Size Exclusion Separation of Protein Mixtures: Comparison of Sepax SRT® SEC Columns and Tosoh TSKgel® Columns

Protein Separation

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Abstract

There are increasing numbers of biological large molecules such as proteins and antibodies manufactured as pharmaceutical therapeutics. It is often a challenge to separate these molecules from lysates and partially purified protein mixes, or differentiate them from variants with different post- translational modifications. The data presented here focus on the use of analytical SRT® SEC 150 and 300Å pore sizes with 5 μm particle size to separate several commercially available proteins with different molecular weights. The separation performance is compared with that of Tosoh TSK G2000SWxl and G3000SWxl respectively.

Introduction

Size exclusion chromatography (SEC) has been shown as an effective way to separate different sizes of proteins, as well as buffer exchanges at the same time. Since SEC technique can be achieved with high resolution with few protein components or partially purified protein mixtures, often researchers use it in the final polishing step in a protein purification scheme or Quality Control departments use it in the detection of the sample uniformity. Samples of interest can be separated from aggregates or variants with different modifications occurred during the process – a crucial step in ensuring the quality of biological products.
Sepax SRT® SEC columns are based on uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized silica. The unique property of the resin minimizes the non-specific interactions between biological samples and the column medium. SRT® SEC provides a wide selection of pore sizes: 100, 150, 300, 500, 1,000 and 2,000Å pore sizes for different molecular weight separation limits. In this application note, the performance of SRT® SEC 150 and 300Å pore sizes with 5 µm particle size is compared with that of Tosoh TSK G2000SWxl and G3000SWxl respectively with the same column dimensions.

Experimental

HPLC system:

Agilent 1200 HPLC with binary pump

SEC columns:

Sepax SRT® SEC-150 (150 Å, 5 µm), Tosoh TSK G2000SWxl (125 Å, 5 µm), 7.8x300 mm
Sepax SRT® SEC-300 (300 Å, 5 µm), Tosoh TSK G3000SWxl (300 Å, 5 µm), 7.8x300 mm

Chemicals and Reagents:

Thyroglobulin, BSA, Ribonuclease A, ply-DL alanine and Uracil were purchased from Sigma-Aldrich. Monobasic and dibasic sodium phosphate were purchased from EMD.

LC Method:

For SRT® SEC-150 and Tosoh TSK G2000SWxl, the flow rate is 1ml/min, mobile phase is 150 mM sodium phosphate, pH 7.0, and the temperature is ambient at 23 degree C. Sample injection volume is 10 µl with following components: Thyroglobulin, BSA, Ribonuclease A at 1.0 mg/ml each and Uracil at 2.5 µg/ml.
For SRT® SEC-300 and TSK G3000SWxl, the column running conditions are the same as that for SRT® SEC-150, except the sample concentration is as follows: thyroglobulin, BSA, ribonuclease A (1 mg/mL); uracil (0.1 mg/mL), injection volume is 5 µl.

Results

In comparison of SRT® SEC-150 and Tosoh TSK G2000SWxl, figure 1 shows the similar separation profiles from both columns under the same LC running conditions. SRT® SEC-150 exhibits a slightly better resolution for Ribonuclease A and Poly-DL-alanine. Table 2 summarizes the retention time for each eluted protein peak. With column dimension at 7.8x300 mm, the retention time for each protein peak is similar using both columns.

SRT® SEC-300 and TSK G3000SWxl have the same column dimension, particle size and porosity. The elution profiles are exhibiting similar chromatographic patterns, under 1 ml/min flow rate and 150 mM phosphate buffer, pH7.0. SRT® SEC-300 shows a better separation resolution with Thyroglobulin and its aggregated forms 9 (figure 2).

Conclusion

In comparison to Tosoh G2000SWxl and G3000SWxl, Sepax SRT® SEC-150 and SRT® SEC-300 demonstrate a similar or slightly better separation with the same protein mixtures respectively. Sepax SRT® SEC columns can be a great alternative for biologics separations, which currently use Tosoh TSK columns.

For more information about these and other products from Sepax Technologies, please visit www.sigmaaldrich.com/sepax

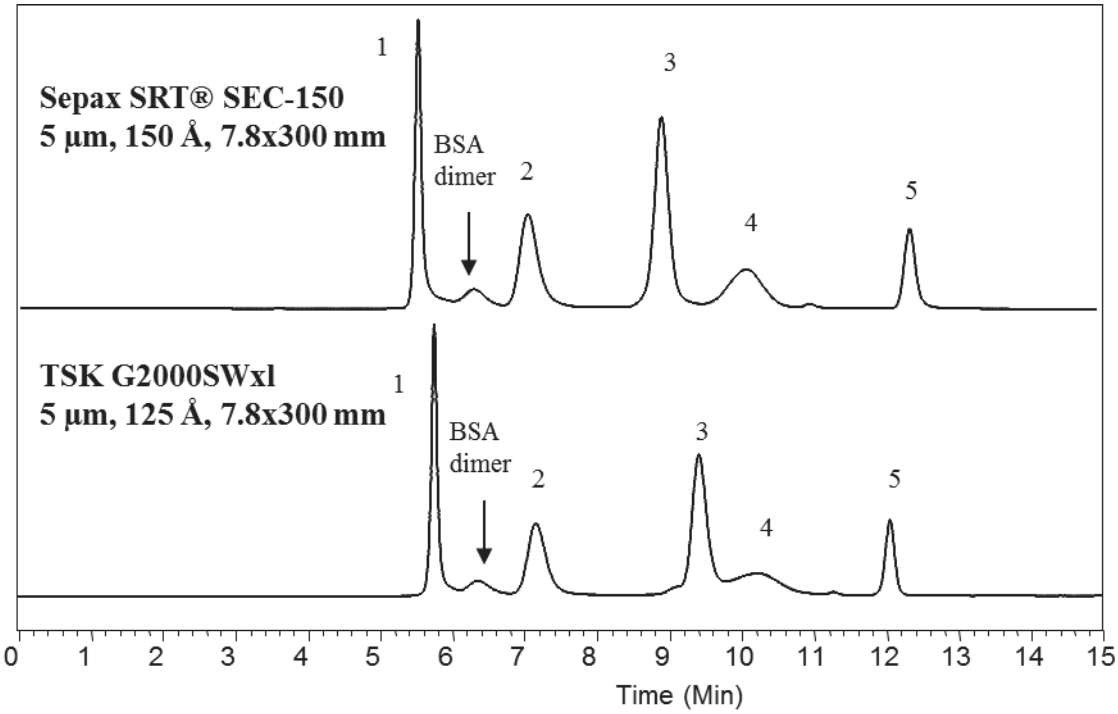

Figure 1. Comparison of chromatograms of SRT® SEC-150 and Tosoh TSK G2000SWxl, 7.8x300 mm using protein mixture 1) Thyroglobulin, 2) BSA monomer, 3) Ribonuclease A, 4) Poly-DL-alanine, 5) Uracil

Peak	Protein	MW (kD)	SRT SEC-150 RT (min)	TSKG2000SWxl RT (min)
1	Thyroglobulin	670	5.43	5.64
2	BSA monomer	66	6.93	7.02
3	Ribonuclease A	13.7	8.74	9.22
4	Poly-DL-alanine	1-5	9.9	10.02
5	Uracil	0.12	12.13	11.81

Table 1. Comparison of retention time of eluted proteins with SRT® SEC-150 and TSK G2000SWxl, 7.8x300 mm.

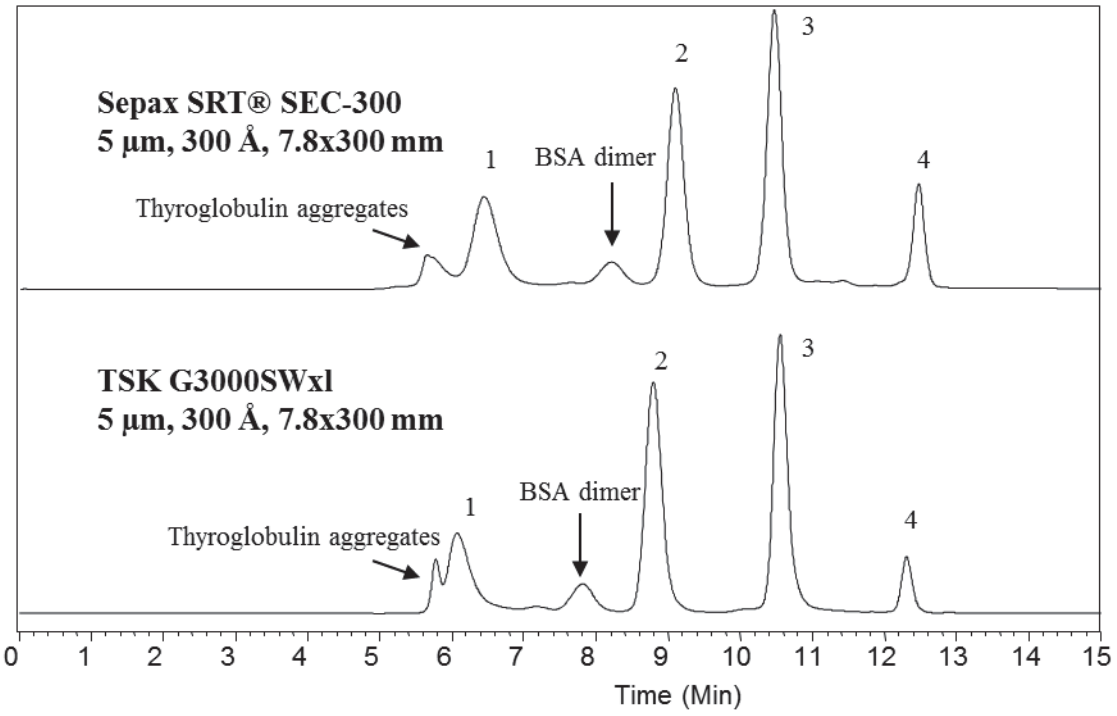

Figure 2. . Comparison of chromatograms of SRT® SEC-300 and Tosoh TSK G3000SWxl, 7.8x300 mm using protein mixture 1) Thyroglobulin, 2) BSA monomer, 3) Ribonuclease A, 4) Uracil

Peak	Protein	MW (kD)	SRT SEC-300 RT (min)	TSK G3000SWxl RT (min)
1	Thyrolobulin	670	6.43	6.08
2	BSA	66	9.08	8.79
3	Ribonuclease A	13.7	10.46	11.07
4	Uracil	0.12	12.46	12.31

Table 2. Comparison of retention time of eluted proteins with SRT® SEC-300 and TSK G3000SWxl, 7.8x300 mm

Sepax Technologies vs. Tosoh Bioscience Cross Reference

Sepax Column Characteristics	TSKgel Column Characteristics
SRT SEC-150, 5µm, 150Å	G2000SWxl, 5µm, 125Å
SRT SEC-300 5µm, 300Å	G3000SWxl, 5µm, 300Å
SRT SEC-500, 5µm, 500Å	G4000SWxl, 8µm, 450Å
Zenix SEC-150, 3µm, 150Å	SuperSW2000, 4µm, 150Å
Zenix SEC-300, 3µm, 300Å	SuperSW3000, 4µm, 300Å

Cross Reference Table

Sepax Column	Sigma-Aldrich P/N	TSKgel Column	Sigma-Aldrich P/N
SRT SEC-150, 7.8 mm x 30 cm, 5µm	Z777045	G2000SWxl, 7.8 mm x 30 cm, 5µm	808540
SRT SEC-300, 7.8 mm x 30 cm, 5µm	Z777051	G3000SWxl, 7.8 mm x 30 cm, 5µm	808541
SRT SEC-500, 7.8 mm x 30 cm, 5µm	Z777057	G4000SWxl, 7.8 mm x 30 cm, 8µm	808542
Zenix SEC-150, 4.6 mm x 30 cm, 3µm	Z777016	SuperSW2000, 4.6 mm x 30 cm, 4µm	818674
Zenix SEC-300, 1.0 mm x 30 cm, 3µm	Z777022	SuperSW3000, 1 mm x 30 cm, 4µm	821845
Zenix SEC-300, 2.1 mm x 30 cm, 3µm	Z777024	SuperSW3000, 2 mm x 30 cm, 4µm	821485
Zenix SEC-300, 4.6 mm x 30 cm, 3µm	Z777028	SuperSW3000, 4.6 mm x 30 cm, 4µm	818675

Please visit www.sigmaaldrich.com/sepax for a listing of these and other SEC columns.