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BETTER PARTICLE SIZE SOLUTIONS

APPLICATION NOTE

Utilizing DLS Flow Mode for High-resolution Size Distribution Measurement of BSA

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Introduction

Traditional nanoparticle size analyzers employ dynamic light scattering (DLS) technique, illuminating samples with a laser beam and detecting scattered light fluctuations caused by Brownian motion of particles suspended in a liquid. The original intensity fluctuation over time is processed via correlation calculations to derive the correlation function. Different mathematical models, such as Cumulants, NNLS or CONTIN, are used to determine size and size distribution.

Batch mode is commonly utilized by DLS analyzers, which refers to traditional testing mode with quartz or plastic cuvettes, and exhibits low resolution for size distribution testing, especially of widely distributed samples. Moreover, the size distribution calculation is extremely algorithm-dependent and its highest resolution can only distinguish between individual components of a narrow distribution with a 2.5-3 times difference in particle size. This greatly limits the quantitative nature of the size distribution results.

In contrast, DLS flow mode, when connected to a front-end separation device, can individually detect the size of each effluent component. Each effluent component is ideally mono-dispersed or closely mono-dispersed, and signals obtained via concentration detectors yield size distribution information independent of algorithms, with a resolution as high as 1.3 : 1.

In this application note, the BeNano 180 Zeta Pro was connected to a SEC (Size Exclusion Chromatography) front-end device, and the size distribution of Bovine Serum Albumin (BSA) was displayed.

Instrumentation

The BeNano 180 Zeta Pro was utilized, employing a 50 mW, 671 nm laser and collecting light scattering signals at 173°. The measurement utilized a 27μL low-volume flow cuvette. Using a BFC-1 signal collector, analog signals outputted from the Refractive Index (RI) detector of the front-end SEC device were collected.

Experiment

BSA solution was prepared in PBS buffer at a concentration of 5 mg/mL, stirred electromagnetically for 10 minutes, and filtered through a 220nm water-based filter before use. Injection and separation were performed through the front-end SEC with an RI detector. Subsequently, individual separated components entered the BeNano for size measurement under 25°C.

Chromatographic Conditions:

- Mobile Phase: PBS Buffer
- Flow Rate: 0.4 mL/min
- Injection Volume: 100 uL

Results and Discussion

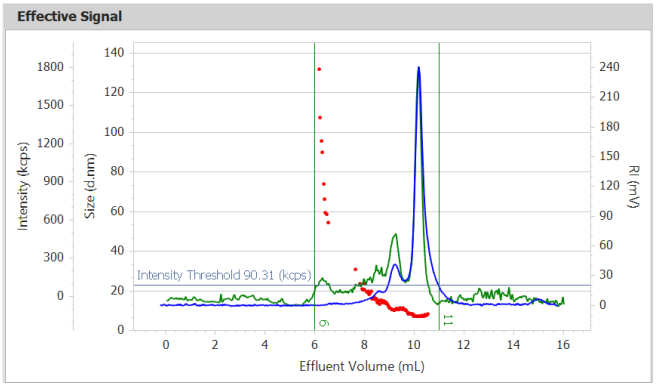

Figure 1. Intensity, RI, and Size Effluent Curves of BSA

The effluent curves in Figure 1 demonstrate multiple peaks for BSA in the total effluent volume of 16 mL. The peaks at around 6 mL represent aggregate peaks, with peaks between 8-10 mL representing separated oligomers. The final major peak at around 10.5 mL represents the monomer of BSA. The larger area of the major peak indicates that the majority of the protein exists as monomers.The decreasing particle sizes detected in the size effluent curve (red dots) correspond well with the principles of SEC separation. This confirms the effectiveness of the front-end SEC in sample separation and validates BeNano as a detector, effectively obtaining particle size information for each effluent component.

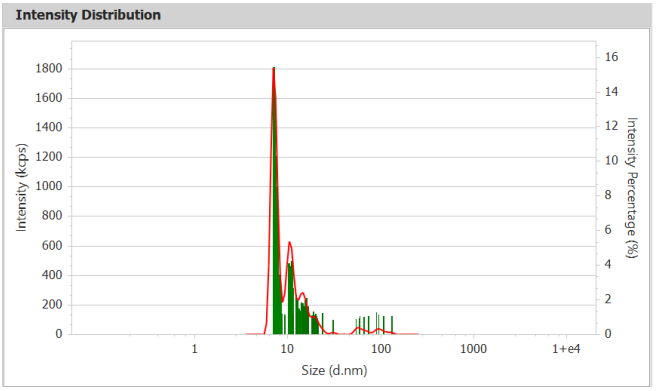

Figure 2. Intensity Distribution Curve of BSA (Bar Chart)

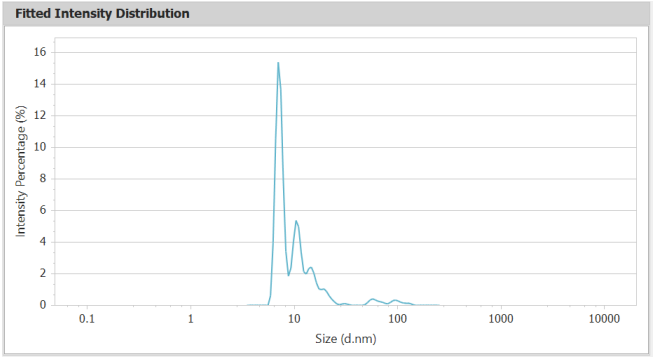

Figure 3. Intensity Distribution Curve of BSA (Line Chart)

Figures 2 and 3 convert the chromatographic effluent curve into intensity distribution curves, clearly identifying separated oligomers, with the monomer at 7.21nm, in excellent agreement with the theoretical size of BSA monomers (~7nm). Sizes and areas of other effluent components are listed in Table 1.

Table 1. Measured distribution Peaks of BSA

	Size (d. nm)	Area (%)	Peak Std Dev (nm)	CV (%)
Peak 1	7.21	57.42	0.61	8.49
Peak 2	10.88	24.31	1.07	14.84
Peak 3	15.49	10.29	1.44	19.98
Peak 4	21.25	3.33	1.87	25.89
Peak 5	31.19	0.35	1.91	26.50

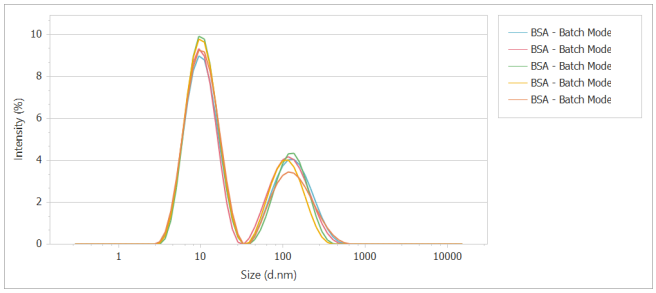

Figure 4. Batch-mode detection of intensity distribution curve of BSA

The intensity distribution curve in batch-mode detection (Figure 4) reveals only two peaks. The smaller peak (around 10nm) corresponds to the size distribution of monomers, dimers, trimers, and other oligomers, while the larger peak (around 100nm) represents larger aggregates.

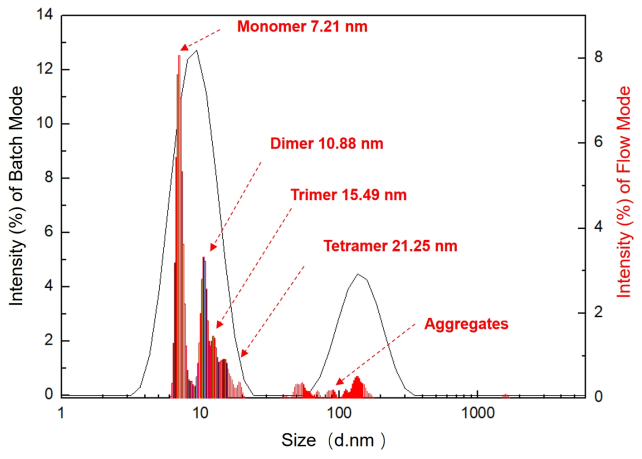

Figure 5. Comparison between batch-mode (line chart) and flow mode (bar chart) intensity distribution curves

The comparison between flow mode and batch-mode detection results in Figure 5 indicates that flow mode can successfully distinguish oligomer components with very small size differences within the small size peaks.

Conclusion

The BeNano with flow mode was used as a detector in conjunction with a front-end separation device, aims to significantly enhance the resolution of size measurement. By measuring BSA, this application note demonstrated the detection capability of BeNano flow mode, effectively showcasing its potential in the field of high-resolution size measurements.

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