Studying Protein Aggregation Using ASEC: Absolute Size Exclusion Chromatography

by
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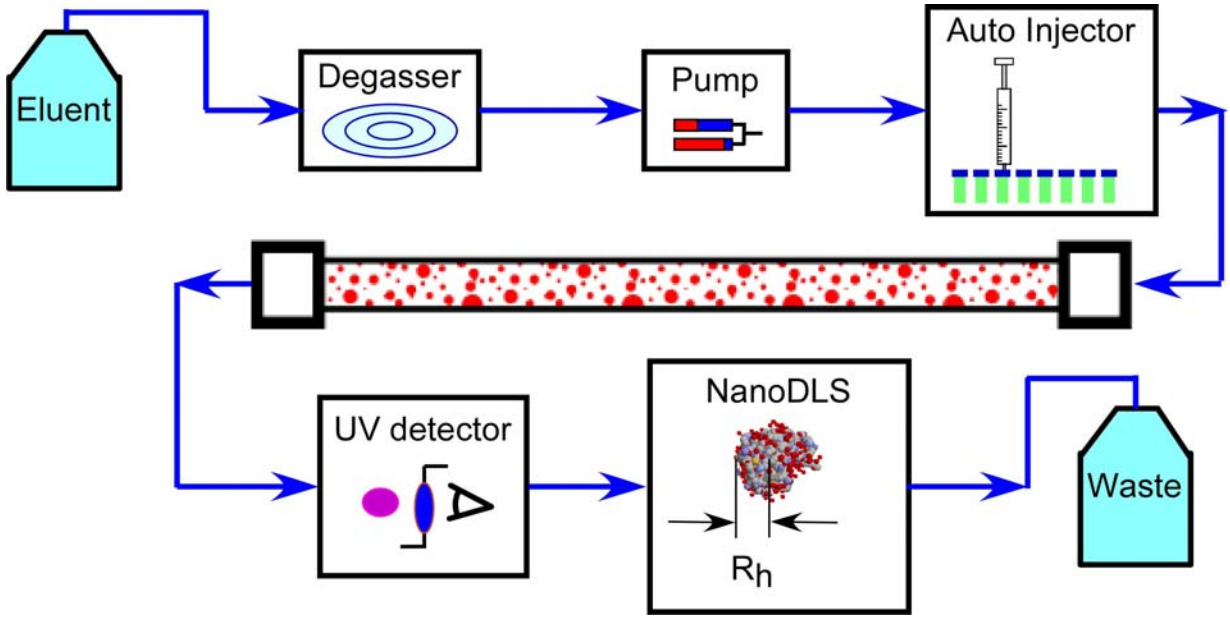
Exploiting the potential of protein therapeutics requires the ability to identify and quantify the protein aggregation that can yield inconsistent activity and provoke immune response. The sensitivity of light scattering to small numbers of large particles (aggregates) makes light scattering an unsurpassed tool for studying aggregation.

Light scattering as a tool for identifying protein aggregates is unsurpassed, because it is so sensitive to a relatively few large particles. There are two forms of light scattering: static (SLS) and dynamic (DLS). SLS provides molecular weight; DLS provides a hydrodynamic size. Either technique may be combined with size exclusion chromatogra-



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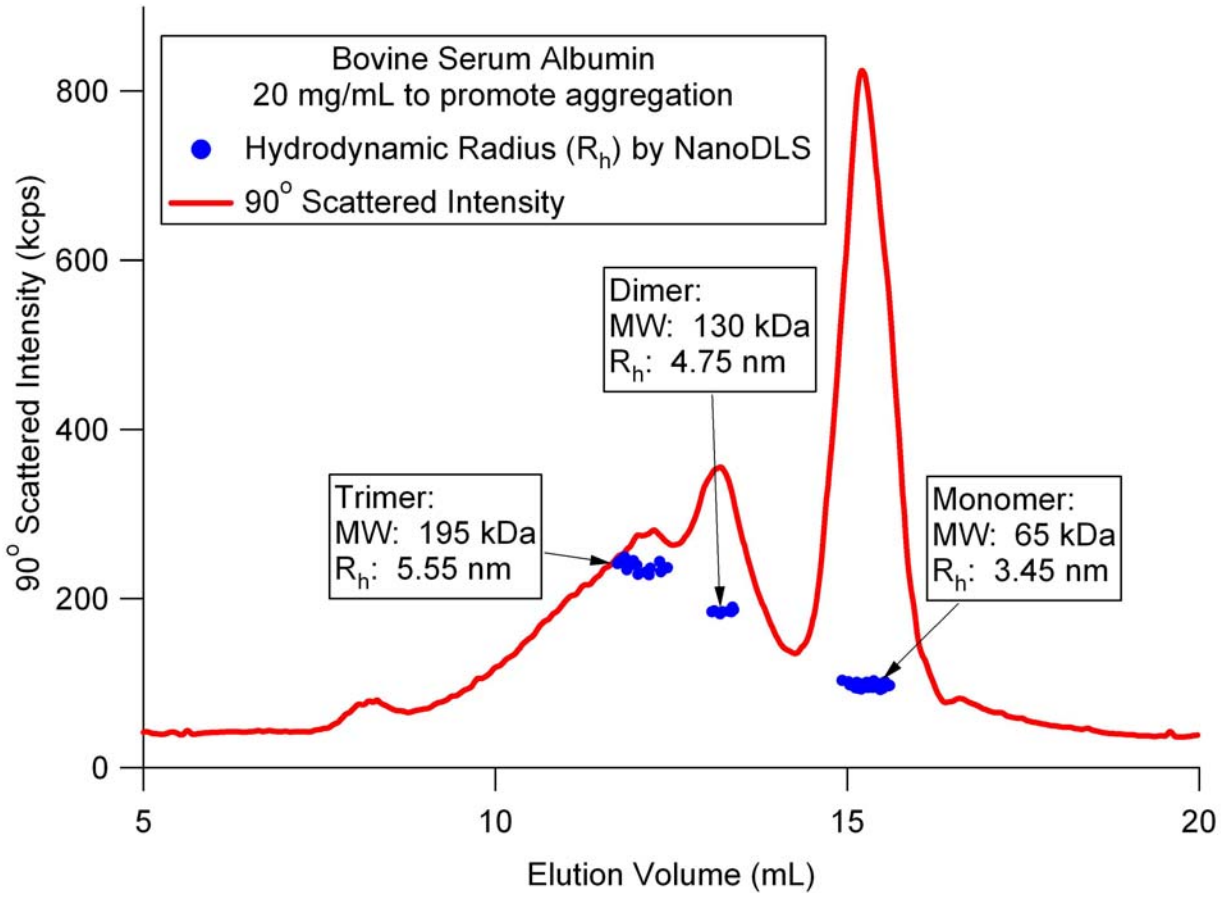
phy to separate aggregates from monomers, yielding quantitative information about the relative abundance of aggregates and monomers, as well as biophysical information about the different species.

SLS requires instrument calibration though is free of column calibration with standards. DLS does not require any calibration. It is truly absolute and deserves the name ASEC. DLS works well on fractionated materials; thus, the ideal situation is to separate proteins and aggregates using column chromatography technology and add a DLS detector. Both the intensity and size information is obtained.

With the DLS measurements made here, molecular weights of monomeric species may be estimated by the Mark-Houwink-Sakurada equation, an empirical relation between hydrodynamic radius and molecular weight. The agreement with literature values is excellent.

A GE Healthcare Superdex 200 column, run at 0.5 mL/min in PBS buffer was used with different proteins, two of which are shown



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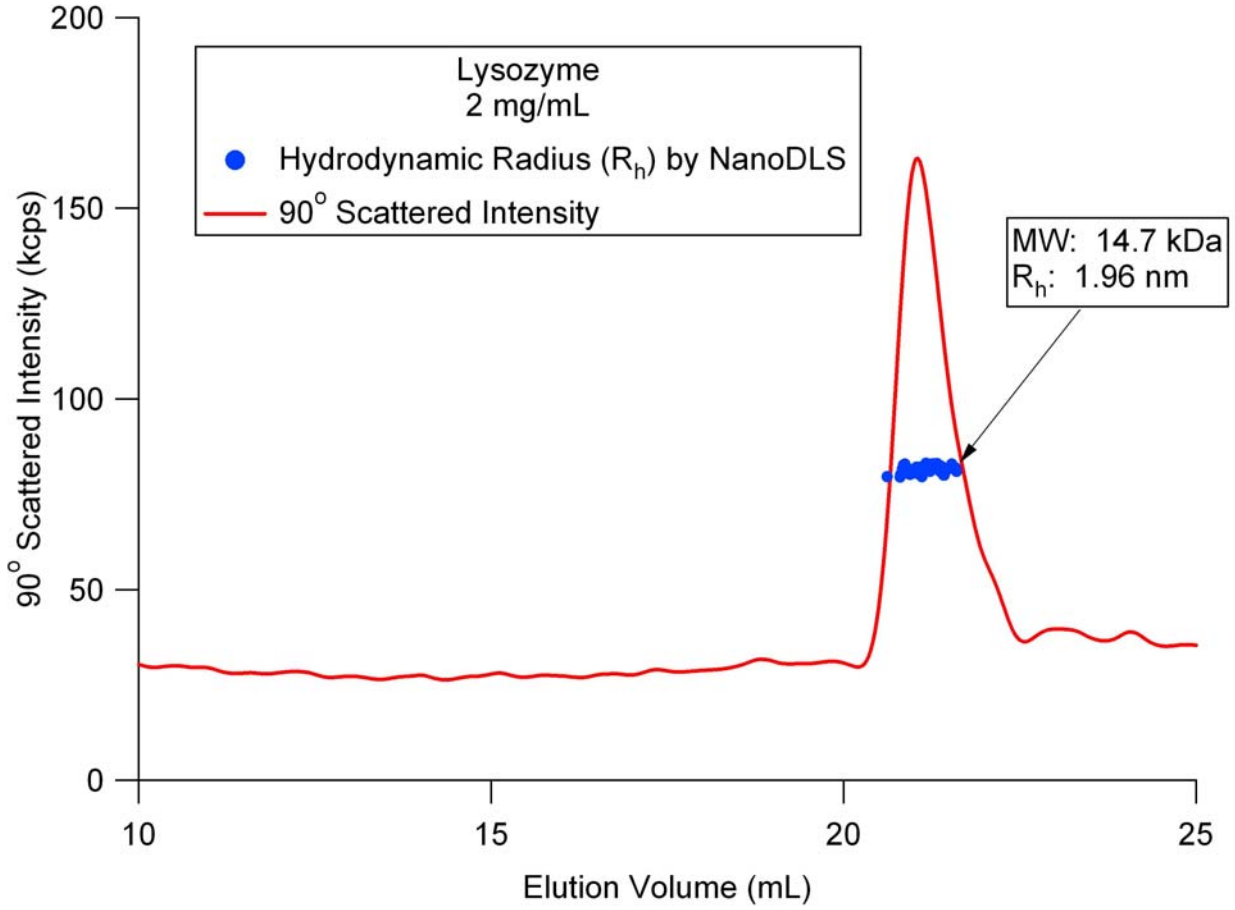
here. Note the solution conditions and concentration for BSA were chosen to promote aggregation to test the resolution of ASEC.

The DLS instrument used in these experiments is the Brookhaven NanoDLS with a patented optical and flow cell design that provides extreme sensitivity, making it an invaluable tool for research with precious biological samples. Results have also been obtained with broader, synthetic polymers and with vaccine purification and production (Max Planck Institute, Magdeburg, Germany).



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