PHYSICAL REVIEW A VOLUME 24, NUMBER 4 OCTOBER 1981

Experimental demonstration of multiple-scattering suppression in quasielastic-light-scattering spectroscopy by homodyne coincidence techniques

George D. J. Phillies
Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

(Received 28 April 1981)

Multiple-scattering artifacts are a not uncommon difficulty in applications of quasielastic-light-scattering spectroscopy (QELSS). An earlier proposal [G. Phillies, J. Chem. Phys. 74,260 (1981)] for a two-beam, two-detector light-scattering spectrometer, which is only sensitive to singly scattered photons, is subject here to an experimental test. The device is found to work essentially as predicted. The diffusion coefficient of 0.091-μm polystyrene spheres in water is concentration independent for solute volume fraction $\lesssim 0.01$. Multiple scattering does not have a significant effect on the QELSS spectrum of bovine serum albumin at a protein concentration of 20 wt. %.

I. INTRODUCTION

In the study of liquid systems, quasielastic-light-scattering spectroscopy (QELSS) measures directly the evanescent fluctuations in an experimentally fixed spatial Fourier component ϵ_k of the local index of refraction. The fluctuation rate Γ of ϵ_k is determined by various transport coefficients, including (for solutions of scattering macromolecules in an optically neutral solvent) the mutual diffusion coefficient D of the macromolecules, namely,

$$\Gamma = Dk^2 \, . \tag{1}$$

It is at best difficult to use light-scattering spectroscopy to study systems which scatter light strongly, since in this case photons may be scattered more than once (as by fluctuations $\epsilon_{\vec{q}}$, $\epsilon_{\vec{k}-\vec{q}}$), before they reach the detector. Calculating the total multiple-scattering spectrum requires a sum over allowed values of the intermediate wave vectors $\vec{q}$, as weighted by the particle scattering factors and the optical geometry of the system. For the ideal case of a spherical scattering volume, Sorenson *et al.*[1] have calculated the double-scattering spectrum of a solution of noninteracting Brownian particles, finding for this geometry that the linewidth for depolarized double scattering from small particles is equal to the linewidth for polarized single scattering at a 180° scattering angle. For a different geometry, a new computation of the double-scattering spectrum would be needed. Furthermore, for optical thicknesses only modestly larger than those at which double scattering becomes important, it is apparently also necessary to take into account the effect of triple, quadruple, etc., scattering.

Given the uncertainties implicit in making mathematical corrections to measured spectra, it would arguably be superior to remove the multiple-scattering effects physically, as by using a small, optically thin sample. However, interesting systems which scatter light strongly also typically have long-range molecular correlations, so the physical properties of a very thin sample can be different than those of the bulk material. Recently, this author[2] proposed an alternate procedure for suppressing multiple-scattering effects. As part of a long-term program to develop a multibeam, multiple-detector instrument for measuring the three-point-density-correlation function,[3] the detailed properties of a two-incident-beam, two-detector scattering experiment (Fig. 1) were considered. In this experiment, one illuminates a sample with two antiparallel laser beams of the same wavelength, positions two detectors on opposite sides of the sample at 90° from the laser beams (so scattering from fluctuations $\epsilon_{\vec{k}}$ and $\epsilon_{-\vec{k}}$ is detected), and studies cross correlations in intensity fluctuations between the two detectors. As has long been known,[4] for single scattering from a large volume, the intensity-intensity cross-correlation function $S_x(\vec{k},t)$ obtained in this way is the same

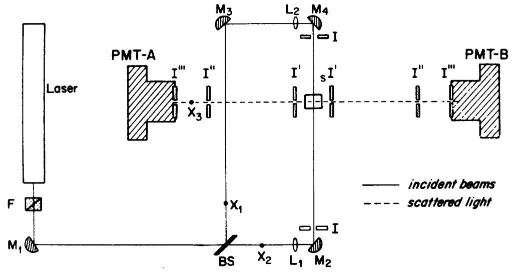

FIG. 1. Optical train of the homodyne coincidence spectrometer. *F* is a neutral density filter, the M_i are mirrors; BS is a beamsplitter; *L* 1 and *L* 2 are focusing lenses; *S* is the (square) sample cell; the *I*, *I'*, *I''*, and *I'''* are collimating irises, X_1 and X_2 are locations for beam stops, X_3 is the beam insertion location for aligning the irises, and PMT-*A* and PMT-*B* are the photomultiplier tubes.

as the function $S(\vec{k},t)$ measured with one beam and one detector. The novel result of Ref. 2 is that there is no double-scattering contribution to $S_x(\vec{k},t)$. While double-scattered photons reach both detectors, the fluctuations in the double-scattering intensities at the two detectors are not cross correlated.

Here an experimental realization of this proposal is demonstrated. After a description of the apparatus, results are presented on two systems: (i) suspensions of 0.091-μm polystyrene latex spheres at concentrations ≤ 1.2 vol % and (ii) bovine serum albumin in 0.15*M* NaCl at a concentration of 20 wt. %.

II. EXPERIMENTAL DEVELOPMENT

Figure 1 shows the optical train actually used in this experiment. The entire apparatus is mounted on a 4×10 ft. vibration isolation table which is floated on commercial airmounts. In operation, laser light (25-mW He—Ne source) is sent through the nondeflecting double-wedge attenuator *F* to the 50% reflection-plate beamsplitter (BS). The two incident beams then pass through converging lenses *L* 1 and *L* 2 (focal length 63 cm) to the sample cell *S*. X_1 and X_2 denote locations at which beam blocks were placed for control experiments. The sample cell was a standard 1-cm fluorimeter cell with four sides polished. For the experiment to work, it was found necessary that BS, *M* 2, *M* 3, and *M* 4 be held in high-precision (nominal settability 6″) angular positioners. The light scattered through 90° was isolated by the pairs of irises *I'* and *I''* and allowed to reach photomultiplier tubes (PMT) *A* and *B* by passing through their front-end irises *I'''*. The photomultipliers used here were an unmatched pair of RCA 7265 tubes with S-20 photosurfaces. This arrangement differs from that proposed in Ref. 3 in that only one laser is used and no spatial filtering of the incident beam is provided.

The major difficulty in operating the instrument comes from the alignment accuracy needed before the cross-spectrum $S_x(\vec{k},t)$ appears. In Ref. 3, it is argued that the required directional accuracy is $\lesssim 0.5$ mrad, i.e., at 1 m from the sample cell, the beam centers must be fixed to within 0.5 mm. For each incident beam, the necessary 4 degrees of positioning freedom were obtained by rotation of the final mirrors and by horizontal and vertical translation of the focusing lenses. The horizontal-beam positions were determined by mounting a pair of pins vertically on the table surface and requiring that the shadows of the pin points be found in the center of the laser beam. The beam heights were determined by allowing the laser beams to pass through a series of aluminum plates containing 0.75-mm holes at the desired height. The collecting irises were positioned by use of a pair of auxiliary mirrors which introduced a laser beam into the optical train at the point X_3. The beam was sent through four irises *I'* and *I''* and the vacant cell mount to PMT-*B*, and the irises and PMT-*B* were positioned to be collinear with the beam. PMT-*A* was positioned by removing the auxilliary mirrors, placing a beamsplitter cube at *S*, and requiring that light which passed through *I'* and *I''* also fall on the entrance iris of PMT-*A*. The irises *I''* were then opened to their maximum. After aligning the optics in this way, a cross spectrum was reliably observed.

Initial efforts to position the collecting irises by simply replacing the sample with a cube beamsplitter gave at best an extremely weak cross spectrum. In some early experiments, the collecting irises were supplemented by collecting lenses placed between *I'* and *I''* or *I''* and *I'''*. The use of collecting lenses never resulted in a satisfactory spectrum, probably because the volumes viewed by the two phototubes overlapped poorly.

The signals from each photomultiplier tube were sent through preamplifier, discriminator, and pulse shaper and sent into the *A* and *B* (cross-correlation) input ports of a 64-channel Langley-Ford Instruments digital correlator. The measured intensity-intensity correlation functions were analyzed by Koppel's cumulants method.[5]

A first concern was to demonstrate that the experiment works in accordance with the theory. Initial measurements were made on dilute (volume fraction ϕ of solute $\lesssim 10^{-4}$) suspensions of 0.091-μm-diameter polystyrene spheres in 0.4 g l^{-1} sodium lauryl sulphate. It was found (cf. Fig. 2) that the diffusion coefficient obtained from a single-detector determination of $S(\vec{k},t)$ and from the two-detector determination of $S_x(\vec{k},t)$ agreed to within experimental error, though the $D = 0.53\times 10^{-7}$ cm^{2}s^{-1} obtained spectroscopically is modestly larger than the 0.50×10^{-7} cm^{2}s^{-1} expected theoretically for 0.091-μm spheres under our operating conditions. In spectra taken with long (multihour) integration times, there was sometimes a difficulty with weak long-time oscillations in the background. These were avoided by doing exponential fits only to the early points (~ 25 channels, $\sim 1/Dk^2$ in time) of the correlation function.

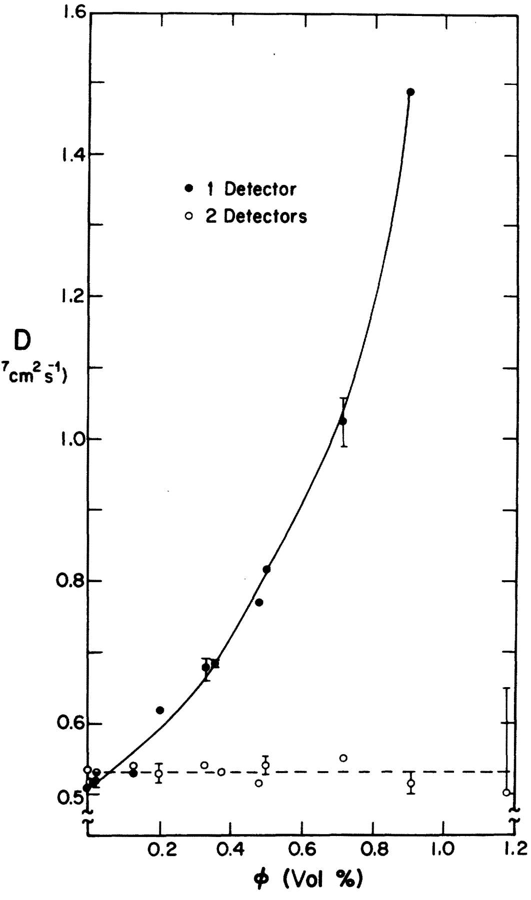

FIG. 2. Diffusion coefficient of polystyrene latex spheres (nominal diameter 0.091 μm) as determined by a conventional one-detector experiment (filled circles) or by homodyne coincidence spectroscopy (open circles). The three left-most pairs of points are at concentrations $\phi = 2.1\times 10^{-5}$, 2.7×10^{-5}, and 8.7×10^{-5}. The one-detector experiment shows a strong concentration dependence for Γ, but the two-detector experiment finds that D is independent of concentration. Representative error bars show the spread in the 2–5 measurements which were averaged to obtain each point.

An extensive search was made for artifacts which might contribute to $S_x(\vec{k},t)$. The experiment works because scattering through wave vectors $\vec{k}$ and $-\vec{k}$ are performed by the same spatial Fourier component of the concentration; light scattered from one incident beam into one detector is cross correlated with light scattered by the other incident beam into the other detector. Light is scattered from a single-incident beam into each of the detectors by different concentration fluctuations, so if only one incident beam were used in the experiment, $S_x(\vec{k},t)$ ought to vanish. To test this prediction about a one-beam, two-detector experiment, beam blocks were alternately placed in the optical train at points X_1 and X_2. Measurement of $S_x(\vec{k},t)$ while either incident beam was blocked showed no indication of a cross spectrum. With the observed signal-to-noise ratios, either beam block must have reduced the amplitude of $S_x(\vec{k},t)$ by more than a factor of 100. The tests with the beam blocks show that the observed $S_x(\vec{k},t)$ does not arise from any of the obvious artifacts caused by laser-power fluctuations, table vibration, or signal leakage between the two correlator input lines.

The simple theoretical analysis of Ref. 2 suggests that $S(\vec{k},t)$ and $S_x(\vec{k},t)$ have the same amplitude. In practice, the rate of increase of amplitude with time of $S_x(\vec{k},t)$ was less than that of $S(\vec{k},t)$. One may define a cross-correlation efficiency E by

$$E = \frac{S_x(\vec{k},0)}{[S^A(\vec{k},0)S^B(\vec{k},0)]^{1/2}} , \tag{2}$$

where the superscripts A and B refer to the two photomultiplier tubes, and all amplitudes are normalized by their integration times. With an optically thin suspension of scatterers, E ranged from 0.04 to 0.14, the efficiency increasing with experience at aligning the instrument.

A further check that the observed $S_x(\vec{k},t)$ corresponds to the predicted phenomenon was made by selecting the polarization of the scattered light.

The incident laser beam and the singly scattered light were vertically polarized; only from multiple scattering does horizontally polarized light appear. A suspension of polystyrene spheres was prepared at a moderate concentration ($\phi=0.006$); horizontal polarizers were placed at the irises I''. On measuring the intensity-intensity autocorrelation function, using either detector, a moderately intense depolarized scattering spectrum was observed. Efforts were then made to observe the depolarized cross spectrum. Even with a 12-h integration time, no cross correlations were found in the depolarized scattering. From the measured cross-correlation efficiency (as obtained from the polarized spectra and cross spectra) and the amplitudes of the depolarized $S(\vec{k},t)$, the absence of a detectable depolarized $S_x(\vec{k},t)$ indicates at least a 30-fold suppression of the depolarized $S_x(\vec{k},t)$ relative to the depolarized $S(\vec{k},t)$. This difference in amplitudes would be expected if only single-scattered light showed cross correlations, but would be difficult to explain if the polarized $S_x(k,t)$ were due to experimental error.

There exists one predictable artifact. If the sample cell is placed so that the incident laser beams are reflected exactly back onto themselves, then light scattered through 90° towards one photomultiplier may be reflected by the side window of the sample cell, back towards the other photomultiplier. In this way, scattering from a single beam by a single fluctuation could reach both detectors. This effect should produce a cross spectrum even when one beam is blocked. After careful alignment of the scattering cell, it was sometimes possible to observe a weak cross spectrum even with one beam block in place. This artifact was reliably eliminated by rotating the scattering cell about the vertical axis by as little as 0.5°, insuring that the scattering volumes seen directly by one photomultiplier tube and by side-window reflection from the other photomultiplier tube do not overlap, even though the volumes seen directly by the two phototubes do overlap.

III. APPLICATIONS

The concentration dependence of D for polystyrene spheres has previously been studied by Bauer,[6] who examined the diffusion of 380-, 1090-, and 2340-Å spheres at concentrations $10^{-5}\leq\phi\leq0.1$. Even at $\phi=1\times10^{-3}$, Bauer found that D as obtained from $S(\vec{k},t)$ differs by more than 20% from its zero-concentration limiting value. In his work, multiple-scattering effects were controlled by using a series of scattering cells of different size (though data on the same sample using different size cells was not reported extensively). The effect of multiple scattering was also checked by comparing the amplitudes of the polarized (VV) and depolarized (VH) spectra, it being found that the VH spectrum was on the order of 1% that of the VV spectrum. Bauer concluded that multiple scattering did not have a significant effect on the observed spectra.

We have used homodyne coincidence spectroscopy to determine the diffusion coefficient of 0.091-μm diameter Dow polystyrene spheres suspended in 0.4 g l^{-1} sodium lauryl sulphate. The data are given in Fig. 2, which also indicates values of D obtained from single-detector determinations of $S(\vec{k},t)$. Measurements were made for $2\times10^{-5}\leq\phi\leq1.18\times10^{-2}$, the higher concentration being above the practical upper limit for determining D by homodyne coincidence techniques using our optical train and a 1-cm scattering cell. The amplitude of $S_x(\vec{k},t)$ and the accuracy of the measurement of D are far less for the sample with $\phi=1.18\times10^{-2}$ than for samples with $\phi<1.0\times10^{-2}$. For $\phi\lesssim10^{-3}$, the two techniques are in good agreement; D is found to be independent of concentration and equal to its zero-concentration limiting value. At higher concentrations, the diffusion coefficients differ. The diffusion coefficient obtained from $S(\vec{k},t)$ increases by more than 250% as ϕ is raised from 1×10^{-3} to 0.9×10^{-2}. In contrast, D from the two-detector experiment is independent of concentration.

The difference between $S(\vec{k},t)$ and $S_x(\vec{k},t)$ is here interpreted as arising from multiple scattering by the sphere suspensions. Multiple scattering should be expected in these systems, whose appearance ranges from slightly lucent to milk-white. With the most concentrated suspensions, the rear face of the scattering cell can only be seen with the aid of back illumination of the cell. The other explanation for the dependence of $S(\vec{k},t)$ on ϕ is that solute-solute interactions are important, but this is not plausible under these conditions. Hard-sphere and hydrodynamic interactions ought to be insignificant at such low ϕ. The presence of $1.6\times10^{-3}M$ sodium lauryl sulphate in all suspensions should serve to screen out any large electrostatic effects. Furthermore, solute-solute interactions ought to have the same effect on $S(\vec{k},t)$ and $S_x(\vec{k},t)$, but it is here observed that $S(\vec{k},t)$ and $S_x(\vec{k},t)$ differ markedly.

Some information on the degree of multiple scattering in these systems may be gleaned from the depolarized (VH) spectrum, which is caused by multiple scattering. Sorenson *et al.* have found for point scatterers, a spherical scattering geometry, and m-fold multiple scattering that the VH linewidth satisfies

$$\Gamma_{\mathrm{VH}}(\text{any angle}) = m\,\Gamma_{\mathrm{VV}}(90^\circ) \ . \tag{3}$$

While our scattering geometry probably does not satisfy their requirement, at $\phi = 3.3\times10^{-3}$, 5×10^{-3}, and 6.6×10^{-3}, we find $\Gamma_{\mathrm{VH}} = 1.6\times10^{-7}$, 2.8×10^{-7}, and $(3.4\pm0.2)\times10^{-7}\ \mathrm{cm^2\,s^{-1}}$, respectively. In contrast, for single scattering (low concentrations) $\Gamma_{\mathrm{VV}}(90^\circ) \simeq 0.53\times10^{-7}\ \mathrm{cm^2\,s^{-1}}$, confirming a strong, ϕ-dependent effect of several-fold multiple scattering on the depolarized spectrum. These results on the VH spectrum are consistent with our interpretation of the high-concentration VV spectrum in terms of m-fold scattering. One notes that Sorenson *et al.* showed that the VH intensity in double scattering is $\frac{1}{8}$ of the VV intensity, so the depolarized, double-scattered spectrum ought to have $\frac{1}{64}$ the amplitude of the polarized double-scattered spectrum. The observation that the VH spectrum is far less intense than the VV spectrum, therefore, does not indicate that multiple-scattering effects are not important.

If one assumes that the theoretical basis for homodyne coincidence spectroscopy is sound, the measurements of $S_x(\vec{k},t)$ reveal the concentration dependence of D for dilute polystyrene sphere suspensions. For $0 \le \phi \le 0.01$, D does not depend strongly on ϕ. If one assumes

$$D = D_0(1 + \alpha\phi) \ , \tag{4}$$

D_0 being the low-concentration limit for D, with our data, $0 \le \alpha \le 5$ would appear acceptable. Even with the scatter in the points, $\alpha < -5$ or $\alpha > 10$ would seem to be unacceptable.

The mutual diffusion coefficient of concentrated protein solutions have been extensively examined by light-scattering spectroscopy and other techniques, a major stimulus for modern work being the substantial disagreement between QELSS values for D and those obtained using a diaphragm diffusion cell.[7] While the weight of the evidence appears to favor the QELSS results, Minton and Ross[8] have argued that multiple scattering or other effects may interfere with QELSS studies of concentrated protein solutions.

We have therefore examined the mutual diffusion of isoionic bovine serum albumin (BSA) in 0.15*M* NaCl at a concentration of 20 wt. %, using both QELSS and homodyne coincidence spectroscopy. Because serum albumin scatters appreciably less light and diffuses far more rapidly than does the polystyrene, it was difficult to obtain accurate values for $S_x(\vec{k},t)$. A conventional one-detector measurement on our sample finds $D = (6.15\pm0.05)\times10^{-7}\ \mathrm{cm^2\ s^{-1}}$. The rather noisier measurements of $S_x(\vec{k},t)$ give $D = (6.0\pm0.5)\times10^{-7}\ \mathrm{cm^2\ s^{-1}}$ which is not significantly different, at least by comparison with the twofold disagreement between QELSS and classical values for D. One concludes (as might be expected from the known Rayleigh ratio of BSA) that in studies on the diffusion of concentrated isoionic serum albumin, multiple scattering does not have a substantial effect on the diffusion coefficient determined by QELSS. These results on BSA corroborate the earlier work of Hall *et al.*,[9] who concluded from the k dependence of $S(\vec{k},t)$ that multiple scattering is not important in concentrated hemoglobin solutions.

ACKNOWLEDGMENTS

The technical assistance of Miss P. L. Neer during the early stages of this work is gratefully acknowledged. This work was supported in part by the National Science Foundation under Grant No. CHE-79-20389. The support of the Donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

---

[1] C. M. Sorenson, R. C. Mockler, and W. J. O'Sullivan, Phys. Rev. A 14, 1520 (1976); 17, 2030 (1978).

[2] G. D. J. Phillies, J. Chem. Phys. 74, 260 (1981).

[3] G. D. J. Phillies, Mol. Phys. 32, 1695 (1976).

[4] B. Crosignani, P. di Porto, and M. Bertolotti, *Statistical Properties of Scattered Light* (Academic, New York, 1975).

[5] D. E. Koppel, J. Chem. Phys. 57, 4814 (1972).

[6] D. R. Bauer, in *Polymer Colloids II,* edited by R. M. Fitch (Plenum, New York, 1980), p. 51; J. Phys. Chem. 84, 1592 (1980).

[7] R. S. Hall and C. S. Johnson, Jr., J. Chem. Phys. 72, 4251 (1980).

[8] A. P. Minton and P. D. Ross, J. Phys. Chem. 82, 1934 (1978).

[9] R. S. Hall, Y. S. Oh, and C. S. Johnson, Jr., J. Phys. Chem. 84, 756 (1980).