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Particle Size Determination by Quasielastic Light Scattering

Sedimentation, microscopy, intensity light scattering, exclusion chromatography, and electrical resistance change are among the techniques routinely used for particle size analysis. Ongoing studies with quasielastic light scattering (QLS) are demonstrating that this relatively new technique offers significant advantages in many particle sizing analyses compared to these more classical approaches. Positive attributes of QLS include rapid analysis time, typically 10 to a few hundred seconds; wide applicable size range, extending from less than 50 Å to 2-μm-diameter particles; minimal sample preparation; and no requisite knowledge of the sample concentration. Of particular interest to the topical fields of solar energy and tertiary oil recovery is the capability of QLS to size "soft" particles such as oil/water microemulsions.

Fundamentally, the QLS experiment is a measurement of the slight (a few kHz) Doppler shift in the frequency of the scattered light that is caused by Brownian motion of the scattering particle. The term quasielastic serves to distinguish the measurement from intensity light scattering (totally elastic), in which no shifts in frequency are considered, and from inelastic techniques such as Raman spectrometry. It must be noted that there is no unanimity on the use of the "quasielastic light scattering" terminology. Names that have been used include dynamic light scattering, photon correlation spectrometry, Rayleigh spectrometry, homodyne spectrometry, heterodyne spectrometry, laser light scattering, and frequency-analyzing light scattering.

QLS Principles

Measurement of the small Doppler shifts that occur in the scattered light

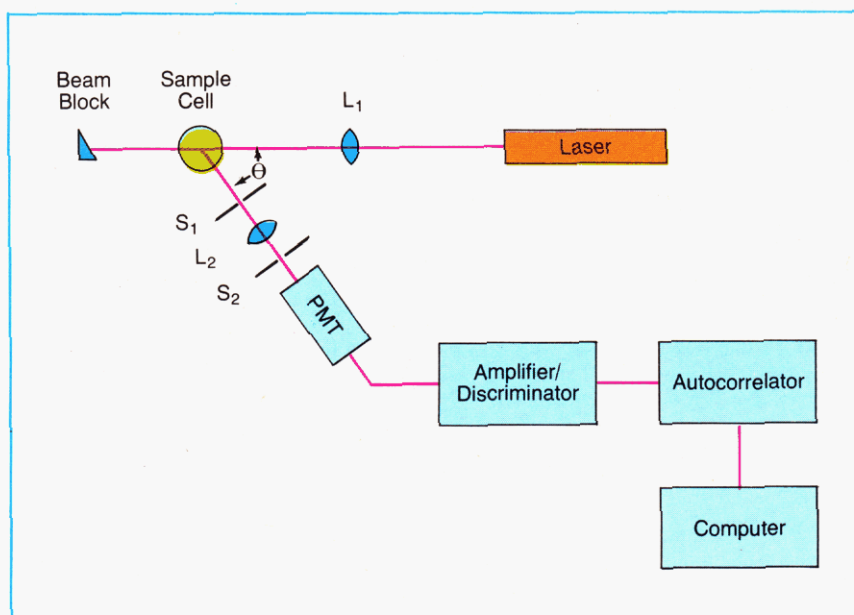


Figure 1. Block diagram of a simple quasielastic light scattering photometer. L_1 , L_2 are lenses; S_1 , S_2 are apertures

due to diffusion of the scattering macromolecules requires the use of "optical mixing" or "light-beating" techniques. Such techniques involve directing scattered light containing two or more frequency components onto a power detector such as a photomultiplier tube (PMT). The output of the PMT will contain a frequency component corresponding to the difference in the frequencies, i.e., the Doppler shift. For example, in the case of scattered light containing two angular frequencies ω_1 and ω_2 , the electric field, E , is given by:

$$E = E_1 \sin \omega_1 t + E_2 \sin \omega_2 t \quad (1)$$

The output of the PMT is proportional to E^2 , thus:

$$\begin{aligned} P &= AE^2 = A(E_1 \sin \omega_1 t + E_2 \sin \omega_2 t)^2 \\ &= A\{E_1^2 \sin^2 \omega_1 t + E_2^2 \sin^2 \omega_2 t \\ &\quad + E_1 E_2 [\cos(\omega_2 - \omega_1)t \\ &\quad - \cos(\omega_2 + \omega_1)t]\} \quad (2) \end{aligned}$$

where A is a constant dependent on the PMT gain.

The frequency components, ω_1 and ω_2 , for light scattered from a He-Ne laser ($\lambda = 6328$ Å) are on the order of 5×10^{14} Hz. A PMT cannot respond directly to such high frequencies, but instead provides a time-averaged power output corresponding to these components. However, the difference frequency term, $\omega_2 - \omega_1$, is a few kHz or less, well within the response range

of the PMT. Thus, the output of the PMT is:

$$P = A \left[\frac{1}{2} E_1^2 + \frac{1}{2} E_2^2 + E_1 E_2 \cos(\omega_2 - \omega_1)t \right] \quad (3)$$

giving a signal with a component equal to the original difference or beat frequency, $\omega_2 - \omega_1$. To observe these small frequency shifts, highly monochromatic incident illumination is necessary. Thus, the high temporal coherence of the laser has been essential to QLS development.

In an actual experiment the various directions and the Boltzmann-distributed speeds of the diffusing molecules create many beat frequencies resulting in a Lorentzian distribution, or spectrum, of output frequencies. Using a spectrum analyzer, it is possible to measure the power spectrum, and from the spectral line-width the average translational diffusion coefficient, D_T , can be calculated. However, D_T generally can be determined more conveniently by measuring the Fourier transform of the power spectrum, the autocorrelation function. The autocorrelation function, $G(\tau)$, of the scattering intensity can be expressed as

$$G(\tau) = \langle n(t) \cdot n(t + \tau) \rangle \quad (4)$$

where $n(t)$ is the scattering intensity at time t , τ is a "delay" time, and the angular brackets indicate that the product term is to be ensemble-averaged over the duration of the experiment. Excellent treatments of autocorrelation theory exist (1-3) and need not be repeated herein. A description of the operating fundamentals of an autocorrelator is given in the following section.

The relationship between D_T and the measured $G(\tau)$ is dependent on the conditions of the optical mixing. When only Doppler-shifted scattered light is mixed on the PMT, the measurement is described as a homodyne or "self-beating" technique. Under this condition and for a monodisperse sample (all particles have an identical diameter),

$$G(\tau) = A(1 + B e^{-2K^2 D_T \tau}) \quad (5)$$

where A is the baseline or square of the average scattering intensity, B is an empirical term related to the collection geometry, and

$$K = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2} \quad (6)$$

where n is the solution refractive index, λ_0 is the incident wavelength, and θ is the observation angle. Conversely, a heterodyne technique involves mixing both incident (unshifted) and shifted scattered light on the PMT. In such cases, the correct form of Equation 5 is

$$G(\tau) = A(1 + B e^{-2K^2 D_T \tau}) \quad (7)$$

provided the intensity of the unshifted light is much greater than that of the frequency-shifted light.

Homodyne techniques are more commonly used in the measurement of D_T , while heterodyne spectrometry is used when a net transport of the scattering macromolecules is measured, such as in electrophoretic light scattering (4). As a comparison of Equations 5 and 7 indicates, a significant error in the calculation of D_T will occur if light of the incident wavelength reaches the PMT in a homodyne experiment. Since scattering from stationary interfaces is unshifted from the incident wavelength, it is imperative that such background scattering be avoided.

As given by Equation 5, the autocorrelation function takes the form of an exponential. To calculate D_T , it is only necessary to determine the exponential time-constant, τ_c , and then

$$D_T = \frac{1}{2K^2 \tau_c} \quad (8)$$

Techniques for computing τ_c are discussed later in this paper.

Computation of particle size from D_T is dependent on the particle shape. For a sphere, the Stokes-Einstein equation gives

$$a = \frac{kT}{3\pi\eta D_T} \quad (9)$$

where a is the particle diameter, k is the Boltzmann constant, T is the temperature (K), and η is the solvent viscosity. Equations analogous to 9 are available for selected nonspherical shapes (3). For comparison studies, it is also feasible to simply calculate a "spherical equivalent" radius.

In some experiments, the measured D_T varies with sample concentration. This can result from physical interactions of the particles directly affecting D_T . Moreover, at high concentrations, light reaching the PMT may be scattered more than once, resulting in additional frequency shifts and a consequent change in the apparent D_T . In either case the measured D_T should be extrapolated to zero concentration prior to employing Equation 9.

Time-dependent phenomena other than translational diffusion can contribute to the measured autocorrelation function; specific examples include rotational diffusion and internal molecular movements (3). However, these phenomena typically have correlation times significantly shorter than those associated with translational diffusion and are unlikely to interfere with particle size measurements.

Instrumentation

The instrumentation essential for QLS measurements is diagramed in

Figure 1. Since monochromatic incident light is required, a laser is the most practical source. Quite adequate measurements can be made with an inexpensive helium-neon laser operating at 6328 Å. Although the particle scattering intensity increases with increasing frequency, no advantage in signal/noise can be gained by operating at shorter wavelengths other than that provided by improved PMT quantum efficiency. This fact results from the increase in background scattering that also occurs at shorter wavelengths. The laser power required is very dependent on the collection optics (S_1 , S_2 , L_2) and scattering angle, θ . With good-quality optics and at a low (near 0°) scattering angle, a 2-mW He-Ne laser is generally satisfactory. It should be noted that excessive laser power is undesirable since heating of the sample will increase particle diffusion.

While the design of the cell is not critical, it is important that surface scattering and dust contamination be minimized. The optical quality of the cell surfaces becomes crucial when operating at low scattering angles.

Apertures S_1 and S_2 and lens L_2 act to define the collection geometry; it is desirable that the viewed scattering volume be well removed from the cell walls. It is also necessary to restrict the cross-sectional area of the viewed scattering volume to ensure that the coherence of the scattered light is maintained. Normally this is accomplished by selecting L_1 to focus the incident beam to a beam diameter of approximately 100 μm and by adjusting the diameter of S_2 to match this beam diameter.

The PMT should be selected for minimum dark count rate. Additional criteria for PMT selection are well documented elsewhere (5).

While QLS measurements can be made at high scattering intensities using analog autocorrelation techniques, far superior results are generally obtained when using photon counting (digital) autocorrelation. The amplifier/discriminator in Figure 1 is required for photon counting. The discriminator passes to the correlator a logic pulse for each incoming photon pulse above a preset amplitude. This amplitude is set so that PMT output pulses that do not originate at the photocathode are ignored. The amplifier/discriminator must be designed for wide bandpass (e.g., 100 MHz) operation to minimize pulse overlap at the correlator.

A variety of commercial digital autocorrelators are available. These devices utilize high-speed shift registers and arithmetic units to perform the computation of Equation 4. Specifically, the correlator counts the photon

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pulses, n_i , in a "sample time" interval ΔT and computes

$$G_1(\tau) = \sum_{i=1}^k n_i n_{i+j} \quad (10)$$

where $j = \tau/\Delta T$ and $k = T/\Delta T$, T being the total analysis time. Typically, the value of $G_1(\tau)$ is simultaneously computed for 20 or more values of τ . Each sum is accumulated in a "channel" of the correlator memory and is transmitted to the computer at the end of the analysis.

The number of correlator channels required depends on the analytical accuracy sought and the size distribution of the sample. For other than essentially monodisperse samples, operation with fewer than 20 channels is ill-advised; 60 or more channels may be appropriate for characterizing polydisperse samples (i.e., samples having a wide size distribution).

Note that the design of the correlator will establish a maximum value of n_i ; larger maximum values require more involved circuitry. In the simplest design the only values allowed for n_i are zero or one. If the number of pulses occurring in an interval ΔT is greater than some preset number, called the clipping level, $n_i = 1$. Otherwise $n_i = 0$. These single-bit or clipped correlators are sufficient for many analyses. However, the clipping level must be correctly set; otherwise the frequency information will be lost. Correlators that utilize larger count registers allow operation at a variety of light levels without loss of information. Depending on the sources of noise encountered in a particular experiment, improved signal-to-noise ratios can often be obtained using the larger register correlators. Correlators using four-bit registers (maximum $n_i = 15$) are commercially available.

Selection of Scattering Angle

In many QLS analyses, little attention is given to the selection of scattering angle; measurements are predominantly made at 90° . However, significant gain in precision and often in accuracy can be obtained by judicious

selection of the scattering angle (6, 7).

Selection of the optimum angle(s) for QLS analysis requires familiarity with particle scattering characteristics as given by Mie scattering theory (8, 9). In addition, consideration must be given to the angular dependence of Equation 6, to the characteristics of the collection geometry, and to problems of dust contamination.

The important aspects of Mie scattering can be summarized as:

- For small particles ($a < \lambda/10$):
 - a) I_θ , the scattering intensity per unit solid angle and per unit scattering volume, is reasonably symmetrical about $\theta = 90^\circ$ when an unpolarized source is used; i.e., I_θ is highest near 0° and near 180° .
 - b) I_θ increases as a function of the sixth power of particle diameter.
- For large particles ($a > \lambda/10$):
 - a) As diameter increases, angular symmetry is lost; the scattering intensity is significantly higher near 0° compared to 180° .
 - b) I_θ oscillates as a function of θ for a given particle diameter; the angles at which the local minima and maxima occur vary with particle size.
 - c) I_θ oscillates as a function of diameter at a given angle. As θ increases, the oscillations have a shorter period and begin at smaller particle sizes.

Consideration of the collection optics indicates a significant increase in collection efficiency can be obtained by operating near 0° or 180° . As noted previously, it is necessary to minimize the cross-sectional area of the viewed scattering volume. Under this constraint, a significantly larger scattering volume is viewed when the collected light path is near coaxial ($\theta = 0^\circ$ or $\theta = 180^\circ$) as opposed to perpendicular ($\theta = 90^\circ$) to the incident beam (3).

A further advantage of operation near 180° is given by Equation 6, in which K is seen to increase with increasing θ . Therefore, from Equation 8, τ_c must decrease with increasing θ for constant a . As τ_c decreases we can use a short ΔT in Equation 10. This allows the use of higher light levels without saturating the correlator count registers, and the counting sta-

tistics are thus improved.

It is also noteworthy that operation near 180° facilitates the analysis of samples demonstrating significant absorbance at the incident wavelength. Finally, observation near 180° minimizes requirements for sample clarification. Dust contaminants, a primary cause of poor precision in QLS measurements, are very large particles that scatter most intensely near 0° , but minimally near 180° . Dust, if present, causes a severe loss of precision in low angle measurements.

A summation of these characteristics yields a guideline for selection of observation angle for small monodisperse samples. Quite simply, the measurement should be made as near 180° as practical, subject to constraints of background scattering. For larger ($a > \lambda/10$) and polydisperse samples, the selection is not as simple. Oscillations in the Mie function at high angles can adversely affect the accuracy of the size measurement. The selection of angle then becomes a compromise between the convenience of minimal sample preparation and the accuracy desired.

A comparison of measurements at low and high angles is shown in Table I. A significant improvement in precision was obtained at 175.5° . Note that the precisions reported at $\theta = 4.5^\circ$ are not the optimum possible at that angle; extending the time of a single measurement (7) allows the relative precision to be reduced to less than $\pm 10\%$ at $\theta = 4.5^\circ$.

The optical diagrams of the systems used for low and high angle measurements are shown in Figures 2 and 3. In Figure 2, the incident laser beam passes through the sample cell from the left, and is then diverted away from the collection optics by the beam block located at the center of the annulus. The attenuators to the left of the cell are used to reduce the incident intensity for highly scattering samples. The annulus and field stop act to restrict collection only to light that is scattered from the center of the cell at a low angle θ . The collected scattered

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Table I. Determination of Particle Diameter of Standard Polystyrene Latex Spheres by QLS

Nominal Diameter ¹	$\theta = 175.5^\circ$			$\theta = 4.5^\circ$		
	d, Å ²	Sample time, μ s	Time of single measurement, s	d, Å ²	Sample time, μ s	Time of single measurement, s
870 Å	866 \pm 11	10	10	—	—	—
1090 Å	1042 \pm 15	10	10	1075 \pm 225	5	20
2600 Å	2689 \pm 78	20	5	—	—	—
3690 Å	3556 \pm 64	20	10	3643 \pm 1200	10	20

¹ Nominal diameter specified by supplier.

² Diameter is average of 10 measurements; precision values are standard deviations of 10 measurements.

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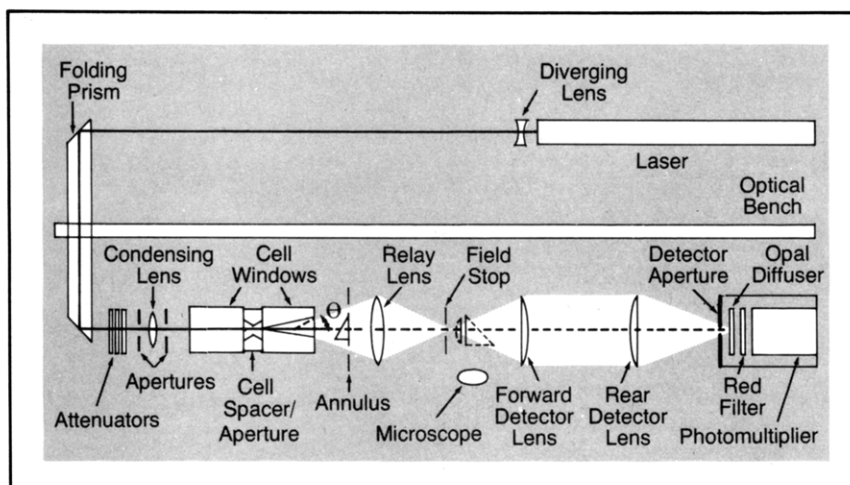


Figure 2. Optical diagram of a QLS photometer for measurement at low scattering angles ($\theta \approx 0^\circ$)

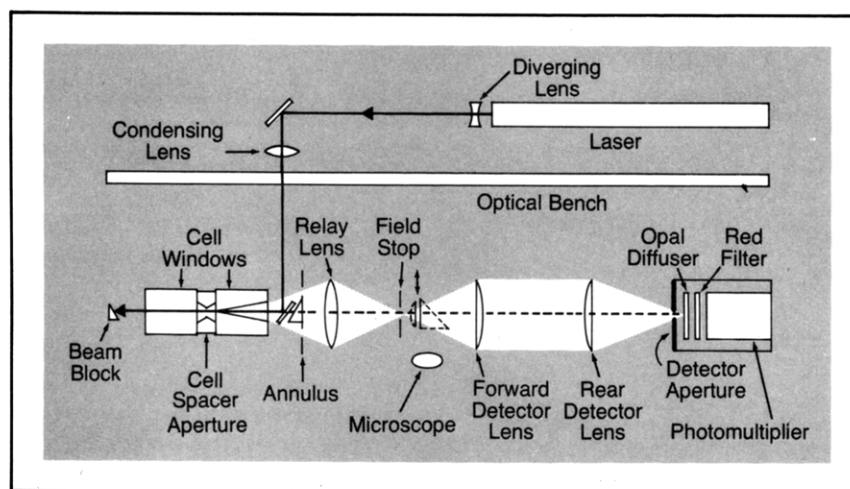


Figure 3. Optical diagram of a QLS photometer for measurement at high scattering angles ($\theta \approx 180^\circ$)

light then passes on to the photomultiplier. Prior to initiating a measurement, the lens and prism (dashed line) may be imposed in the light path to divert the light to the microscope, allowing the positioning of the cell and the degree of sample clarification to be examined.

To obtain measurements near 180° (Figure 3), two mirrors can be imposed in the incident beam. The incident beam now enters the cell from the right, and is diverted away by a repositioned beam block at the left end of the cell. The collection optics are unchanged, and thus now view light that is scattered in a direction nearly opposite to the incident beam.

Sample Preparation

The primary aspect of sample preparation for QLS is dust removal. As discussed in the previous sections, the requirements for clarification vary with the observation angle. At low angles, it is essential that the sample be

filtered or centrifuged to remove dust contaminants. Near 180° , precision of $\pm 2\%$ can often be obtained without filtration.

Samples can be prepared in any solvent that will suspend the sample; consideration should be given to the possibility of particle aggregation. Acceptable concentration ranges vary according to the scattering behavior of the sample. As a guideline, polystyrene latex spheres of diameter $0.1 \mu\text{m}$ to $0.3 \mu\text{m}$ can be satisfactorily measured in the range of $5 \mu\text{g/mL}$ to $100 \mu\text{g/mL}$.

Data Analysis

The determination of the exponential time constant, τ_c , from the QLS data can involve sophisticated computational techniques, a variety of which have been reviewed elsewhere (10). When the sample is monodisperse, $G(\tau)$ is a single exponential, and a simple nonlinear least squares analysis generally provides optimum results. In

practice, this technique is used for most narrow size distribution samples.

When the sample is polydisperse, the resulting $G(\tau)$ is a summation of exponentials. The most common technique for determining τ_c in such cases is the method of cumulants developed by Koppel (12). The cumulant analysis provides a measure of the moments of the intensity-weighted size distribution. The first and second moments (the z -average size and the variance) can often be reliably calculated; higher-order moments can be calculated but are highly imprecise. The accuracy of the moments is reduced at higher angles and/or higher particle sizes at which Mie function oscillations distort the relative scattering intensities. The cumulant analysis is generally inadequate for analyzing multimodal distributions.

For applications where the cumulant analysis is of limited utility, the histogram technique of Chu et al. (10), may be used. In this approach, the size distribution (sample fraction vs. particle size) is approximated by an equally or unequally segmented histogram. An autocorrelation function is computed from the histogram, and the histogram is adjusted until the error of fit of the calculated $G(\tau)$ and measured $G(\tau)$ is minimized. The histogram technique has been shown to be especially useful for bimodal distributions.

The techniques described above involve measurements at a single angle. An assortment of techniques have also been developed for using the angular variations in the measured D_T that result from Mie function oscillations as a semi-quantitative determinant of the particle size distribution (13, 14).

Applications

Because of their ready availability and well-characterized size distribution, much of the development of the QLS technique has employed standard polystyrene latex spheres. The sizing of polymer lattices and resins continues as a common QLS measurement; by avoiding the need for extensive sample preparation and drying, QLS offers a convenient alternative to electron microscopy. Specific applications include the monitoring of particle growth during emulsion and non-aqueous dispersion polymerization processes (15) and the sizing of fluorocarbon "vesicles" (6), currently under investigation as possible artificial blood components.

It is in those applications where the nature of the analysis or the particle precludes desolvating the sample or subjecting it to the shear forces involved in several alternative techniques that QLS has the greatest application. For example, variation in particle size induced by solvent

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changes can be easily studied (15, 16).

Micelles and microemulsions, classic examples of "soft particles" that inherently exist only in solution and can degrade under shear, are conveniently sized by QLS. A wide variety of micelle applications have been reported, particularly in the biochemical literature (17, 18). Investigations of the enhanced cleavage of water by visible light in microemulsion systems have employed QLS to size the microemulsion particles (19, 20), and the demonstrated capability of QLS techniques to size water-oil microemulsions (21) may yield a useful tool for characterizing micelles and microemulsions used in tertiary oil recovery (22).

QLS is also a useful method for observing changes in particle size during association/aggregation phenomena.

Reported applications include monitoring the aggregation of antigen-coated latex spheres and a corresponding antibody (23), the aggregation of bacterial ribosomes (24), and possible secondary aggregation of sodium dodecyl sulfate micelles (25).

Finally, it should be noted that the QLS technique has utility beyond the measurement of particle size. The wide range of biochemical applications has been reviewed elsewhere (26). QLS techniques have been utilized for determination of molecular conformation (27, 28), molecular weight and molecular weight distributions (29, 30), and molecular rotation (31). Polymer branching has been characterized (32), and QLS has been applied to the study of latex surfaces (33) and polymeric soft gels (34).

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