Application of photon correlation spectroscopy to a macromolecular system

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Photon correlation spectroscopy was used to determine the diameter of polystyrene spheres suspended in water. Two separate trials yielded diameter measurements of 293.5± 5.4 nm and 297.0 ± 5.2 nm respectively. The two values agree well with one another and are within one standard deviation of the given manufacturer's sphere diameter of 304 ± 6 nm. These results support the use of photon correlation spectroscopy as an effective method to measure the structural properties of macromolecular solutions.

INTRODUCTION

The use of dynamic light scattering as an investigative tool began around 1914 when Leon Brillouin published theoretical findings on the light scattered from an excited isotropic body.[1] Experimental results of frequency shifts in the light scattered from a liquid followed shortly after the theoretical predictions, but it was the advent of the laser in the 1960's that allowed small frequency changes to be measured and thus provided an impetus for the application of light scattering to a wide variety of systems including solids, gels, membrane vesicles and colloidal dispersions.[2]

Structural properties of a material, such as size and shape, can be determined from a light scattering experiment. In 1964, Robert Pecora developed a technique called photon correlation spectroscopy that was particularly useful in the study of macromolecular systems.[1] Photon correlation spectroscopy is utilized in this experiment to determine the radius of polystyrene spheres suspended in water.

THEORY

In light scattering experiments, the electric field of the incident light imparts an oscillating polarization on the particles in the sample.[2] Those particles whose polarity differs from the surroundings scatter the incident light. Within the sample, the particles are in constant motion, and it is the movement of particles that causes fluctuations in the detected intensity signal. According to Pecora,[1] the duration of the fluctuations contains physical information about the particles, including size.

Diffusivity of a particle suspended in a liquid is opposed by the liquid's viscosity,[3] so that the particles spread throughout the liquid more slowly as the thickness of the liquid increases. For small motion, laminar or steady-state flow is a good assumption. The Stokes-Einstein equation for the diffusivity of particles in a liquid defines the diffusion coefficient, D, of the particles as

$$D = \frac{k_B T}{6 \pi \eta r} \qquad (1)$$

where k_B is Boltzmann's constant, T is the temperature in Kelvin, η is the viscosity of the liquid in which the particles are suspended and r is the radius of the particles.

Since the fluctuation of the intensity signal is caused by the motion of particles, those particles that diffuse more quickly are characterized by a smaller fluctuation time. As seen in Eqn. (1), the diffusion coefficient is inversely proportional to the size of the particles, and, consequently, the smaller the particle, the shorter the fluctuation time.

A correlation equation can be used to determine the correlation or relationship between the measurements of a fluctuating signal. To measure the correlation of an intensity signal, the time average of the signal is taken,[1] called an autocorrelation function

$$g_1(t) = \langle I(t) I(t + \tau) \rangle = \exp(-t\tau) \qquad (2)$$

For monodisperse system in Brownian motion, the diffusion of the particles in erratic motion causes the intensity autocorrelation
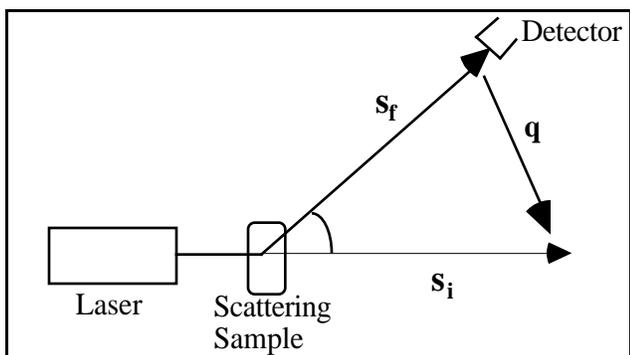
FIG. 1. The incident wave vector, s_i, is scattered in all directions upon reaching the scattering sample, but only the light of the wave vector s_f is detected. The geometry of the apparatus defines the scattering vector q where the angle is called the scattering angle.

equation to decay exponentially where Γ, the linewidth of the spectrum, is related to the diffusion coefficient by $\Gamma = Dq^2$, and the scattering vector, q, is given by

$$q = \frac{4\pi n}{\lambda_o}\sin\frac{\theta}{2} \qquad (3)$$

in which n is the index of refraction of the liquid medium, θ is the scattering angle and λ_o is the laser wavelength in air. Geometrically, the scattering vector is defined as the difference between the scattered wave vector and the incident wave vector (see Fig. 1). By combining Eqn. (1) and the equation for the linewidth, Γ, an equation for the radius of the scattering particle is obtained as

$$r = \frac{k_B T q^2}{6\pi\eta\Gamma} \qquad (4)$$

Photon correlation spectroscopy digitally measures the intensity fluctuations of a signal at the level of the photon. Multiple measurements are taken of the number of photons that reach a detector in a sample time. For each sample time, the autocorrelation function is computed by an automated correlator. When the logarithm of the autocorrelation function is graphed versus time, the slope of the resulting line is the linewidth, Γ. Using Eqn. (4), the size of the scattering particles can then be determined.

EXPERIMENT

The sample cell was a 20mL cylindrical glass cell that contained one drop of polystyrene spheres (Duke Scientific diameter 304±6nm) suspended in approximately 16mL of filtered distilled water. The sample cell sat in the sample cell assembly of a computer controlled BI-200 SM Goniometer. A He-Ne laser beam was

focused to the sample cell, and the scattering angle was set at 90° by adjusting the rotating rigid arm. The pinhole was set to 200μm and the filter to 633nm. The sample cell was surrounded by Decalin to reduce the amount of stray light that reached the detector. A water bath attached to the goniometer controlled the temperature of the Decalin at 25.3±0.1°C and a micropump removed dust from this fluid.
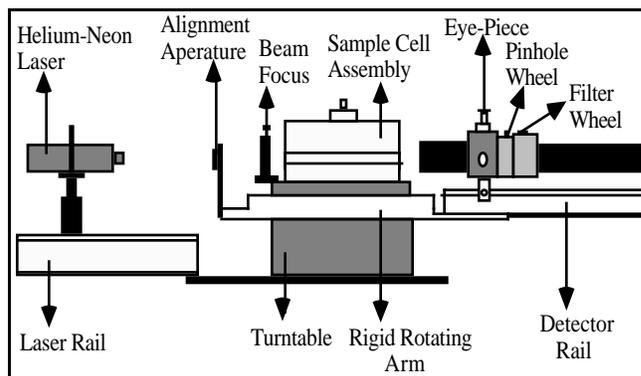

FIG. 2. Diagram of the BI-200 SM Goniometer with the major features labeled.

The BI9IST program was used to record the angle and pinhole sizes. A Toluene sample was used to test the laser stability and intensity signal as it is a pure liquid that is easily measured. In the BI9 data acquisition program, the scattering angle was set to 90.0 ±0.1°. The temperature was measured as 25.3±0.1°C, the viscosity as 0.8844cP,[4] the wavelength was set to 632.8nm and the fluid index of refraction to 1.33. Two data runs were taken with the sample time interval set at 0.5μs, and the total acquisition time was 7500μs for Run 1 and 7500μs for Run 2. The raw data was transferred to Igor Pro for analysis. The count rate for both runs was about 2 x 10^5 counts/second, and a dark count rate of 10 counts/second was found by turning the filter wheel to the closed position and initiating a data run.

ANALYSIS AND INTERPRETATION

The calculated base values from the BI9 software, 507925 for Run 1 and 468251 for Run 2, were subtracted from the autocorrelation functions. This subtraction causes the correlation function to decay to zero instead of a nonzero value; it does not affect the shape of the curve. A graph was made of the autocorrelation function (arbitrary units) versus time (μs). An exponential

Table I: Fitting coefficient values and resulting Γ for Runs 1 and 2

	C_o	C_1	C_2 (μs^{-1})	Γ (s^{-1})
Run 1	-205 ± 193	93893 ± 213	$9.98 \times 10^{-4} \pm 6 \times 10^{-6}$	588.6 ± 3.5
Run 2	246 ± 199	80755 ± 244	$1.04 \times 10^{-3} \pm 4 \times 10^{-6}$	582.7 ± 2.2

fit was done only to those points in the 0-6000μs time range as this range was long enough to show a definite decrease and stabilization to 0 on the autocorrelation scale while eliminating the most erratic portions of the data sets (see Fig. 3 and Fig. 4).

To fit the autocorrelation function, an exponential equation of the form

$$g_1(t) = C_o + C_1 * \exp(-C_2 * x) \qquad (5)$$

was used with equal weight given to all data. The fitting coefficients (see Table I) and their appropriate error are reported by the fitting function where $C_2 = \Gamma$/Samples. For each data run, the BI9 software records a numerical Samples value, and it was given as 589824 for Run 1 and 557056 for Run 2. The inclusion of the Samples value may arise from the manner in which the program calculates the autocorrelation function. Instead of taking one photon count per sample time, the BI9 software may break the set sample time into multiple sections and count the photons within those sections as well. In doing so, the correlator achieves a total number of sample times that resembles a run of a longer duration. Thus, the Samples value may account for the "short-cut" that the correlator uses to include a large number of sample times within one data run, but whatever the reason, the Samples value must be used to calculate Γ from C_2.
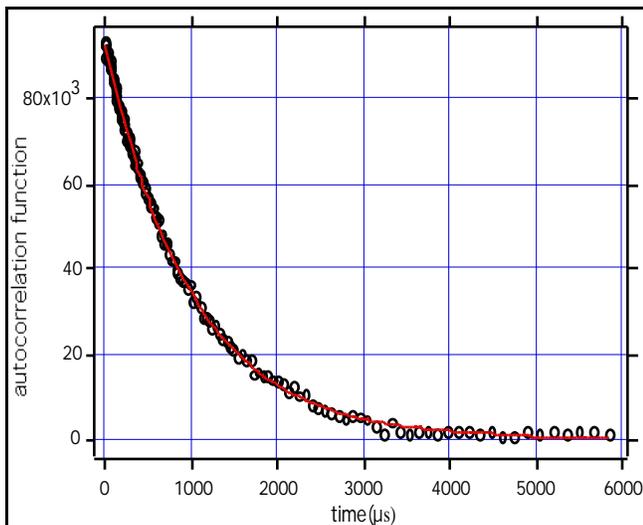

FIG. 3. Graph of the autocorrelation function (arbitrary units) versus time (μs) for Run 1 of the polystyrene mixture. The dots represent the experimental data while the solid line is the theoretical fit.
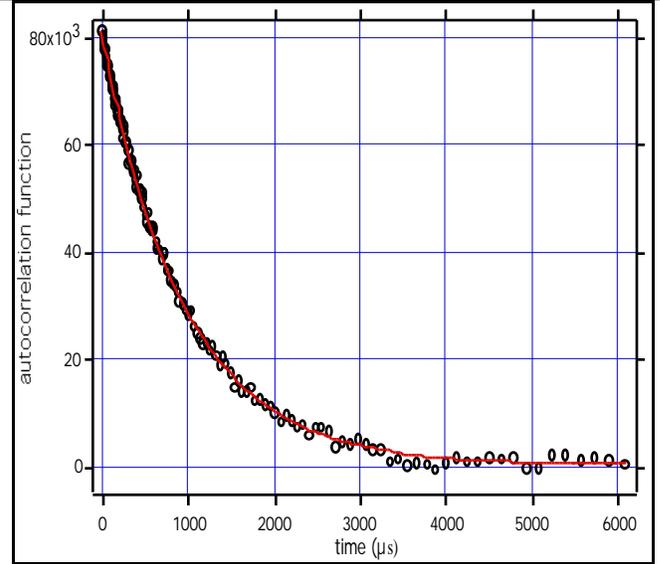

FIG. 4. Graph of the autocorrelation function (arbitrary units) versus time (μs) for Run 2 of the polystyrene mixture. The dots represent the experimental data while the solid line is the theoretical fit.

Once the fitting coefficients were obtained, the linewidth, Γ, was found. For Run 1, $\Gamma = 588.6 \pm 3.5 s^{-1}$, and for Run 2 $\Gamma = 582.7 \pm 2.2 s^{-1}$. Next, Eqn. (3) was used to determine the value for the scattering vector as $q = (1.87 \pm 0.02) \times 10^5 cm^{-1}$. To then determine the radius of the polystyrene spheres, Eqn. (4) was used with $k_B = 1.381 \times 10^{-6} \frac{erg}{K}$. For Run 1, the radius was determined to be $r = 146.8 \pm 2.7$ nm, and for Run 2, $r = 148.5 \pm 2.6$ nm. Both experimental values are within one standard deviation of the manufacturer's radius value of 152 ± 3 nm. Additional error not included in the error propagation includes the dark count rate and the possibility of spheres sticking together within the sample.

CONCLUSION

Photon correlation spectroscopy has successfully been used to determine the diameter of polystyrene spheres with two separate data runs yielding results of (293.6 ± 5.4)nm and (297.0 ± 5.2)nm respectively. These values are within one standard deviation of each and within one standard deviation of the manufacturer's value of (304 ± 6)nm. This experiment can act as a preliminary test of the spectroscopy system with

many possibilities for further experiments. Future applications include the study of polymer interactions in gels and a measure of the translational diffusion coefficients of proteins and nucleic acids. With further advancements in laser and digital technology, the applicability of light scattering techniques will continue to grow and to provide a better understanding of previously unmeasurable quantities.

[1] R. Pecora, editor, *Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy*, (Plenum Press, New York, 1985).

[2] B.J. Berne and R. Pecora, *Dynamic Light Scattering with Applications to Chemistry, Biology and Physics*, (Wiley-Interscience, New York, 1976).

[3] C. Kittel and H. Kroemer, *Thermal Physics*, Second Edition, (W.H. Freeman and Company, New York, 1980).

[4] J. White, Summer 1996 Laboratory Notebook, Vol. 1.