Abstract for an Invited Paper
for the MAR09 Meeting of
The American Physical Society

Characterizing Protein Complexes with UV absorption, Light Scattering, and Refractive Index Detection.

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Many modern pharmaceuticals and naturally occurring biomolecules consist of complexes of proteins and polyethylene glycol or carbohydrates. In the case of vaccine development, these complexes are often used to induce or amplify immune responses. For protein therapeutics they are used to modify solubility and function, or to control the rate of degradation and elimination of a drug from the body. Characterizing the stoichiometry of these complexes is an important industrial problem that presents a formidable challenge to analytical instrument designers. Traditional analytical methods, such as using florescent tagging, chemical assays, and mass spectrometry perturb the system so dramatically that the complexes are often destroyed or uncontrollably modified by the measurement. A solution to this problem consists of fractionating the samples and then measuring the fractions using sequential non-invasive detectors that are sensitive to different components of the complex. We present results using UV absorption, which is primarily sensitive to the protein fraction, Light Scattering, which measures the total weight average molar mass, and Refractive Index detection, which measures the net concentration. We also present a solution of the problem inter-detector band-broadening problem that has heretofore made this approach impractical. Presented will be instrumentation and an analysis method that overcome these obstacles and make this technique a reliable and robust way of non-invasively characterizing these industrially important compounds.