be excluded from entering the pores and elute in the void volume as a narrow concentrated peak. Columns packed with conventional packing materials such as dextran, cellulose and polyacrylamide have limited physical stability and cannot withstand the pressure needed to speed up the desalting process. Recently, using a continued crosslinking process, Tosoh scientists have successfully increased the mechanical strength of polyacrylamide gel by four-fold over that of conventional gels thereby reducing the timescale of desalting to less than 10 minutes.

TSKgel BioAssist DS columns contain spherical 15 µm polyacrylamide particles* that are packed in 4.6 mm ID x 15 cm and 10 mm ID x 15 cm PEEK columns. These TSKgel columns are designed for the desalting and buffer exchange of proteins and polynucleotides at analytical (4.6 mm ID) and semi-preparative (10 mm ID) scale. Either column can be operated safely up to 4 MPa (40 bar or 600 psi).

Figure 1 shows the calibration curve of a 6 mm ID x 15 cm TSKgel BioAssist DS column using polyethylene glycol standards and a water mobile phase. As in all self-respecting SEC columns, the pore volume is larger than the volume in between the particles. The molecular mass cut-off (exclusion limit) for PEGs is about 2500. Results similar to those shown in Figure 1 can be obtained on the commercially available 4.6 mm x 15 cm and 10 mm ID x 15 cm TSKgel BioAssist DS columns.

Figure 1. Calibration curve of TSKgel BioAssist DS desalting column



Column:	Prototype TSKgel BioAssist DS column, 6 mm ID x 15 cm
Mobile phase:	DI H_2O
Flow:	0.5 mL/min
Samples:	ethylene glycol, PEGs

Experimental Conditions

HPLC System: Agilent 1200 with Chemstation (ver B.04.01)
Column: : TSKgel BioAssist DS, 15 µm, 4.6 mm ID x 15 cm and 10 mm ID x 15 cm
Mobile phase: 10 mmol/L KH_2PO_4/Na_2HPO_4, pH 6.7, 10 mmol/L Na_2SO_4 + 0.005% NaN_3
Flow rate: 0.8 mL/min (4.6 mm ID) and 1.0 mL/min (10.0 mm ID)
Detection: UV @ 280 nm and RI
Temperature: ambient
Injection vol.: 10 µL

All chemicals and standards were of electrophoretic or analytical grade and were obtained from Sigma-Aldrich. Before injection, standards and samples were filtered through a 0.45 µm PVDF filter.

* US Patent 7,659,348,

Table 1.

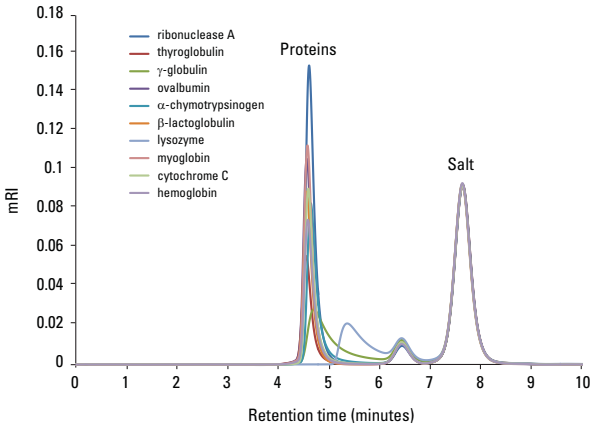
Protein	MW (kDa)	Concentration* (g/L approx.)
ribonuclease A	14.7	19.5
thyroglobulin	670	11.3
γ-globulin	150	14.5
ovalbumin	45	13.1
α-chymotrypsinogen	25.6	13.1
β-lactoglobulin	18.4	10.8
lysozyme	14.7	11.6
myoglobin	16.7	14.5
cytochrome C	12.3	11.0
hemoglobin	68	11.9

*in 100mmol/L Phosphate buffer, pH 6.7

Results and Discussion

Figure 2 demonstrates the rapid and reproducible desalting of a large number of proteins *(see Table 1)* at semi-preparative scale using a TSKgel BioAssist DS, 10 mm ID x 15 cm column. In this application the salt concentration of the proteins was reduced 10-fold from 0.1 to 0.01 mol/L. The reproducibility of the separation was determined by measuring the plate number of the ribonuclease A peak for four injections of various sample loads. The % RSD value (n=4) was less than 5% for a 1.5 mg injection. At this load, the resolution between ribonuclease A and the salt peak was larger than 6. At 1.95 mg load of ribonuclease A, the resolution between the protein and salt peak was 4.3. Note that the analysis is complete within 10 minutes.
In a similar study performed on a 4.6 mm x 15 cm TSKgel BioAssist DS column the resolution for a 1.95 mg load of ribonuclease A was larger than 2 at the high flow rate of 0.8 mL/min.

Figure 2. Desalting of proteins using a 15 µm, 10 mm ID x 15 cm TSKgel BioAssist DS column



Proteins in 0.1 mol/L phosphate buffer, pH 6.7
Mobile phase: 0.01 mol/L KH_2PO_4/Na_2HPO_4, pH 6.7, 0.01 mol/L Na_2SO_4 + 0.05% NaN_3

Conclusions

TSKgel BioAssist DS columns are designed for desalting of proteins and polynucleotides at analytical and semi-preparative scale. The novel highly cross-linked, hydrophilic, polyacrylamide beads showed excellent mechanical strength compared with conventional polyacrylamide beads and cross-linked dextran beads. This study demonstrated that the TSKgel BioAssist DS columns can be effectively used for desalting a large sample load in less than 10 minutes.

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