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Review

Recent applications of light scattering measurement in the biological and biopharmaceutical sciences

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Abbreviations used: SLS, static light scattering; DLS, dynamic light scattering; MALS, multi-angle light scattering; CG–SLS, composition gradient static light scattering; CG–MALS, composition gradient multi-angle light scattering; CG–DLS, composition gradient dynamic light scattering; SEC, size-exclusion chromatography; FFF, field-flow fractionation; UV, ultraviolet; CD, circular dichroism; SAXS, small angle X-ray scattering; SANS, small angle neutron scattering; ELS, electrophoretic light scattering; mAb, monoclonal antibody; NP, nanoparticle; Hb, hemoglobin; BSA, bovine serum albumin; SE–UHPLC, size exclusion–ultra high-pressure liquid chromatography; MW, molecular weight; HSA, human serum albumin.

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Introduction

Measurement of the average intensity of excess scattering, termed static light scattering (SLS), has become one of the most widely used techniques for the determination of molar mass and, in some cases, the size of macromolecules in solution. Similarly, development of the theory of fluctuations in light scattering intensity and commercially available instrumentation for experimental measurement of these fluctuations on a microsecond time scale, termed dynamic light scattering (DLS) or quasi-elastic light scattering, has led to widespread use of this technique to measure the short-time diffusion coefficient and apparent size of macromolecules in solution. For a comprehensive review of the theory and practice of SLS, see Refs. [1]; for DLS, see Ref. [2].

The current introduction to light scattering methodology and review of recent literature – 2013 to present – was undertaken for several reasons: (i) to bring the techniques of SLS and DLS to the attention of a broad audience of researchers in the biological and biopharmaceutical sciences, (ii) to illustrate the various purposes to which these techniques have recently been put, and (iii) to suggest ways in which future studies of various phenomena can be made more informative.

We begin with a summary of the principles underlying each type of measurement discussed here and common relations used in analysis of experimental data. Details can be found in the cited references. Next, various research objectives are introduced, and citations to the literature are grouped according to these objectives. Most of the articles cited report studies of proteins in solution, but we also cite studies of synthetic peptides and nanoparticles, which have recently become of interest to the biopharmaceutical industry as potentially targetable drug carriers. Finally, we address several topics of concern to the reviewer.

The number of light scattering-related publications in the biological and biopharmaceutical science fields from 2013 onward is very large and appears in an extraordinarily broad range of journals. It is likely that some possibly important contributions have been inadvertently missed, and apologies are offered in advance to the authors of the missing citations.

Methods

Static light scattering of macromolecules in dilute solution

A laser-based light scattering photometer instrument, schematically indicated in Fig. 1, measures the average intensity of light $I(w,\theta)$ scattered by sample in the scattering volume at w/v concentration w and scattering angle θ and reports the excess Rayleigh ratio R, calculated according to

$$R(w,\theta) = k_{inst}[I(w,\theta) - I(0,\theta)], \tag{1}$$

where k_{inst} is an instrumental constant determined by calibration with a scattering standard and $I(0,\theta)$ is the intensity of light scattered by pure solvent. Data collected from a solution of a single species of macromolecule are conventionally analyzed in the context of the Zimm–Debye formulation of static light scattering. In the dilute limit, the dependence of R on w and θ is given by

$$R(w,\theta) = K_{opt}\left(\frac{d\tilde{n}}{dw}\right)^2 MwP(\theta)\left[1 - 2A_2P(\theta)w + O\left(w^2\right)\right]. \tag{2}$$

Here K_{opt} is an optical constant calculated according to

$$K_{opt} = \frac{4\pi^2\tilde{n}_0^2}{\lambda_0^4 N_A}, \tag{3}$$

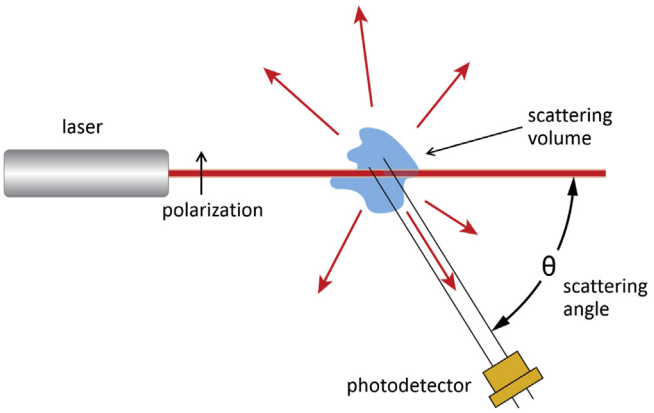

Fig. 1. Schematic of an apparatus for measurement of static light scattering. Collection of angle-dependent data may be implemented either by varying the angular position of a single detector or by positioning detectors at multiple angles, as in modern MALS instruments.

where $\tilde{n}_0$ denotes the refractive index of solvent, λ_0 is the wavelength of incident light in vacuum, and N_A is Avogadro's number. $(d\tilde{n}/dw)$ denotes the specific refractive increment of solute, M is the molar mass of solute, A_2 is a measure of the effective interaction between scattering molecules, and $P(\theta)$ is a structure factor calculated according to

$$P(\theta) = 1 - \frac{16\pi^2\tilde{n}_0^2}{3\lambda_0^2}\left\langle r_g^2\right\rangle \sin^2(\theta/2) + O\left[\sin^4(\theta/2)\right], \tag{4}$$

where the quantity $\langle r_g^2\rangle$ denotes the mean square radius of the scattering solute (discussed below). If the largest dimension of the scattering particle is much less than the wavelength of the incident light (<~35 nm), as in the case of most proteins and small protein complexes, $P(\theta) \approx 1$ (i.e., angular dependence of excess scattering is negligible). Given independently determined values of K_{opt} and $(d\tilde{n}/dw)$, the values of M and A_2 (and $\langle r_g^2\rangle$ in the case of a sufficiently large scattering solute) are obtained by extrapolation of data obtained at various solute concentrations and scattering angles to infinite dilution and zero scattering angle.

The quantity A_2 is conventionally identified as the second virial coefficient in the expansion of the osmotic pressure in powers of solute concentration. Different laboratories may express concentration using different units, most commonly g/L or mol/L and less commonly the number density ρ (molecules/cm^3) or ϕ (unitless volume fraction). Thus, the initial concentration dependence of light scattering may be specified as values of other parameters, the most common of which is $B_{22} = A_2/M$. It should be kept in mind that all of these parameters are proportional to A_2 and are readily interconvertible by appropriate scaling of concentration units.

Although a more rigorous interpretation of A_2 has been presented [3,4], it is not obvious whether or when the distinction between the more rigorous interpretation and the conventional one is qualitatively or quantitatively significant in aqueous solutions of macromolecules. Therefore, we adopt the conventional interpretation of A_2 for the current review. Statistical thermodynamics provides an exact relation between A_2 and an orientationally averaged potential of mean force $U(r)$ acting between two molecules of the scattering species separated by center-to-center distance r in a dilute solution [5]: