

A laser light scattering apparatus

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The use of a low power helium-neon laser for light scattering measurements is described. The Rayleigh and Cabannes theories have been modified for polarized incident light. A new procedure is recommended for data when the solvent correction is relatively high. Using this method a precision of 4.7% was obtained for the molecular mass of bovine plasma albumin in water, compared with 6.3% for the more usual method.

The chief advantages of using a laser for light scattering are the high degree of monochromaticity and the well-defined collimated beam obtainable without the use of ancillary lenses slits and filters.

It has been shown (Kielich, 1968) that high intensity laser beams such as are obtained from ruby crystals, produce orientation of the scattering particles, and that the scattered intensity is not proportional to the incident intensity. For low energy neutral gas lasers these complications of the Rayleigh theory do not arise (Bothorel & Lalonne, 1968) and this has been confirmed using a helium-neon laser for scattering studies on liquids by Rudder & Bach (1967) and on polymer solutions by Block (1968) and by Anderson (1968).

In the visible spectrum the helium-neon gas laser giving light of 632.8 nm wavelength is generally used. The light output is plane polarized since the end windows of the tube are set at the Brewster angle to minimize reflection losses.

MATERIALS AND METHODS

The apparatus was similar to that of Attwood (1965) except that the mercury lamp, filters, slits and lenses for the incident light beam were replaced by a Scientifica-Cook B17/S laser (0.5 mW at 632.8 nm).

The scattered light intensity was measured by an E.M.I. 6097 B photomultiplier, which could be rotated horizontally for angular measurements; this was connected to a Techmation model 451NS digital voltmeter.

According to Heller (1965), Rayleigh's theory of light scattering applies to the scattering of unpolarized light of wavelength λ , by a particle of maximum dimension smaller than $\lambda/6$. The Rayleigh ratio is given by

$$R_{\theta} = \frac{ir^2}{I} = \frac{8\pi^2 p^2 (1 + \cos^2 \theta)}{\lambda^4} \quad \dots \quad (1)$$

where i = intensity of scattered light at angle θ ; I = intensity of incident light; r = distance of scattering particle from detector; p = polarizability of the particle; λ = wavelength of light.

In the bracketed term the 1 represents the *vertically* polarized component of the scattered light, which is independent of θ ; the $\cos^2 \theta$ term represents the *horizontally*

polarized output which is thus zero at $\theta = 90^\circ$. In practice there is always some horizontally polarized scatter at 90° due to the anisotropic nature of the scattering particles and Cabannes & Rocard (1929) have shown that the observed value of R_θ must be multiplied by $(6 - 7d)/(6 + 6d)$ to give the true Rayleigh ratio where d , the depolarization at 90° , is the ratio of the intensity of horizontally polarized scattered light divided by the intensity of the vertically polarized component. This is measured using a polaroid sheet in front of the photomultiplier and measuring the ratio of photomultiplier output readings with the polaroid set for passing horizontally and then vertically polarized scattered light.

In practice $i \ll I$ and direct comparison is difficult. It is, however, possible to calculate the fraction of light scattered in terms of the scatter of a standard Perspex block calibrated by measurements of turbidities t , of solutions. The relation between turbidity and Rayleigh ratio is

$$t = 16\pi R_{90}/3 \dots \dots \dots (2)$$

So that measurement of the turbidity using a spectrophotometer enables R_{90} to be calculated. Ludox, a colloidal preparation of silicic acid is used for this purpose since dilute sols act as Rayleigh scatterers provided large aggregates are removed by centrifugation. The quantity measured in the light scattering apparatus is S_θ defined as

$$S_\theta = \frac{\text{Scatter of solution at angle } \theta}{\text{Scatter of standard block of Perspex at } 90^\circ}$$

and S_θ is directly proportional to R_θ

$$R_\theta = CS_\theta \dots \dots \dots (3)$$

where C is the calibration constant for a given Perspex standard. In the case of He-Ne laser the incident light is polarised at ϕ to the horizontal. The vertically polarized component is proportional to $\sin^2\phi$, and the horizontal component to $\cos^2\phi$. The $(1 + \cos^2\theta)$ term of Rayleigh's theory then becomes

$$(\sin^2\phi + \cos^2\phi \cos^2\theta) = \sin^2\phi(1 + \cos^2\theta \cot^2\phi) \dots \dots (4)$$

in which ϕ is a constant for a given apparatus, and since R_θ is calculated during the calibration procedure, using the polarized light to measure scatter from a Perspex block, the $\sin^2\phi$ term becomes incorporated into the calibration constant C (Pugh, 1970).

Cabannes' factor must also be modified for the case of polarized incident light. The relation between the observed depolarization, $d(\phi)$ and the corresponding depolarization, d , for an unpolarized source is given by

$$d = 2 \sin^2\phi(1 - 2 \cos^2\phi + 1/d(\phi)) \dots \dots (5)^*$$

Equations (1), (2), (3), (4) show that S_θ is related to S_{90} by the equation

$$S_\theta = S_{90}(1 + \cos^2\theta \cot^2\phi) \dots \dots (6)$$

A correction must be applied however since the scattering volume viewed by the photomultiplier is proportional to $\sin \theta$. Equation (6) thus becomes

$$\begin{aligned} \text{or } S_\theta &= S_{90}(1 + \cos^2\theta \cot^2\phi)/\sin \theta \\ S_\theta \sin \theta &= S_{90} + S_{90} \cos^2\theta \cot^2\phi \dots \dots (7) \end{aligned}$$

* Copies of the proof for this formula are available from the Editorial Department, Journal of Pharmacy and Pharmacology, 17, Bloomsbury Square, London, WC1A 2NN.

A plot of $S_{\theta} \sin \theta$ against $\cos^2 \theta$ should therefore give a straight line of intercept S_{90} and slope $S_{90} \cot^2 \phi$. Ludox sols were used for testing this equation since they give scattering envelopes which are symmetrical about 90° . It was found that the plot was not exactly linear because of a small constant error in the angle scale markings, of the order of 10–20 minutes of arc. This was eliminated by taking the means of supplementary scatter readings on either side of 90° as being the true reading at exactly θ . This gave a good straight line relation, Fig. 1, from which ϕ was found to be $39^\circ 45'$.

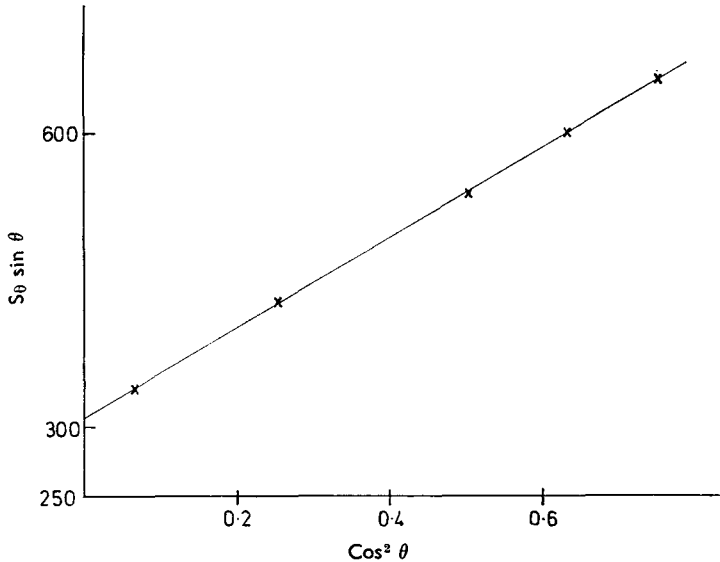


FIG. 1. Plot of $S_{\theta} \sin \theta$ against $\cos^2 \theta$ for Ludox.

Equation (1) has been developed by Debye (1947) and Zimm (1948) to enable calculation of molecular masses of solutes,

$$\frac{1}{M} = \frac{2\pi^2 n_0^2 (dn/dc)^2}{\lambda^4 N_A C} \left(\frac{c}{S} \right) \quad \dots \quad (8)$$

$c \rightarrow \text{zero}, \theta \rightarrow \text{zero}$

M = weight average molecular mass; n_0 = refractive index of solvent; c = concentration in g cm^{-3} ; λ = wavelength of light *in vacuo*; N_A = Avogadro number; $S'_{\theta} = S_{\theta} \sin \theta / (1 + \cos^2 \theta \cot^2 \phi)$.

The extrapolation to zero angle is made to eliminate destructive interference.

For small particles it is only necessary to measure S'_{90} , S'_{45} , and S'_{135} . The ratio S_{45}/S_{135} is called the dissymmetry Z , and for a given particle shape a quantity $P^{-1}(\theta)$ can be calculated such that

$$S'_{90} P^{-1}(\theta) = S'_0$$

Tables of $P^{-1}(\theta)$ and Z have been given for various particle shapes by Beattie & Booth (1960).

Normally S_{θ} is measured by subtracting the scatter for pure solvent from the solution reading, this procedure introduces inaccuracies particularly when the scattering of solutions is low, the correction for solvent scatter then being relatively large and any error in the solvent scattering measurement is introduced into all the other measurements (Pugh, 1970). It is considered that a better result is obtained from a

plot of Δn against S'_θ at low concentrations. When, as with bovine plasma albumin, the plot is linear, the slope of the line gives the value of $\Delta n/(S'_{\text{soln}} - S'_{\text{soln}})$, otherwise the curve is fitted by a polynomial and the linear coefficient (the slope at low scatter) is taken as the measure of this quantity.

Substitution of $c = \Delta n/(dn/dc)$ in equation (8) for dilute solutions removes the need for squaring the (dn/dc) term

$$\frac{1}{M} = \frac{2\pi^2 n_0^2 (dn/dc)}{\lambda^4 NC} \left(\frac{\Delta n}{S'_\theta} \right) \quad \dots \quad \dots \quad \dots \quad (9)$$

$\Delta n \rightarrow \text{zero}, \theta \rightarrow \text{zero}$

To use this second method values of S' are plotted as abscissa against refractive index differences between solvent and solution, Δn , determined with a Rayleigh refractometer at the same wavelength as the laser for each solution used in the scattering measurements, as ordinate.

The molecular mass of bovine plasma albumin in water was determined by both methods. The data are given in Table 1. The graph of Δn against S'_{90} was a straight line and so $(\Delta n/S'_{90})_{\Delta n \rightarrow 0}$ was simply the slope of this line.

Table 1. *Light scattering data for BPA in water.*

$\Delta n \times 10^3$	$c \times 10^3$ (c, g cm ⁻³)	S'_{90}	$S'_{90} - 0.419$	$\frac{c}{S'_{90} - 0.419} \times 10^3$
3.207	17.240	9.390	8.971	1.922
1.703	9.156	5.337	4.918	1.862
0.977	5.253	3.194	2.775	1.893
0.816	4.387	2.432	2.013	2.179
0.457	2.457	1.704	1.285	1.912
0.457	2.457	1.622	1.203	2.042
0	0	0.419	0	—

$n_0 = 1.332$; $\lambda = 6.328 \times 10^{-5}$ cm; calibration constant, $C = 1.595 \times 10^{-5}$; $dn/dc = 0.1860$; $Z = 1.042$; $d_0 = 0.0328$; $\phi = 39^\circ 45'$; $P^{-1}(\theta) = 1.029$; Cabannes factor = 1.060.

The mean molecular mass was calculated using the normal procedure giving $M_w = 66\ 100$ with $P = 0.95$ limits of error of 6.3%; the modified method gave $M_w = 65\ 700$ with $P = 0.95$ limits of error of 4.7%. The statistical methods are described by Saunders & Fleming (1966). The mean results are in agreement with the value given by Riddiford & Jennings (1966).

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