LASER LIGHT SCATTERING FROM LIQUIDS

✚2586

B. J. Berne
Department of Chemistry, Columbia University, New York, New York 10027

R. Pecora
Department of Chemistry, Stanford University, Stanford, California 94305

INTRODUCTION

Many detailed reviews of laser scattering from fluids (1–16) have appeared since the comprehensive review by Chu (1) in this series. Among these, we would like to call attention to those by Swinney (14) on critical phenomena, by Gelbart (13) on collision-induced phenomena, by Pecora (7) on macromolecules in solution, and by Fleury & Boon (12) on the overall field of scattering from fluids.

Over the past ten years, laser light scattering has become a major tool for studying the dynamical properties of gases, liquids, and solids. The literature has grown so rapidly that in one of the reviews cited above (12) there are about 513 references covering the period from 1964 to the beginning of 1972, and these do not even include Raman scattering or the scattering from solids (5). Obviously, the field has become so vast as to be impossible to offer either a critical or didactic review of all of its interesting applications in the limited space available. In any case, much of this material is amply reviewed elsewhere (1–16). Consequently, we limit ourselves to a description of some recent advances in subjects of interest to us. Specific attention is paid to the application of light scattering to the study of macromolecules, bacterial motion, gels, collision-induced anisotropies, and rotational motion of small molecules in liquids. The important fields of critical scattering (14), polarized (Rayleigh-Brillouin) scattering from liquids and gases composed of small molecules (12), and scattering from solids (4, 5) and liquid crystals (12) are not discussed in this review.

SUMMARY OF PRINCIPLES

In a light scattering experiment a beam of light is focused onto a region of a fluid and is scattered into a detector. Polarizers and analyzers are used to define the polarizations of the incident and scattered light beams, respectively. Physically, the instantaneous scattered field can be regarded as the superposition of waves scattered from the individual scattering centers. This scattered field therefore fluctuates in response to the molecular motions of the scatterers. A variety of detection schemes are used to analyze the time dependence of these fluctuations. The detection method used in a particular experiment depends upon the time scale of these fluctuations.

One such method called the "filter method" involves the spectral decomposition of the scattered light by a diffraction grating or a Fabry-Perot interferometer (2, 12). These devices act as filters which pass a single frequency component of the scattered light for a given setting. The filter output is then incident upon the cathode of a photomultiplier. The average dc output of the photomultiplier is proportional to the spectral density of the scattered electric field at the filter frequency. The filter is then swept through a range of frequencies. The spectral density can be related by theory to time correlation functions of molecular properties (2). Because of the resolution limitations of currently available gratings and interferometers this method can only be used to study relatively rapid molecular dynamic processes, that is, those that occur on a time scale faster than 10^{-6} sec.

For molecular dynamic processes that occur on time scales slower than 10^{-6} sec, other methods must be used. The methods used are called "optical mixing" techniques (4). Optical mixing techniques are the optical analogs of beating techniques developed in radiofrequency spectroscopy. One such method is the heterodyne beat method in which the scattered field is mixed with a "local oscillator" field on the surface of a photomultiplier. If the amplitude of the local oscillator field is sufficiently large compared to that of the scattered field, the photocurrent output of the photomultiplier tube will, to a good approximation, be a linear function of the scattered field amplitude. This current is then passed into a hard wire computer called an autocorrelator, which computes the time correlation function of the photocurrent. This correlation function is proportional to the real part of the autocorrelation function of the scattered electric field. Thus, optical heterodyne techniques can be used to measure the same kind of properties measured by filter techniques albeit on a slower time scale.

Another optical mixing technique is the homodyne (or self beat) technique (4). In this method only the scattered field impinges on the photocathode. No local oscillator is used. Since the instantaneous photocurrent is proportional to the intensity of the light impinging on the photocathode, the output of the photomultiplier is proportional to the square of the amplitude of the scattered field. This signal is passed into an autocorrelator which then evaluates the time autocorrelation function of the intensity of the scattered light. This method is sometimes also called "intensity fluctuation spectroscopy."

Thus, all these methods determine various time correlation functions of the scattered electric field amplitude $E_s(t)$. In particular, the filter, heterodyne, and homodyne techniques respectively give, $\langle E_s^*(o)E_s(t)\rangle$, $\mathrm{Re}\,\langle E_s^*(o)E_s(t)\rangle$, and $\langle |E_s(o)|^2 |E_s(t)|^2\rangle$.

In general the homodyne and heterodyne methods determine different time correlation functions. In the event that there are flows in the scattering medium (for instance, by virtue of convection, sedimentation, or electrophoresis), the heterodyne method can be used to determine the velocities of the flows, whereas the homodyne method is insensitive to these flows. These effects are discussed more fully in the section on electrophoretic light scattering. However, in many applications not involving flow, these two methods give almost the same information (14). Exceptions to this are discussed below.

In order to analyze the results of light scattering experiments, it is first necessary to relate $E_s(t)$ to the molecular properties of the fluid. The solution of Maxwell's equations in the Born approximation gives the well-known result that $E_s(t)$ is proportional to the quantity $\delta\alpha_{if}(\mathbf{q},t) \equiv \hat{n}_f \cdot \boldsymbol{\delta\alpha}(\mathbf{q},t) \cdot \hat{n}_i$, where $\hat{n}_i$ and $\hat{n}_f$ are the polarization directions defined by the polarizer and analyzer, respectively; $\boldsymbol{\delta\alpha}(\mathbf{q},t)$ is the Fourier transform of the local polarizability fluctuation $\boldsymbol{\delta\alpha}(\mathbf{r},t)$; and $\mathbf{q}$ is the scattering vector. For the small frequency shifts observed in light scattering the length of $\mathbf{q}$ is related to the scattering angle θ, the incident light wavelength λ, and the index of refraction of the scattering medium n

$$q = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2} \qquad 1.$$

Substitution of these results into the time correlation functions of the electric field amplitude that are measured by the techniques described above shows that the filter and heterodyne experiment is determined by

$$I_{if}^{(1)}(\mathbf{q},t) = \langle \delta\alpha_{if}^*(\mathbf{q},o)\delta\alpha_{if}(\mathbf{q},t)\rangle \qquad 2.$$

and the homodyne experiment is determined by

$$I_{if}^{(2)}(\mathbf{q},t) = \langle |\delta\alpha_{if}(\mathbf{q},o)|^2 |\delta\alpha_{if}(\mathbf{q},t)|^2\rangle \qquad 3.$$

In a filter experiment the spectral density of $I_{if}^{(1)}(\mathbf{q},t)$ is the quantity that is directly measured

$$I_{if}^{(1)}(\mathbf{q},\omega) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} dt e^{-i\omega t} I_{if}^{(1)}(\mathbf{q},t) \qquad 4.$$

where ω is the difference between the frequencies of the incident and scattered beams. In optical mixing experiments a spectrum analyzer is sometimes used instead of an autocorrelator. In this eventuality, the spectral densities of $I_{if}^{(1)}$ and $I_{if}^{(2)}$ are the quantities directly measured.

Theoretical treatments of light scattering can be divided into two classes: phenomenological and molecular. In the phenomenological theories the polarizability fluctuation is expanded in terms of local fluctuations of hydrodynamic

variables and the correlation functions are determined using the theory of irreversible thermodynamics (see for example 2). In the molecular theories $\delta\alpha$ is expressed as a superposition of contributions from each molecule and the correlation functions are evaluated using models or, in some cases, more complicated many-body techniques (see for example 7 and 13). Unfortunately there is as yet no molecular theory of light scattering that reduces to the results of the phenomenological theories. For a detailed discussion of these attempts see the review by Gelbart (13) and the recent work of Harris (17) and Pasmanter et al (18).

DIFFUSION OF MACROMOLECULES

In dilute macromolecular solutions where the dimensions of the macromolecule are small compared to q^{-1} (so that intramolecular interference effects can be ignored), the fully polarized scattering will consist of several terms, a rapidly decaying part due to local pressure and temperature fluctuations at constant concentration and a slowly decaying part due to fluctuations of concentration at constant temperature and pressure (19) and, in the event that the molecules are nonspherical, rapidly decaying parts due to molecular tumbling (20). The slowly decaying concentration fluctuations are temporally separable from the remaining rapidly decaying fluctuations and give rise to a term (19) which decays exponentially with time constant

$$\tau_q = \frac{1}{q^2 D} \qquad 5.$$

where D is the diffusion coefficient of the macromolecules in a volume-fixed frame of reference (11). The quantity τ_q is roughly the time it takes a particle to diffuse a distance q^{-1}. This time is typically of the order 10^{-1} to 10^{-3} sec and, thus, in the experiments to be discussed below optical mixing techniques are used. It is important to note that D may be concentration dependent and only in the limit of infinite dilution can it be unambiguously related to the self-diffusion coefficient at infinite dilution, D_s. The main object of much of the light scattering work on diffusion is to determine the dimensions and molecular weight of a macromolecule. For this purpose it is necessary to determine D_s, since it is this quantity which is relatively easily related to the dimensions and molecular weight of the macromolecule. For example for a sphere of radius a, D_s is given by the well-known Stokes-Einstein result, $D_s = kT/6\pi\eta a$, where η is the solution viscosity. In the event that D_s can be determined it can be combined with sedimentation velocity and specific volume measurements to give the molecular weight (21)

$$M = \frac{RTS}{D_s(1 - \rho\bar{v})} \qquad 6.$$

where S is the sedimentation coefficient at infinite dilution, ρ is the mass density

of solvent, and $\bar{v}$ is the partial specific volume of the macromolecule. The combination of these techniques provides a rapid and precise method for determining molecular weights of large macromolecules and viruses (22–24).

The concentration dependence of the diffusion coefficient, D, in the volume-fixed frame of reference has been the subject of several experimental studies (25–27). First it should be noted that hydrodynamic and molecular calculations usually determine diffusion coefficients, $D^{(s)}$, in a reference frame in which macromolecules move relative to the solvent. The relationship between D^s and D is given by the well-known formula (27)

$$D = D^{(s)}(1 - \bar{v}c) \qquad 7.$$

where $\bar{v}$ is the specific volume and c the mass concentration of the macromolecule. $D^{(s)}$ is itself a function of concentration (24–26).

Measurements of macromolecular diffusion coefficients are now common. Many groups are engaged in using these diffusion coefficient measurements to study problems of chemical and biological interest (28–32). We mention here some of the more novel recent studies. Earlier studies are referenced in the review articles by Pecora (7) and Ford (11).

Koppel has combined the light scattering method with zonal sedimentation in a sucrose gradient to study the ribosomes of *Escherichia coli* (33). During centrifugation in a sucrose gradient, the macromolecules separate into physically distinct bands according to their sedimentation properties. The light scattering analysis is then carried out immediately after centrifugation directly on the different bands and thus on different macromolecular components. By using the sedimentation coefficient, the tabulated partial specific volume and the diffusion coefficient in Equation 5, Koppel obtained the molecular weight of the *E. coli* 70S ribosomes and the 50S and 30S ribosome subunits.

Thus, in a single experiment Koppel was able to determine the diffusion coefficients, sedimentation coefficients, molecular weights, and relative concentrations of each of the components of a complex system.

In a dilute polydisperse macromolecular solution, the total scattered light time correlation function is a superposition of those of each of the species in the mixture. Unless the decay constants for each of these time correlation functions are very different from each other, one can measure only an "average" diffusion coefficient from the light scattering experiment (34–38). Koppel (39) has developed a cumulant method for obtaining a well-defined average D as well as a measure of the spread of the polymer distribution by analyzing the logarithm of the normalized light scattering time correlation function at small times. He shows how to obtain the "z-average" diffusion coefficient and the mean square value of the fluctuation of D from the z-average value.

Other authors have used the light scattering method to study aggregation (40–43). Since the diffusion coefficient of a molecular aggregate is smaller than that of the monomer and the aggregate should in fact contribute more to the total time correlation function because of its higher molecular weight, we expect light scattering to be very sensitive to small amounts of aggregate in a system.

Wilson et al (43) have used this fact to detect the onset of aggregation of hemoglobin S molecules. The erythrocyte sickling phenomenon of sickle cell anemia is caused by this aggregation.

Most macromolecules, when dissolved in salt solutions, acquire charges which are shielded by an atmosphere of counterions. This ion atmosphere affects the diffusion coefficient of the macromolecule and hence the light scattering time correlation function. Stephen (44) has presented a simple model of this effect in the limit where the Debye shielding length is very much larger than the interparticle spacing. Lee & Schurr (45) have studied the light scattering from poly-L-lysine-HBR in the presence of added salt. They find that they cannot reproduce the previously obtained results of Jamieson, Mack & Walton (46) on the same system, and in a future work they promise to present a detailed experimental and theoretical study of charge effects on diffusion in this system. Schleich & Yeh (47) have performed similar studies on poly-L-proline.

In an interesting study Pusey et al (24) have observed an angularly dependent diffusion coefficient and an angular dependence in the integrated scattering from a dilute solution consisting of highly charged R17 viruses and their counterions. When additional salt is added the angle dependence mentioned above disappears. These results cannot be explained by the Stephen theory (44) and await a full theoretical explanation. Nevertheless, in a recent note, Schaefer & Berne (48) have suggested that this angular dependence is due to the long range repulsive interaction between the highly charged virus particles. They derive a relationship between the observed diffusion coefficient and the integrated intensity.

ROTATIONAL AND INTRAMOLECULAR RELAXATION OF MACROMOLECULES

When the macromolecules are not small compared to q^{-1} intramolecular interference is important in determining the scattered light intensities. Furthermore if the fluctuations in intramolecular segmental distances are of the order q^{-1} the time constants for these motions are important in determining the form of the polarized scattered field-time correlation functions. The theory of these effects is reviewed by Pecora (7).

Huang & Frederick (49) and King, Knox & McAdam (50) have investigated intramolecular motions in polystyrenes of molecular weights $> 10^6$. Both groups report values for the longest intramolecular relaxation times that are in qualitative agreement with the results of the Rouse-Zimm theory. Fujime has continued his theoretical and experimental work on scattering from large semiflexible macromolecules and biological structures (51–55). Most of this work has been recently reviewed by Fujime (8). Schmidt reports having observed the translational diffusion coefficient and internal bending mode in N1-DNA (56).

Recent theories (57–59) have focused on the limit of very large molecules where $qR_G \gg 1$ (R_G = radius of gyration). Most of these theories predict that the half width at half height of the scattered spectrum varies as q^{ν}, where ν varies

from 3 to 4. Huang & Frederick (60) have measured homodyne spectral half widths of the light scattered from dilute solutions of polystyrenes having molecular weight 4.4×10^7 in tetrahydrofuran. They find that the power v is highly concentration dependent and could not reliably extrapolate the data to infinite dilution to make quantitative comparisons with the various theories.

Since the original article by Cummins et al (61) several groups have studied the polarized dynamic light scattering from tobacco mosaic virus (TMV) solutions (62–65). Fujime (62) and Schaefer et al (64) have attempted to detect terms in the TMV scattering spectrum dependent on the anisotropy of the translational diffusion coefficient of this rod-like molecule. The interpretation of Fijume's results (62) was complicated by his use of polydisperse samples. Schaefer et al (64) used monodisperse samples but obtained essentially a zero value for the translational diffusion coefficient anisotropy—a result in disagreement with the usual simple hydrodynamic models.

Rotational diffusion coefficients and intramolecular relaxation times may also be studied by observing the dynamic depolarized light scattering from macromolecular solutions (66–68). These quantities may affect the depolarized time correlation function even if there is no structural change comparable to q^{-1}. The only criterion is that there be a change in optical anisotropy associated with the rotation or intramolecular motion.

Schurr & Schmitz (69) and King et al (65) have repeated the original experiment of Wada et al (67) on the low angle depolarized TMV scattering. Schmitz & Schurr criticize the experiment of Wada et al but they, along with King et al, find agreement of their rotational diffusion coefficients with those of Wada et al (67).

Maeda & Saito (70) have calculated the spectrum of light scattered from optically anisotropic rods which are not small compared to q^{-1}. They include the coupling between rotational and translational motion and provide computed spectra for a rod with the TMV parameters.

Schmitz & Schurr (71) have applied the depolarized scattering technique to examine intramolecular relaxation times of calf thymus DNA. They report finding three relaxation times in the depolarized spectrum. The longest of these ($\tau \approx 18$ msec) is close to what one would predict on the basis of flow-dichroism results. The temperature profile of the longest relaxation time was investigated and found to exhibit a spike near the helix-coil denaturation temperature. This spike was interpreted as resulting from an increase in the molecular weight of the DNA in the denaturation temperature region.

DYNAMICS OF GELS

Light scattering is a promising technique for the study of gels. Prins, Rimai & Chompff (72) have observed oscillatory correlation functions in the scattering from agarose and polyvinyl alcohol gels. Tanaka, Hocker & Benedek (73), in the most thorough work to date, have studied the dynamic polarized scattering of 5 and 2.5% polyacrylamide gels as a function of scattering angle and temperature.

They present a theory for thermal displacements of the gel fiber network and analyze their experimental results in terms of this theory. The predicted light scattering electric field time correlation function is a single exponential with relaxation time

$$\tau_q = \frac{f}{q^2 G_1} \qquad 8.$$

where f is the frictional force per unit volume on the fiber network as it moves with unit velocity relative to the gel liquid, and G_1 is the longitudinal compressional modules for longitudinal displacements of the fiber network. Tanaka et al (73) obtained τ_q from light scattering and also performed macroscopic measurements to independently measure G_1 and f. They find good agreement between theory and experiment if their light scattering data are fit to a single exponential, although cumulant analysis of the experimental data indicated nonexponential decay.

McAdam, King & Knox (74) have performed light scattering measurements on chemically crosslinked polystyrene-tetralin gels where the number of monomers between chemical crosslinks was varied. Linewidth studies on uncrosslinked polymer solutions of similar concentrations differed considerably. This implies that molecular motion in the chemically crosslinked network is different from that in a simple concentrated polymer solution. The results on the gels were interpreted using the model of Brownian motion in a harmonic well. The size of the well depends on the number of effective crosslinks and physical entanglements. Neither the physical model nor the experimental results gives exponential light scattering time correlation functions.

Lee & Schurr (75) have proposed a simple "mean force" model of a large polymer or gel which exhibits a q^2 dependence of the decay constant at large qR_G.

CHEMICAL REACTIONS

Much of the early enthusiasm for using light scattering as a probe of macromolecular reaction kinetics has waned (see, for example, the review in 7). It appears that the polarizability changes that occur in chemical processes are probably too small in most cases to have substantial effects on the light scattering time correlation functions. Bloomfield & Benbasat (76) have, however, extensively analyzed theoretical light scattering spectra for reactions of the form $A \leftrightarrows B$ and $2A \leftrightarrows A_2$. They conclude that for many macromolecular reactions of this type, the changes in diffusion coefficient should be sufficient to render the reaction rates visible to light scattering.

Jamieson, Mack & Walton (46) report having measured the rate of the helix to extended coil transition in poly-L-lysine HBR. Lee & Schurr (45) have studied the same system but have seen no evidence of the reaction component in the scattering time correlation function. Jamieson, Downs & Walton (77) report a

rate constant for a conformation change of elastin aggregates. This rate constant was, however, obtained from a three Lorentzian fit to an experimental spectrum.

Alms et al (78) have repeated the previous experiment of Yeh and Keeler on dilute $ZnSO_4$ solutions and find no evidence for reactive broadening.

Uzgiris & Golibersuch (79) investigated the light scattered from hemoglobin solutions. The heterodyne time correlation function was measured and fit to a single exponential. The decay constant of this exponential contained a diffusion part proportional to q^2 and a q-independent part. The q-independent part was found to depend on hemoglobin concentration and solvent condition in the same way as the reciprocal relaxation time of the hemoglobin tetramer-dimer reaction. The authors thus concluded that the q-independent part of the heterodyne decay constant is due to the hemoglobin tetramer-dimer reaction rate. The results are, however, not in accord with the Bloomfield & Benbasat model light scattering calculations (76).

ELECTROPHORETIC LIGHT SCATTERING (ELS)

Ware & Flygare (80) have developed a method for studying solutions of macroions that combines standard electrophoretic techniques with heterodyne beat laser light scattering. They have demonstrated that under certain conditions, electrophoretic mobilities and diffusion coefficients of macromolecules can be measured simultaneously and rapidly by this technique. This method is based on the fact that macroions are accelerated by an applied electric field $\mathbf{E}$ to a terminal velocity $\mathbf{v} = \mu\mathbf{E}$, where μ is the electrophoretic mobility. Light scattered by such a moving body should experience a Doppler shift $\omega(\mathbf{q}) = \mathbf{q} \cdot \mathbf{v} = \mu(\mathbf{q} \cdot \mathbf{E})$ which increases with E. [$\omega^{-1}(q)$ can be regarded as the time it takes a particle to drift the distance q^{-1}.] When the correlation function measured in the heterodyne method is time Fourier transformed, or when the experiment is done with a spectrum analyzer, the spectral density is a pair of Lorentzian bands at the Doppler frequencies $\pm\omega(q)$ each of half width at half-maximum q^2D. Measurement of the positions of the peaks gives $\omega(q)$ and thereby μ. Measurement of the width gives D.

Ware & Flygare (80) have measured the electrophoretic spectrum of an aqueous solution of bovine serum albumin (BSA) using an adaptation of the Tiselius electrophoresis cell and have verified the results of their theoretical predictions. According to theory (81) $\mu/D = (Ze/k_BT)f'(\kappa)$, where Ze is the magnitude of the charge on BSA in esu, κ is the inverse debye screening length (which is a function of $I^{\frac{1}{2}}$ where I is the ionic strength), and $f'(\kappa)$ is a complicated decreasing function of κ and thereby $I^{\frac{1}{2}}$ which arises because the counterions screen the macroion, which consequently feels a reduced electric field. The charge Ze on BSA is an increasing function of the pH. Ware & Flygare (80) report that measurements of μ are consistent with these considerations; that is, the mobility increases with ionic strength and increases with pH. More experiments of this type should increase our understanding of colloidal solutions.

The full force of this method becomes apparent when we consider a dilute solution containing several species of macroions. Each species of macroion β will be accelerated to a different terminal velocity $\mathbf{v}_\beta = \mu_\beta \mathbf{E}$ and will thereby Doppler shift the scattered light by different amounts $\omega_\beta(q) = \mu_\beta(\mathbf{q} \cdot \mathbf{E})$. The resulting spectrum is then a superposition of symmetric Lorentzian doublets, one doublet for each species. The doublet corresponding to species β is located at $\pm\omega_\beta(q)$ with half width $q^2 D_\beta$ and area proportional to $\alpha_\beta^2 C_\beta$, where α_β and C_β are the polarizability and concentration of species β, respectively. Thus if the differences between the shifts $\{\omega_\beta(q)\}$ are large compared to the diffusion widths $\{q^2 D_\beta\}$ it should be possible to resolve the bands and thus determine the electrophoretic mobilities, the diffusion coefficients, and the relative concentrations of the various species. Because the widths $(q^2 D_\beta)$ are quadratic functions of q and the shifts $\{\omega_\beta(q) = \mu_\beta(\mathbf{q} \cdot \mathbf{E})\}$ are linear functions of q, the resolving power goes up as q is decreased (correspondingly as the scattering angle is decreased). The resolving power also increases with increasing E.

Ware & Flygare (82) have reported the observation of a resolved spectrum in mixtures of BSA monomers and BSA dimers and fibrinogen.

In addition to the foregoing, Flygare & Ware (83) have reported preliminary data on ELS analysis of human blood plasma. Their spectra show resolution equal to or better than the best available from moving boundary electrophoresis; nevertheless, a positive assignment of the many bands has not yet been made. The ELS data must be combined with standard biochemical analyses for positive identification. These spectra are promising because only a few minutes are required for the determination, compared to the slow times of moving boundary electrophoresis. The clinical possibilities of the method should be obvious.

Uzgiris and his co-workers (84, 85), using a cell of a different design than Ware & Flygare, have performed the first ELS experiments on micron-sized particles (84), namely polystyrene latex spheres (PLS), the bacterium staphylococcus epidermidis, and human erythrocytes. The accuracy of the method was confirmed by comparing the results on erythrocytes with their well-known mobilities. The mobility of PLS was found to be proportional to $I^{-\frac{1}{2}}$ for low ionic strengths (in agreement with Ware & Flygare on BSA). However at ionic strengths of less than 0.001 N KCl, the mobility ceased to increase as $I^{-\frac{1}{2}}$ but rather leveled off. This is the first experimental observation of such an effect because ordinary electrophoresis cannot be applied at such low ionic strengths. This effect has yet to be explained. This work demonstrates the utility of ELS in determining the mobilities of very large particles in a matter of minutes. By comparison, conventional electrophoresis is a painstaking method.

Uzgiris & Costaschuk (85) have studied by ELS the mobility of PLS as a function of the concentration of the cationic polymer, polyethyleneimine. Since the PLS are negatively charged over a wide range of pH, the cationic polymer binds to the surface of the spheres, thus reducing their charge and concomitantly their mobility. At high concentrations, the charge becomes positive with an attendant change in sign of the mobility. This was the observed behavior. Uzgiris & Costaschuk present a simple method for determining the sign of the charge.

Electrophoretic light scattering has been subjected to an exhaustive critique and review by Ware (86). This review should be very valuable to anyone interested in these experiments. The design of scattering cells and electrodes is discussed. Estimates of heating due to high field strengths are given, and convective instabilities and electro-osmosis are discussed. The method is compared with conventional electrophoresis and possible future applications are mentioned.

Recently Bennett & Uzgiris (87) have devised a scheme for pulsing the applied field which differs from the scheme used by Ware & Flygare. These authors claim that their method involves relatively simple electronics and "perfect" data collection efficiency.

Uzgiris & Kaplan (88, 89) have used ELS to measure the mobility of lymphocytes and erythrocytes, and have found that by coating Pt electrodes with BSA, irreversible electrode effects are eliminated. De Blois et al (90) have applied the technique to the study of human plasma low density lipoproteins. Uzgiris (91) has given a detailed review of the methods used in his laboratory and considers the details of cell and electrode design.

Berne & Gininger (92) have proposed a method for determining chemical rate constants using ELS. Each macroionic species in a solution has a characteristic Doppler shift and a corresponding band. If the macroions undergo chemical reactions, the bands can be broadened and shifted. Berne & Gininger have computed the expected spectrum for a dimerization reaction $2A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A_2$ and for the simple reaction involving a conformation change $A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} B$. They show how the line shape—the shifts and widths—can be used to determine the reaction rates, and they present a simulated spectrum for a typical globular protein. This method is very similar in spirit to NMR determinations of exchange rates. It does not suffer from the same weaknesses discussed in connection with attempts to measure rate constants by ordinary light scattering. There is considerable hope that the experiment will succeed and several groups are now attempting to measure these rate coefficients.

ELS is now an active field. It offers the possibility of studying ionic solutions rapidly, economically, and at ionic strengths too low for previous study. ELS has, as discussed above, potential applications to chemical kinetics, biochemistry, and clinical medicine.

INTENSITY FLUCTUATION SPECTROSCOPY

In a light scattering experiment the intersection between the focused incident beam and the beam visible to the detector defines the scattering volume, V. In most cases the average number of molecules, $\langle N \rangle$, in this volume is sufficiently large that the scattered field (which results only from these molecules) is the superposition of a large number of terms, $N(\sim \langle N \rangle)$. Then it is usually assumed that the central limit theorem applies, so that E_s can be regarded as a Gaussian random process, in which case homodyne and heterodyne techniques are both determined by the same correlation function $\langle E_s^*(o)E_s(t)\rangle$. When $\langle N \rangle < 100$,

however, there are too few terms in the superposition, E_s, and the central limit theorem should not apply. This is sometimes the case for very dilute solutions of large scatterers (e.g. particles with characteristic dimension $\geq 1\ \mu$). In such dilute solutions these particles move independently of each other. Schaefer & Berne (93) have shown that in this case the correlation function of the fluctuations in the scattered intensity arriving at the detector is

$$I^{(2)}(q,t) = \langle N\rangle^2 [\underbrace{l + |F_s(q,t)|^2}_{\text{interference}}] + \underbrace{\langle \delta N(o)\delta N(t)\rangle}_{\text{number}} \qquad 9.$$

where $\delta N(t) \equiv N(t) - N(o)$ is the fluctuation in the number of particles in the scattering volume. Thus the intensity of the scattered light from such a solution fluctuates on two widely different time scales. The slow fluctuations arise because of changes in the total number of scatterers in the scattering volume (number fluctuations), and the fast fluctuations result from the superposition (at the detector) of the randomly fluctuating phases of the light scattered from each particle in the scattering volume (interference fluctuations). The time scale of the number fluctuations, τ_N, is determined by the time that it takes the scatterer to cross V, whereas the time scale of the interference fluctuations, τ_q, is determined by the time it takes the scatterer to traverse the much shorter distance q^{-1}. Number fluctuations can be studied in the absence of the interference fluctuations by making the sampling interval large compared to τ_q but short compared to τ_N. Alternatively, the interference fluctuations could be averaged spatially by using a detector that is large compared to the coherence solid angle of the scattered field.

In this connection it is important to note that the study of number fluctuations does not require a coherent source of radiation. Very simple devices can be used for its study. It should also be noted that the interference scattering is proportional to $\langle N\rangle^2$ whereas the number fluctuations are proportional to $\langle \delta N^2\rangle = \langle N\rangle$ so that the relative contributions of the number fluctuations, $1/[\langle N\rangle + 1]$, is significant only for $\langle N\rangle$ small.

It is important to recognize that the number fluctuations give a slowly varying background term. Thus, if this background is ignored when it makes important contributions to $I^{(2)}$, inaccurate values for diffusion coefficients may result.

Koppel & Schaefer (94) have shown that when the number of photocounts in an interval is prescaled, a clipped autocorrelator can be used to determine the intensity autocorrelation function in the case where the Gaussian approximation breaks down. This is important because without prescaling a clipped autocorrelator gives very complicated information. In addition, Schaefer & Pusey (95, 96) have shown how the distribution of photocounts reflects the non-Gaussian nature of the scattered light. Schaefer (97) has recently reviewed some of this material.

Number fluctuations can in principle be used to study velocity fields in convective and turbulent flow, electrophoresis, sedimentation, and any other phenomenon in which there is an average flux of particles. The interference term in

Equation 9 provides no such information about flows, so that except in very dilute solutions homodyne scattering cannot be used for such studies. In the following section we show how number fluctuations have been used to study swimming speeds and mean free paths of motile microorganisms.

Another exception to the Gaussian approximation involves systems in which there are long range correlations in the structure such as occur near critical points. The range of the correlations may be characterized by a "correlation length" ξ. If we divide the scattering volume into $N(\xi)$ subregions of volume ξ^3, the scattered field amplitude can be loosely regarded as the superposition of scattered amplitudes from these $N(\xi)$ "independent" regions. Then if $N(\xi)$ is small enogh a straightforward application of Equation 9 shows that a term like $\langle \delta N_\xi(o)\delta N_\xi(t)\rangle$ should appear in the homodyne correlation function. This term is of order $\langle N\rangle$. For the conditions under which critical scattering has been observed, this is a negligible contribution. In a recent article Tartaglia & Chen (98) have considered this problem and have also shown by an application of the hydrodynamic fluctuation theory of Fox & Uhlenbeck (99) that the Gaussian approximation applies to hydrodynamic fluctuations away from the critical point.

There are techniques other than light scattering which also probe number fluctuations. Magde et al (100) have shown how fluctuations in the fluorescence intensity of certain species are directly related to number fluctuations and thus to dynamic processes.

In this technique a continuous wave (cw) beam of laser light is focused on a system containing molecules that absorb this radiation. When the molecules are in a certain "state" they fluoresce. The fluorescent light is viewed at right angles through a lens which accepts only light from a very small region in the incident beam. The experimental arrangement therefore defines a scattering volume. The light falls on a photocathode which puts out a current proportional to the instantaneous fluorescence intensity. This current is then autocorrelated. This gives the autocorrelation function of the fluctuations in fluorescence intensity, δI_f. Since $\delta I_f(t)$ is proportional to $\delta N_f(t)$, the number fluctuation of fluorescent species in V, what is determined is the number fluctuation $\langle \delta N_f(o)\delta N_f(t)\rangle$. This can be used to determine diffusion coefficients, flow velocities, motilities, etc of labeled species. It can also be used to study chemical reaction rates.

Magde et al (100) have studied the intercalation of ethydium bromide, Et, into DNA by this method

$$\text{DNA} + \text{Et} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} (\text{DNA} - \text{Et})$$

In this case unbound Et absorbs the incident light but only intercalated DNA fluoresces. Thus the fluctuations in the fluorescence reflect two processes, the diffusion processes that takes DNA – Et into and out of V and the chemical rate processes for the binding and unbinding of the complex. Magde et al (100) have analyzed the results using the same theory applied in light scattering to chemically reacting systems (7) and have determined the rate constants for these

reactions. This method is described in great detail by Elson et al (101, 102). Because of the specificity of the labeling process, fluorescence correlation spectroscopy should be very useful in future studies of transport through membranes, motility, electrophoresis, chemical kinetics, and turbulence.

Nonoptical techniques can also be used to study number fluctuations. Feher (103) and Feher & Weissman (104) have determined the relaxation time of the ionization reaction of berylium sulfate by observing electrical conductivity fluctuations.

Fluctuation spectroscopy will undoubtedly experience much development in the next several years.

STUDIES OF MOTILE MICROORGANISMS

The spectrum of light scattered from motile microorganisms is quite different from the spectrum of particles executing Brownian motion. Bergé et al (105) were the first to perform such experiments. They studied the spectrum of motile spermatozoa and observed that as the motility decreased, the spectrum narrowed. Combescot (106) calculated the spectrum on the basis of a model of spermatozoa motility. Although spermatozoa have dimensions which are not negligible compared to the wavelength, this calculation ignores intramolecular scattering and computes the spectrum associated with an isotropic ensemble of point particles, each of which traverses a helix of given pitch and diameter with constant speed. Combescot finds that the spectrum probes the translational and rotational motion of the particles, and that for the experimental conditions only the translations are easy to observe.

Nossal (107) has also calculated the spectrum to be expected from living microorganisms. He too ignores intramolecular scattering, but uses a model of motility not applicable to spermatozoa but applicable to other kinds of microorganisms. For example, motile strains of *E. coli* K_{12} bacteria, when viewed under a microscope, appear to move at constant speed in straight lines. These motions persist for distances much greater than q^{-1} ($\sim$1000 Å) before the microorganism changes direction; that is, the mean free path, Λ, of the bacteria is such that $q\Lambda \gg 1$, and from the point of view of light scattering, a microorganism can be treated as a freely swimming particle of velocity $\mathbf{V}$. Nossal (107) has computed the spectrum of light scattered from an aqueous dispersion of microorganisms, implicitly assuming that these particles are spherical. He finds that the heterodyne correlation function is proportional to

$$I^{(1)}(q,t) = \int_0^\infty dV w(V) \frac{\sin(qVt)}{(qVt)} \qquad 10.$$

where $w(V)$ is the distribution of swimming speeds in the sample.

In a sequence of experiments, Nossal et al (108–110) have measured the homodyne scattering from bacterial dispersions of motile *E. coli* K_{12} and from this determined $I^{(1)}(q,t)$. Their experiments seem to confirm the theoretical

expectation based on Equation 10 that $I^{(1)}(q,t)$ is a function of qt. This was tested by showing that when the measured $I^{(1)}(q,t)$ are plotted versus qt for different scattering angles (or q) the results fall on the same curve (see Figure 1 of 108). Nossal et al then Fourier inverted their data and used the model embodied in Equation 10 to determine a distribution of swimming speeds. Their interpretation therefore depends on the adequacy of their theoretical model.

Nossal & Chen (109) have observed that when $CuCl_2$ is added to the dispersion, the microorganisms stop swimming and in effect become Brownian particles whose motion is determined by a diffusion coefficient which they measure. They have also devised a method for determining what fraction of microorganisms in the dispersion are motile. In a related study, Nossal & Chen (110) have studied the chemotactic response to oxygen *E. coli* by performing an optical heterodyne experiment on the dispersion when an oxygen gradient was introduced into their system. They detected a response of the bacteria to the oxygen gradient.

An important parameter in samples of motile microorganisms is the "mean free path" Λ. Because $\Lambda \gg q^{-1}$ the optical mixing techniques used by Nossal & Chen (108, 110) do not give values of Λ. Nevertheless it is possible to determine values of Λ in the following way: Schaefer & Berne (93) have shown that for particles as large as *E. coli* and for typical scattering volumes, V, involved in these experiments, it is possible to work at concentrations for which the average number of bacteria $\langle N \rangle$ in V is small. Then the homodyne correlation function contains the additional term $\langle \delta N(o) \delta N(t) \rangle$ (see Equation 9). The number of bacteria in V fluctuates on a time scale determined by the time it takes a microorganism to swim through V. If the dimensions of V, say L, are small compared to Λ, $\langle \delta N(o) \delta N(t) \rangle$ should decay on a time scale determined only by the swimming speed; whereas if $L \approx \Lambda$, the bacterium has a chance to turn around before leaving V so that its residence time should be longer than if it swims straight through. Then a measurement of $\langle \delta N(o) \delta N(t) \rangle$ gives Λ. We call these cases a and b respectively. Schaeffer (111) has performed an experiment for case b on *E. coli* and has determined Λ. Banks et al (112) have performed an experiment on a strain of *E. coli* with very large Λ where case a applies and have determined that the root mean square swimming speed of *E. coli* increases with temperature for temperatures from 25° to 35°C.

It is important to note that in the absence of convection, when case a applies, $\langle \delta N(o) \delta N(t) \rangle$ unambiguously gives information about translational swimming. Unfortunately, this is not true of the methods used by Chen & Nossal as we now discuss.

E. coli (and for that matter spermatozoa) are not spheres as implicitly assumed in Nossal's model but are rods about 1 μm long and 0.3 μm wide. For the qs involved in the experiments of Nossal & Chen (108–110), it is not possible to ignore intramolecular interference in such rods. Assuming that a given microorganism always swims along its principal axis and does not rotate, Berne & Nossal (113) have shown that an isotropic (randomly oriented) sample has a correlation function that behaves quite differently from Equation 10 in that it

does not scale with qt. The question immediately arises: Why does the experimental result scale with qt? Several answers are possible. The one we find most compelling is that the sample contains both motile and nonmotile microorganisms. The correlation function is then a sum of two functions, one due to the diffusion of the nonmotile microorganisms that scales as q^2t and the other due to the motile microorganism that does not scale as q^2t or as qt. The superposition however can scale as qt under certain conditions. There are indeed many experimental conditions under which scaling is not observed.

When *E. coli* are observed in a microscope they seem to wobble perhaps by virtue of their flagellar motion; this wobble is very likely a projection on the microscope slide of helical motion about the axis of motion. Helical motion of such large objects should lead to a modulation of the intramolecular scattering [see for example, Pecora (114, 115)]. In some recent experiments, Schaefer et al (116) find evidence based on the viscosity dependence of the measurements that this helical motion effects the spectrum. (It should be pointed out that when helical motion of the rod is present, there are no values of the rotational and translational speed for which scaling with qt is possible.) These authors evaluate a parameter they call the root mean square rotational speed, and compare it with the translational root mean square velocity determined from number fluctuations. These numbers turn out to be quite different. Banks et al (112) show that their ratio is a constant as a function of temperature, leading to the reasonable idea that these quantities should scale. Thus the measurement of one quantity might provide a means of determining the other.

The use of light scattering to study motility is reviewed by Nossal et al (109) and by Schaefer (117). This is a very promising application of light scattering which should in the near future contribute to our understanding of chemotaxis and phototaxis.

COLLISION-INDUCED LIGHT SCATTERING

Levine & Birnbaum (118) first pointed out that collisions distort the electronic structure of the interacting particles and give rise to short lived anisotropies in the polarizability so that even monatomic fluids should exhibit depolarized light scattering. This paper was followed by the first experimental observation of the spectral distribution of this collision-induced depolarized scattering (119, 120) in the dilute rare gases argon and krypton. The depolarized spectra obtained in these experiments have inverse widths which are of the order of the duration of a collision in these gases. These initial experiments were interpreted on the basis of simple models (121, 122). Since then there has been a great deal of activity on three broad fronts: (*a*) the line shapes have been studied as a function of temperature and density, from the dilute gas into the liquid state for argon and neon (123) and more recently for molecular liquids (124); (*b*) microscopic theories of depolarized scattering have been developed (17, 18, 125) and collision-induced polarizabilities have been calculated (126) for helium and argon by ab initio

methods; and (*c*) computer simulations have been carried out to delineate the underlying atomic collective motions that contribute to the observed spectra (127), and to see whether the simple dipole-induced-dipole approximation to the pair polarizability suffices for a description of the density dependence of the integrated depolarized intensity (128) and for the precise spectral frequency dependence (129).

In the foregoing we have only sampled characteristic references. Our omissions do not reflect any criticism of the remaining papers which the interested reader can find discussed in the very comprehensive and detailed review of this subject by Gelbart (13).

DEPOLARIZED LIGHT SCATTERING FROM LIQUIDS

The spectrum of light scattered from liquids containing optically anisotropic molecules contains information about the rotational motion of the molecules (130). Only some of the more recent work is reviewed here. Fleury & Boon (12) give an extensive list of references to earlier work.

The depolarized component of several liquids (e.g. nitrobenzene, quinoline, ethylene dibromide, aniline) has been shown to contain a doublet, symmetric about zero frequency with a splitting of about 0.5 GHz (131–134). The existence of this doublet had been predicted by Leontovich (135) using a hydrodynamic model. Several investigators have reformulated theories of this doublet (and the rest of the "reorientational Rayleigh wing") using phenomenological (136–139) and statistical mechanical theories (140–146). The statistical mechanical theories make use of the Mori formalism (147) for treating irreversible processes. These theories are not all equivalent and there has been much discussion over which is most appropriate to describe the experimental spectra (145, 146, 148).

Alms et al (148) conclude that the Mori-type theory most compatible with physical intuition and the experimental spectra is a "two variable" theory of the scattering in which one of the variables is a symmetric second rank tensor associated with the reorientation of the molecule and the other is the transverse velocity. Thus, the doublet may be viewed as the result of coupling a transverse velocity fluctuation to the molecular reorientation. Alms et al (148) also have experimentally studied the depolarized doublet in anisaldehyde as a function of temperature and show that the two-variable theory fits the data quite well in a range of theoretical parameters not previously investigated for any liquids. Keyes & Kivelson (142, 144) obtain the same formal expression for the scattering spectrum as Alms et al (148), but when expressing a major parameter of the theory in microscopic terms obtain incorrect results which, as Alms et al have pointed out, predict no depolarized doublet.

The liquids in which the depolarized doublet has been observed are composed of relatively large molecules. Enright & Stoicheff (149) thus set out to observe such spectra in simple molecular liquids. They have observed the doublet in liquid CS_2 and have compared their spectra to the theoretical spectra predicted

by Rytov (136) and Keyes & Kivelson (142, 144). Enright & Stoicheff find agreement with both theories, although it should be pointed out that they merely used the form of the Keyes & Kivelson result and not their detailed molecular theory. This form is the same as that used by Alms et al (148) so that Enright & Stoicheff's conclusions are in agreement with those of Alms et al.

Measurements of the depolarized component have been used by Pecora and co-workers (78, 150–152) to study molecular reorientation in liquids. These authors studied both neat liquids and solutions where the solvent contributed negligibly to the depolarized spectrum. They first showed (150) that for a series of benzene derivatives in solution the reorientation times obtained from light scattering depended only on the solution viscosity and not on the molecular composition of the solvent as long as the solute concentration was not very high and there were no strong solute-solvent interactions (e.g. hydrogen bonding). They then investigated solutions of carboxylic acids (78) in water and in CCl_4 and showed that the strong hydrogen bonding in the water solutions strongly affected the solute reorientation time. They also studied (78) the reorientation of the carboxylic acid dimers in the CCl_4 solutions by depolarized light scattering.

By combining depolarized light scattering with carbon 13 spin lattice relaxation measurements these authors were able to study both the different components of the rotational diffusion tensor for a series of benzene derivatives (152) and also to measure both the collective and single particle reorientation times for $CHCl_3$ and nitrobenzene (151).

The viscosity dependences of the two single particle reorientation times of the symmetric top molecules, benzene and mesitylene, and the three single particle reorientation times for the asymmetric tops, toluene and nitrobenzene, were found (152) to be linear in solvents which exhibited no strong solute-solvent interactions. For $CHCl_3$ the single molecule reorientation time at constant viscosity obtained from the C^{13} NMR measurements at 40 and 100% $CHCl_3$ concentrations was shown to agree with the constant viscosity light scattering relaxation time extrapolated to zero $CHCl_3$ concentration (151). Light scattering thus measures the collective reorientation time at high concentrations of solute and the single molecule reorientation time at low concentrations. For $CHCl_3$ the collective reorientation time is larger than the single molecule time. Similar results (151) were obtained for the more complicated case of nitrobenzene solutions.

Pecora and co-workers (78, 150–152) used a Fabry-Perot interferometer as the filter in their experiments. They could not detect any deviation of the scattered field time correlation from exponential shape. It is clear, however, that at short enough times these correlation functions cannot be exponential (153). Van Konynenburg & Steele (153) and Dardy et al (154), using diffraction gratings as filters, have tried to observe the short time behavior of the time correlation function by observing the scattered spectrum with inclusion of the far wings. The complication arises in that collision-induced scattering (see the previous section) also contributes to the far wings. Both groups (153, 154) thus assumed functional forms for the collision-induced contributions to the spectra and devised

techniques for subtracting them from the experimental spectra. They obtained rotational correlation functions for short times.

These studies illustrate the important information about molecular reorientation in liquids that may be obtained from depolarized light scattering.

Literature Cited

1. Chu, B. 1970. *Ann. Revs. Phys. Chem.* 21:145
2. Benedek, G. B. 1968. In *Brandeis Lectures in Theoretical Physics, 1966*, ed. M. Chretien et al. New York: Gordon and Breach
3. Cummins, H. Z., Swinney, H. L. 1970. *Progr. Opt.* 8:133
4. Cummins, H. Z. 1971. In *Light Scattering in Solids*, ed. M. Balkanski. Paris: Flammarion
5. Fleury, P. A. 1970. *Phys. Acoust.* 6:1
6. Mountain, R. D. 1970. *CRC Crit. Rev. Solid State Sci.* 1:5
7. Pecora, R. 1972. *Ann. Rev. Biophys. Bioeng.* 1:257
8. Fujime, S. 1972. *Advan. Biophys.* 3:1
9. Jamieson, A. M., Maret, A. R. 1973. *Chem. Soc. Rev.* 2:325
10. Ford, N. C., Gabler, R., Karasz, F. E. 1973. *Advan. Chem.* 125:25
11. Ford, N. C. 1972. *Chem. Scr.* 2:193
12. Fleury, P. A., Boon, J. P. 1973. *Advan. Chem. Phys.* 24:1
13. Gelbart, W. 1974. *Advan. Chem. Phys.* In press
14. Swinney, H. L. 1974. In *Photon Correlation and Light Beating Spectroscopy*, ed. H. Z. Cummins, E. R. Pike. New York: Plenum.
15. Pusey, P. N. 1974. Ibid
16. Ware, B. R. 1974. *Advan. Colloid Interface Sci.* In press
17. Harris, R. A. 1974. *Chem. Phys. Lett.* 19:49
18. Pasmanter, R. A., Samson, R., Ben-Reuven, A. 1972. *Chem. Phys. Lett.* 16:470
19. Mountain, R. D., Deutch, J. M. 1969. *J. Chem. Phys.* 50:1103
20. Pecora, R. 1968. *J. Chem. Phys.* 49:1036
21. Khatchalsky, A., Curran, P. F. 1967. *Nonequilibrium Thermodynamics in Biophysics.* Cambridge, Mass.: Harvard Univ. Press
22. Foord, R. et al 1970. *Nature* 227:242
23. Dubin, S. B. et al 1970. *J. Mol. Biol.* 54:547
24. Pusey, P. N. et al 1972. *J. Phys. Paris* 33(C1):163
25. Ford, N. C., Owen, J. E. M., Karasz, F. E. 1970. *Discuss. Faraday Soc.* 49:228
26. King, T. A., Knox, A., Lee, W. I., McAdam, J. D. G. 1973. *Polymer* 14:151
27. King, T. A., Knox, A., McAdam, J. D. G. 1973. *Polymer* 14:293
28. Reed, T. F., Frederick, J. E. 1971. *Macromolecules* 4:72
29. Kramer, O., Frederick, J. E. 1972. *Macromolecules* 5:69
30. Lin, S. H. C., Dewan, R. K., Bloomfield, V. A., Morr, C. V. 1971. *Biochemistry* 10:4788
31. French, M. J., Angus, J. C., Walton, A. G. 1971. *Biochim. Biophys. Acta* 251:320
32. Sellen, D. B. 1973. *Polymer* 14:359
33. Koppel, D. E. 1973. PhD thesis. Columbia Univ., New York
34. Pecora, R., Tagami, Y. 1969. *J. Chem. Phys.* 51:3298
35. Frederick, J. E., Reed, T. F., Kramer, O. 1970. *Macromolecules* 4:242
36. Kramer, O., Frederick, J. E. 1971. *Macromolecules* 4:613
37. Reed, T. F. 1972. *Macromolecules* 5:771
38. Thompson, D. S. 1971. *J. Chem. Phys.* 54:1411
39. Koppel, D. E. 1972. *J. Chem. Phys.* 57:4814
40. Rimai, L., Hickmott, J. T., Cole, T., Carew, E. B. 1970. *Biophys. J.* 10:20
41. Herbert, T. J., Carlson, F. D. 1971. *Biopolymers* 10:2231
42. Wilson, W. W., Johnson, C. S. 1972. *J. Phys. Chem.* 76:2744
43. Wilson, W. W., Luzzana, M. R., Penniston, J. T., Johnson, C. S. 1974. *Proc. Nat. Acad. Sci. USA* 71:1260
44. Stephen, M. J. 1971. *J. Chem. Phys.* 55:3878
45. Lee, W. I., Schurr, J. M. 1974. *Biopolymers.* In press
46. Jamieson, A. M., Mack, L., Walton, A. G. 1972. *Biopolymers* 11:2207
47. Schleich, T., Yeh, Y. 1973. *Biopolymers* 12:993
48. Schaefer, D. W., Berne, B. J. 1974. *Phys. Rev. Lett.* 32:1110
49. Huang, W.-N., Frederick, J. E. *Macro-*

molecules. In press
50. King, T. A., Knox, A., McAdam, J. D. G. 1973. *Chem. Phys. Lett.* 19:351
51. Ishiwata, S., Fujime, S. 1972. *J. Mol. Biol.* 68:511
52. Fujime, S., Maruyama, M., Asakura, S. 1972. *J. Mol. Biol.* 68:347
53. Fujime, S., Ishiwata, S., Maeda, T. 1972. *Biochim. Biophys. Acta* 283:351
54. Fujime, S., Hatano, S. 1972. *J. Mechanochem. Cell Motility* 1:81
55. Fujime, S., Maruyama, M. 1973. *Macromolecules* 6:237
56. Schmidt, R. L. 1973. *Biopolymers* 12:1427
57. de Gennes, P. G. 1967. *Physics* 3:37
58. Dubois-Violette, E., de Gennes, P. G. 1967. *Physics* 3:181
59. Silbey, R., Deutch, J. M. 1972. *J. Chem. Phys.* 57:5010
60. Huang, W.-N., Frederick, J. E. 1973. *J. Chem. Phys.* 58:4022
61. Cummins, H. Z., Carlson, F. D., Herbert, T. J., Woods, G. 1969. *Biophys. J.* 9:518
62. Fujime, S. 1970. *J. Phys. Soc. Jap.* 29:416
63. Wada, A., Ford, N. C., Karasz, F. E. 1971. *J. Chem. Phys.* 55:4
64. Schaefer, D. W., Benedek, G. B., Schofield, P., Bradford, E. 1967. *J. Chem. Phys.* 55:3884
65. King, T. A., Knox, A., McAdam, J-D. G. 1973. *Biopolymers* 12:1917
66. Pecora, R. 1968. *J. Chem. Phys.* 49:1036
67. Wada, A., Suda, N., Tsuda, T., Soda, K. 1969. *J. Chem. Phys.* 50:31
68. Dubin, S. B., Clark, N. A., Benedek, G. B. 1971. *J. Chem. Phys.* 54:5158
69. Schurr, J. M., Schmitz, K. S. 1973. *Biopolymers* 12:1021
70. Maeda, H., Saito, N. 1973. *Polymer J.* 4:309
71. Schmitz, K. S., Schurr, J. M. 1973. *Biopolymers* 12:1543
72. Prins, W., Rimai, L., Chompff, A. J. 1972. *Macromolecules* 5:104
73. Tanaka, T., Hocker, L. O., Benedek, G. B. 1973. *J. Chem. Phys.* 59:5151
74. McAdam, J. D. G., King, T. A., Knox, A. 1974. *Chem. Phys. Lett.* 26:64
75. Lee, W. I., Schurr, J. M. *Chem. Phys. Lett.* In press
76. Bloomfield, V. A., Benbasat, J. A. 1971. *Macromolecules* 4:609
77. Jamieson, A. M., Downs, C. E., Walton, A. G. 1972. *Biochim. Biophys. Acta* 271:34
78. Alms, G. R., Bauer, D. R., Brauman, J. I., Pecora, R. 1973. *J. Chem. Phys.* 59:5321
79. Uzgiris, E. E., Golibersuch, D. C. 1973. *Phys. Rev. Lett.* 32:37
80. Ware, B. R., Flygare, W. 1971. *Chem. Phys. Lett.* 12:81
81. See, for example, Tanford, C. 1961. *Physical Chemistry of Macromolecules*, 412. New York, Wiley
82. Ware, B. R., Flygare, W. 1972. *J. Colloid Interface Sci.* 39:670
83. Flygare, W. H., Ware, B. R. 1973. In *Molecular-Electro-Optics*, ed. C. T. O'Konski. New York: Dekker
84. Uzgiris, E. E. 1972. *Opt. Commun.* 6:55
85. Uzgiris, E. E., Costaschuk, F. M. 1973. *Nature Phys. Sci.* 242:77
86. Ware, B. R. 1974. *Advan. Colloid Interface Sci.* 4:1
87. Bennett, A. J., Uzgiris, E. E. 1973. *Phys. Rev. A* 8:2662
88. Uzgiris, E. E., Kaplan, J. H. *Biochem. Biophys. Res. Commun.* Submitted
89. Uzgiris, E. E., Kaplan, J. H. 1974. *Rev. Sci. Instrum.* 45:120
90. De Blois, R. W., Uzgiris, E. E., Devi, S. K., Gotto, A. M. 1973. *Biochemistry* 12:2645
91. Uzgiris, E. E. 1974. *Rev. Sci. Instrum.* 45:74
92. Berne, B. J., Gininger, R. 1973. *Biopolymers* 12:1161
93. Schaefer, D. W., Berne, B. J. 1972. *Phys. Rev. Lett.* 28:475
94. Koppel, D. E., Schaefer, D. W. 1973. *Appl. Phys. Lett.* 22:36
95. Schaefer, D. W., Pusey, P. N. 1972. *Phys. Rev. Lett.* 29:843
96. Schaefer, D. W., Pusey, P. N. 1972. In *Coherence and Quantum Optics*, ed. L. Mandel, E. Wolf. New York: Plenum
97. Schaefer, D. W. 1974. In *Laser Applications to Optics and Spectroscopy*, ed. S. F. Jacobs, et al. Reading, Mass.: Addison-Wesley
98. Tartaglia, P., Chen, S. H. 1973. *J. Chem. Phys.* 58:4389
99. Fox, R. F., Uhlenbeck, G. E. 1970. *Phys. Fluids* 13:1893
100. Magde, D., Elson, E., Webb, W. W. 1972. *Phys. Rev. Lett.* 29:705
101. Elson, E. L., Magde, D. 1974. *Biopolymers* 13:1
102. Magde, D., Elson, E. L., Webb, W. W. 1974. *Biopolymers* 13:29
103. Feher, G. 1970. *Biophys. J. Abstr.* 10:118
104. Feher, G., Weissman, M. 1973. *Proc. Nat. Acad. Sci. USA* 70:870
105. Bergé, P., Volochine, B., Billard, R., Hamelin, A. 1967. *C. R. Acad. Sci. D* 265:889

106. Combescot, R. 1970. *J. Phys. Paris* 31:767
107. Nossal, R. 1971. *Biophys. J.* 11:341
108. Nossal, R., Chen, S. H., Lai, C.-C. 1971. *Opt. Commun.* 4:35
109. Nossal, R., Chen, S. H. 1972. *J. Phys. Paris* 33(C1):12
110. Nossal, R., Chen, S. H. 1972. *Opt. Commun.* 5:117
111. Schaefer, D. W. 1973. *Science* 180:1293
112. Banks, G., Alpert, S. S., Schaefer, D. W. *Nature.* Submitted
113. Berne, B. J., Nossal, R. *Biophys. J.* Submitted
114. Pecora, R. 1968. *J. Chem. Phys.* 48:4126
115. Pecora, R. 1968. *J. Chem. Phys.* 49:1032
116. Schaefer, D. W., Banks, G., Alpert, S. 1974. *Nature.* 248:162
117. Schaefer, D. W. 1974. See Ref. 97
118. Levine, H. B., Birnbaum, G. 1968. *Phys. Rev. Lett.* 20:439
119. McTague, J. P., Birnbaum, G. 1968. *Phys. Rev. Lett.* 21:661
120. McTague, J. P., Birnbaum, G. 1971. *Phys. Rev. A* 3:1376
121. Volterra, V., Bucaro, J. A., Litovitz, T. A. 1971. *Phys. Rev. Lett.* 26:55
122. Lallemand, P. 1972. *J. Phys. Paris* 33(C1):257
123. Fleury, P. A., Daniels, W. B., Worlock, J. M. 1971. *Phys. Rev. Lett.* 27:1493
124. Bucaro, J. A., Litovitz, T. A. 1971. *J. Chem. Phys.* 54:3846
125. Gelbart, W. M. 1973. *Mol. Phys.* 26:873
126. O'Brien, E. F., Gutschick, V. P., McKoy, V., McTague, J. P. 1973. *Phys. Rev. A* 8:690
127. Berne, B. J., Bishop, M., Rahman, A. 1973. *J. Chem. Phys.* 58:2696
128. Alder, B. J., Strauss, H. L., Weis, J. J. 1973. *J. Chem. Phys.* 59:1002
129. Alder, B. J., Strauss, H. L., Weis, J. J. 1973. *Phys. Rev. A* 7:281
130. Pecora, R., Steele, W. A. 1965. *J. Chem. Phys.* 42:1872
131. Starunov, V. S., Tiganov, E. V., Fabelinskii, I. L. 1967. *Zh. Eksp. Teor. Fiz. Pis'ma Red.* 5:317 (1967. *JETP Lett.* 5:260)
132. Stegeman, G. I. A., Stoicheff, B. P. 1968. *Phys. Rev. Lett.* 21:202
133. Enright, G. D., Stegeman, G. I. A., Stoicheff, B. P. 1972. *J. Phys. Paris* 33(C1):207
134. Stegeman, G. I. A., Stoicheff, B. P. 1973. *Phys. Rev. A* 7:1160
135. Leontovich, M. A. 1941. *Izv. Akad. Nauk SSSR Ser. Fiz.* 5:148 (1941. *Bull. Acad. Sci. USSR Phys. Ser.* 4:499)
136. Rytov, S. M. 1957. *Zh. Eksp. Teor. Fiz.* 33:514 (1958. *Sov. Phys. JETP* 6:401)
137. Rytov, S. M. 1970. *Zh. Eksp. Teor. Fiz.* 59:2130
138. Volterra, V. 1969. *Phys. Rev.* 180:156
139. Chung, C. H., Yip, S. 1971. *Phys. Rev. A* 4:928
140. Ben-Reuven, A., Gershon, N. D. 1971. *J. Chem. Phys.* 54:1049
141. Ailawadi, N. K., Berne, B. J., Forster, D. 1971. *Phys. Rev.* 3:1472
142. Keyes, T., Kivelson, D. 1971. *J. Chem. Phys.* 54:1786
143. Andersen, H. C., Pecora, R. 1971. *J. Chem. Phys.* 54:2584
144. Keyes, T., Kivelson, D. 1972. *J. Chem. Phys.* 56:1876
145. Keyes, T., Kivelson, D. 1972. *J. Phys. Paris* 33(C1):231
146. Gershon, N. D., Oppenheim, I. 1973. *Physica* 64:247
147. Mori, H. 1965. *Progr. Theor. Phys.* 33:423; 34:399, and references cited therein
148. Alms, G. R., Bauer, D. R., Brauman, J. I., Pecora, R. 1973. *J. Chem. Phys.* 59:5304
149. Enright, G., Stoicheff, B. P. 1974. *J. Chem. Phys.* 60:2536
150. Alms, G. R., Bauer, D. R., Brauman, J. I., Pecora, R. 1973. *J. Chem. Phys.* 58:5570
151. Alms, G. R., Bauer, D. R., Brauman, J. I., Pecora, R. 1973. *J. Chem. Phys.* 59:5310
152. Bauer, D. R., Alms, G. R., Brauman, J. I., Pecora, R. 1974. *J. Chem. Phys.* In press
153. Van Konynenburg, P., Steele, W. A. 1972. *J. Chem. Phys.* 56:4776
154. Dardy, H. D., Volterra, V., Litovitz, T. A. 1973. *J. Chem. Phys.* 59:4491

CONTENTS