ecule XY as compared to those of the free molecule, we know that the products are "real" molecules of definable stoichiometries, with conventional chemical bonding and structures, and not, for example, just weakly interacting van der Waals species. As mentioned in the introduction, the only differences between the "matrix" synthetic method and "normal" syntheses are the choice of a lower temperature and of a rigid inert "solvent."

The advantages of the technique include control of the stoichiometry of the products, the cryogenic stabilization of normally unstable chemical species, the study of chemical reactions under diffusion-controlled conditions, and the synthesis of entirely new compounds. The applications of these studies can be found in any system where the interaction between a metal atom and a gaseous molecule is of importance, i.e., catalysis, chemisorption, and biological-fixation

processes. The future of "matrix" synthesis holds many possibilities as there are yet many interactions to be studied. Perhaps the most exciting chemical possibilities include the chemistry of many of the reactive species formed: hydrogenation (e.g., $M-N_2$ + H_2, M–olefin + H_2), simultaneous cocondensation of two high-temperature species (SiO (monomer) + M (atom)), reactions of metal atoms with free radicals (M (atom) + CH_2), etc. The possibilities are real since, although experimentally unusual, the technique has now been made quite routine.

Chemical and Biological Applications of Laser Light Scattering

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In a light-scattering experiment, light from a source impinges on a sample and is scattered into a detector placed at a given angle, θ, with respect to the direction of the incident radiation, as depicted in Figure 1. A photon that is scattered by a molecule can either gain or lose energy to translational, rotational, vibrational, and electronic degrees of freedom, thereby suffering frequency shifts. The frequency spectrum of the scattered light will exhibit resonances corresponding to these transitions, and the total light-scattering spectrum is called the *Raman spectrum*.

In order to obtain a resolved Raman spectrum it is necessary, among other things, that the spectral distribution of the incident light have a narrow width compared to the frequency shifts and widths that occur in the Raman process. Before the discovery of the laser, the spectral widths of conventional light sources were such that only electronic, vibrational, and rotational Raman effects with their corresponding large frequency shifts could be studied. With the advent of the laser, it is now not only possible to study the above Raman processes routinely, but it is

also possible to observe the translational Raman process with its much smaller frequency shifts and widths. In fact, frequency shifts as low as 1 Hz can be detected in modern light-scattering laboratories. What makes this all possible is the laser—a light source which delivers very monochromatic light at high intensity.

In the last decade there has been considerable activity in the field of light scattering. Light scattering has been used to probe collective modes[1] in solids, liquids, and gases. It has been particularly useful in the study of critical phenomena and has been used to study the kinetic theory of gases.

In this Account, we shall attempt to outline only a few of the important applications of laser light scattering to problems of a purely chemical and biological interest. In all of these applications intensity-correlation spectroscopy is used. We begin by outlining the techniques involved in these applications.

Methods of Detection

In a light-scattering experiment, a beam of laser light of frequency ω_0, wave vector $\mathbf{k}_i$ (the wave vector points in the direction of propagation of the light and has a magnitude $2\pi/\lambda_i$ where λ_i is the wavelength of the light), and field strength $\mathbf{E}_i$ first passes through a polarizer, then impinges on a sample from which it is scattered, then passes through an analyzer, and finally enters a detector. The position of the

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(1) See, for example, the review by P. A. Fleury and J. P. Boon, *Advan. Chem. Phys.*, in press.
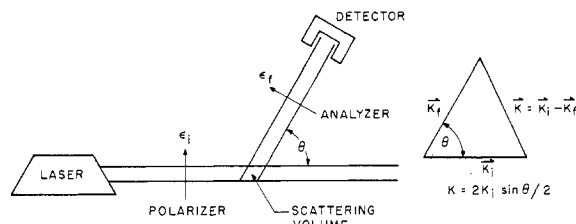
Figure 1. A schematic representation of the light-scattering experiment.

detector defines the scattering angle θ. In addition, the intersection of the incident beam and the beam intercepted by the detector defines a scattering region of volume V. All of this is illustrated in Figure 1.

Each atom and molecule in the scattering volume V scatters some of the light that impinges on it so that the total electric field, E_s, scattered into the detector is a superposition of the amplitudes scattered from each of the molecules in V. For spherical molecules, simple scattering theory gives[2]

$$E_s(t) \propto \frac{\epsilon_i \cdot \epsilon_f}{R} k_f{}^4 E_i e^{i(kR - \omega_0 t)} \sum_j \alpha_j e^{i\mathbf{k} \cdot \mathbf{R}_j(t)} b_j(t) \qquad (1)$$

where ϵ_i and ϵ_f are polarization directions defined by polarizer and analyzer, R is the distance between V and the detector, $\mathbf{k}$ is the scattering vector defined in Figure 1, whose magnitude is

$$k = 2k_i \sin(\theta/2) \qquad (2)$$

This follows from the law of cosines if, as is usually the case, $|\vec{k}_i| \simeq |\vec{k}_f|$. ω_0 is the incident light frequency, α_j is the polarizability of the jth molecule at frequency ω_0, $\mathbf{R}_j(t)$ is the center of mass position of the jth molecule, and $b_j(t)$ is a quantity which is either 1 or 0 when particle j is respectively inside or outside the scattering volume V at time t. The sum, therefore, goes over all molecules in the system.

Atoms and molecules are in continuous thermal motion so that $\mathbf{R}_j(t)$, $b_j(t)$ and thereby $E_s(t)$ fluctuates in time. In a light-scattering experiment the fluctuations in $E_s(t)$ are measured, and then analyzed to give information about the dynamics of molecular motion. In order to understand how this is done, we must review some of the main features of the detection.

The detector can either be an interferometer or a detector that utilizes the photoelectric effect. An interferometer gives the frequency spectrum directly but is only useful for an analysis of fluctuations that occur on the time scale 10^{-8} sec. or shorter. Photoelectric detectors, on the other hand, can detect much slower processes, anywhere from 1 sec to 10^{-7} sec. In a photoelectric detector, the number of electrons ejected at any time t, and concomitantly the photoelectron current, are proportional to the intensity of the light that arrives at the detector; that is, the output of the detector is proportional to $|E_s(t)|^2$. This is a typical "square-law detector."

There are two kinds of light-scattering experiments that utilize square-law detectors.

Homodyne Experiments. Here the only light that

(2) R. Pecora, *J. Chem. Phys.*, 40, 1604 (1964).

enters the detector is the scattered light. The photocurrent is processed through a correlator which computes the time correlation function $\langle |E_s(0)|^2 |E_s(t)|^2 \rangle$. In the special case of spherical scattering centers, eq 1 applies, and we find that $\langle |E_s(0)|^2 |E_s(t)|^2 \rangle$ is proportional to the molecular correlation function

$$F_2(k,t) \equiv \langle |\sum_j \alpha_j e^{i\mathbf{k} \cdot \mathbf{R}_j(0)} b_j(0)|^2 \sum_l \alpha_l e^{i\mathbf{k} \cdot \mathbf{R}_l(t)} b_l(t)|^2 \rangle \qquad (3)$$

with a proportionality constant only dependent on the geometry of the scattering experiment.

Heterodyne Experiment. Here a part of the incident field, $E_i'(t)$, is reflected from a piece of Teflon inserted in the sample so that the total field that arrives at the detector is the superposition of the scattered and reflected field, $E_s(t) + E_i'(t)$. The output photocurrent is proportional to $|E_s(t) + E_i'(t)|^2$. Since the scattered field E_s is small compared to the reflected field $E_i'(t)$, the photocurrent is to a very good approximation linear in $E_s(t)$. This output is processed through a correlator, and the correlation function of the photocurrent is determined. The time-dependent part of this correlation function is proportional to $\langle E_s^*(0) E_s(t) \rangle$ which on substitution of eq 1 is proportional to the molecular correlation function

$$F_1(k,t) = \langle \sum_{j=1} \alpha_j e^{-i\mathbf{k} \cdot \mathbf{R}_j(0)} b_j(0) \sum_l \alpha_l e^{i\mathbf{k} \cdot \mathbf{R}_l(t)} b_l(t) \rangle \qquad (4)$$

Interferometers

If the scattered field is passed through an interferometer it can be shown that the spectrum is proportional to the time–Fourier transform of the correlation function $\langle E_s(0) E_s(t) \rangle$ and thereby for our special case to the time–Fourier transform of $F_1(k,t)$.

Thus the three basic methods of detection essentially determine time correlation functions, albeit different time correlation functions.

Polymer Dynamics

One of the important applications of light scattering is to the dynamics of polymer solutions. In this case the polarizability of a polymer molecule, α_p, is much larger than the polarizability of a solvent molecule, so that the sum $\sum_j \alpha_j b_j(t) \exp(i\mathbf{k} \cdot \mathbf{R}_j(t))$ reduces from a sum over all molecules to a sum only over the polymer molecules—they account for most of the scattering.

In very dilute polymer solutions, the motion of one polymer molecule rarely affects the motion of the other polymer molecules. In this eventuality cross-terms in eq 3 and 4 can be neglected. The heterodyne correlation function (we shall discuss the homodyne function later) is then proportional to

$$g_1(k,t) = \langle N \rangle F_s(\mathbf{k},t) \qquad (5)$$

where $\langle N \rangle$ is the average number of polymer molecules in V, and the very important function $F_s(\mathbf{k},t)$ is

$$F_s(\mathbf{k},t) \equiv \langle e^{i\mathbf{k} \cdot \Delta \mathbf{R}(t)} \rangle \qquad (6)$$

where $\Delta \mathbf{R}(t)$ is the displacement of a polymer molecule in time t. The probability distribution of a displacement $\Delta \mathbf{R}$ in time t is, according to diffusion

theory, $W(\Delta\mathbf{R},t) = [4\pi Dt]^{-3/2} \exp(-\Delta R^2/4Dt)$, so that

$$F_s(k,t) = \int d^3(\Delta R)e^{+ik\cdot\Delta R}W(\Delta\mathbf{R},t) = e^{-k^2Dt} \qquad (7)$$

Thus, from elementary considerations we see that $F_s(k,t)$ and $g_1(k,t)$ decay exponentially with a time constant

$$1/\tau_k = k^2D \qquad (8)$$

Since k^2 ($=4k_i^2 \sin^2(\theta/2)$) depends on the scattering angle in a predictable way, a series of experiments at different scattering angles allows a plot of $1/\tau_k$ *vs.* k^2 to be constructed. The slope of this plot then gives the self-diffusion coefficient of the polymer, and through the Stokes–Einstein relation for a sphere, $D = k_BT/6\pi\eta a$, the particle radius a.

This experiment was first suggested and carried out in 1964 by a group at Columbia University,[3] but by a slightly different method. Recognizing the utility of this kind of experiment, several groups have in the intervening years developed this method to the point where it is now possible to determine diffusion coefficients to an accuracy of a few per cent in a matter of minutes.[4] Prior to this development, it was difficult to determine D for polymers. When these data are combined with sedimentation data, polymer molecular weights can be determined, to high accuracy.[5]

Another method for analyzing the data is to time-Fourier-transform the heterodyne correlation function, using fast Fourier transform programs. Since the Fourier transform of an exponential function is a Lorentzian, we find from eq 5 and 7 that the transform is proportional to eq 9, where c is the concentration of polymer. The half-width of this spectrum is k^2D.

$$S(k,\omega) = \pi^{-1}\left[\frac{k^2D}{\omega^2 + [k^2D]^2}\right]\alpha_p{}^2c \qquad (9)$$

So far we have considered spherical polymers. We have tacitly assumed that the polymer was sufficiently small that light scattered from one region of the polymer would not interfere with light scattered from another region; that is, we assumed that the polymer was small compared with the distance k^{-1}. In many applications, the particles are sufficiently large that intramolecular interference is important. Then the light scattered from the molecule cannot be characterized by the polarizability α_p. Equation 9 then becomes for spherical polymers[6]

$$S(k,\omega) = \pi^{-1}cS(k)\left[\frac{k^2D}{\omega^2 + [k^2D]^2}\right] \qquad (10)$$

where $S(k)$ is an angle-dependent factor called the polarizability structure factor which reaches a maximum for small scattering angles.

In a similar manner it can be shown that the spectrum, $S(k,\omega)$, of a dilute solution of macromolecules is a superposition of Lorentzian bands

$$S(k,\omega) = \pi^{-1}\sum_v c_vS_v(k)\left[\frac{k^2D_v}{\omega^2 + [k^2D_v]^2}\right] \qquad (11)$$

where c_v, D_v, and $S_v(k)$ are respectively the concentration, self-diffusion coefficient, and polarizability structure factor of macromolecular component v. In general it is difficult to determine the diffusion coefficients from this superposition unless they all differ substantially, which is seldom the case.

Ware and Flygare[7] have devised a scheme for studying the separate components in a mixture of charged macroions. Their method is based on the following observation. When an applied electric field, E, is switched on, the macroions are accelerated to different terminal velocities $\mathbf{V}_v$, depending on their respective mobilities μ_v (eq 12). The light scattered

$$\mathbf{V}_v = \mu_v\mathbf{E} \qquad (12)$$

from each charged component v will then be Doppler shifted by frequency

$$\omega_v(\mathbf{k}) = \mu_v(\mathbf{k}\cdot\mathbf{E}) \qquad (13)$$

where $\mathbf{k}$ is the scattering vector of the light. The Doppler shift is $\omega = \omega_0(1 + (\hat{\mathbf{k}}\cdot\hat{\mathbf{V}}_v/c))$, where c is velocity of light and $\hat{\mathbf{k}}$ is the direction of scattering vector, $\Delta\omega = \omega_v(\mathbf{k}) = \omega_0\mathbf{k}\cdot\mathbf{V}_v/c = \mu_v(\mathbf{K}\cdot\mathbf{E})$. Thus in an applied field the spectrum becomes

$$S(k,\omega) = \pi^{-1}\sum_v c_vS_v(k)\left\{\frac{k^2D_v}{[\omega - \omega_v(k)]^2 + [k^2D_v]^2}\right\} \qquad (14)$$

If the difference in Doppler shifts $\omega_v(k)$ is larger than the diffusion widths, it is possible to resolve $S(k,\omega)$ into separate components, and it is thereby possible to measure relative concentrations, diffusion coefficients, and mobilities of the macromolecular components. This is best accomplished at small scattering angles because the widths k^2D_v diminish faster as a function of k than do the shifts $\omega_v(k)$ which depend linearly on k. Because of its strong similarity to moving boundary electrophoresis, this method has been dubbed "electrophoretic light scattering."

So far we have been discussing nonreactive mixtures. Berne and Gininger[8] have suggested that electrophoretic light scattering can be used to probe chemical kinetics. Let us suppose in the above that some of the components can react to form other of the components. In the limit that the reaction rates are smaller than the shifts Berne, *et al.*, find that the spectrum has the simple form

$$S(k,\omega) = \pi^{-1}\sum_v c_vS_v(k)\left\{\frac{k_v + k^2D_v}{[\omega - \omega_v(k)]^2 + [k_v + k^2D_v]^2}\right\} \qquad (15)$$

where k_v is the chemical rate at which molecules leave component v through all possible chemical channels. In this simple case we see that the width of each resolved Lorentzian is $k_v + k^2D_v$, so that a plot of this half-width *vs.* k^2 has slope D_v and intercept k_v. Berne and Gininger[8] have calculated the reactive spectrum for general classes of reactions. In most applications the spectrum has a more complicated shape than eq 15, which is only valid for widths small compared to Doppler shifts. Nevertheless, it

(3) See, for example, the review by H. Cummins and H. L. Swinney, *Progr. Opt.*, **9**, 1 (1970).

(4) See, for example, P. N. Pusey, D. W. Schaefer, D. E. Koppel, R. D. Camerini-Otero, and R. M. Franklin, *J. Phys. (Paris)*, **33**, ci-163 (1972).

(5) S. B. Dubin, J. H. Lunacek, and G. B. Benedek, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1164 (1967).

(6) B. J. Berne and R. Pecora, "Introduction to the Molecular Theory of Light Scattering," manuscript of book in preparation.

(7) B. R. Ware and W. H. Flygare, *J. Colloid Interface Sci.*, **39**, 670 (1972); B. R. Ware and W. H. Flygare, *Chem. Phys. Lett.*, **12**, 81 (1971).

(8) B. J. Berne and R. Gininger, *Biopolymers*, **12**, 1161 (1973).
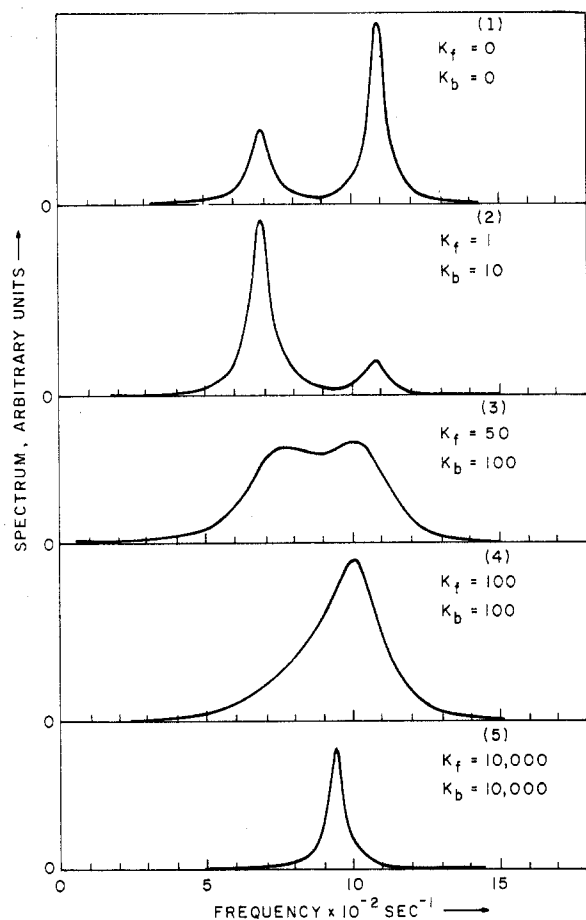
Figure 2. The light-scattering spectrum of a chemically reacting system. In this case the spectrum is computed for association-dissociation equilibrium of chymotrypsin $2A_1 \rightleftarrows A_2$, k_f', k_b, where k_f' and k_b are the forward and backward rate constants. The constant in the figure is $k_f = 2k_f'c_1^0$, where c_1^0 is the concentration of monomer. This graph is taken from ref 8.

Figure 3. the distribution of swimming speeds of motile *E. coli* determined from light scattering experiments; see ref 10.

appears that the rates can still often be determined from light scattering. For example, the dimerization of chymotrypsin looks like a good candidate for study. The computed spectrum for this reaction is presented in Figure 2. There are many other possibilities for further study.
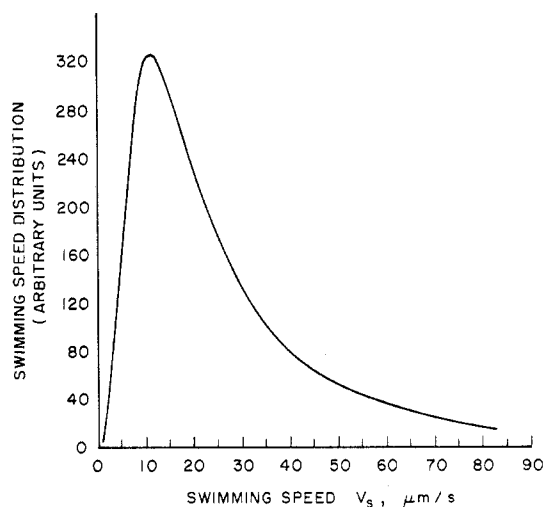
This method is different in principle from previous suggestions for using laser light scattering to measure reaction rates,[9] but it reduces to these other methods in zero field. It is analogous to the study of exchange rates in nmr.

Motility of Microorganisms

An important biological application of light scattering is to the study of the motility of microorganisms. Microorganisms swim along linear paths for distances long compared to the incident wavelength, λ_i, before changing their direction. The displacement of a microorganism along one of these trajectories is $\Delta R(t) = Vt$, where V is the swimming velocity. If $W(V)$ is the velocity distribution function, it follows from eq 6 that

$$F_s(\mathbf{K}, t) = \int d^3V W(\mathbf{V}) e^{i\mathbf{k}\cdot\mathbf{V}t} \qquad (16)$$

Thus $F_s(\mathbf{k},t)$—the function measured in light scattering—can be regarded as the Fourier transform of

$W(\mathbf{V})$ with Fourier variable $\mathbf{k}t$. It is possible to Fourier-invert the measured $F_s(\mathbf{k},t)$ to get $W(\mathbf{V})$ or the speed distribution function $4\pi V^2 W(\mathbf{V})$. The speed distribution of *E. coli* determined by light scattering is shown in Figure 3. These curves are based on the experiments of Chen and Nossal.[10] A current problem in biology is the study of bacterial chemotaxis. Light scattering can be used to give information about how various chemotactic agents can affect the swimming speed distribution.

As cells die, they stop swimming and simply diffuse. In any sample we expect there to be some dead cells. $F_s(k,t)$ is then

$$F_s(k,t) = X_L \int d^3V e^{i\mathbf{k}\cdot\mathbf{V}t} W(\mathbf{V}) + X_D e^{-K^2 Dt} \qquad (17)$$

where X_L and X_d are the fractions of living and dead cells, respectively. Light scattering can be used to measure X_L and X_D and thereby give information about the viability of a sample. This has been suggested as a means for studying the viability and potency of sperm cells.

Thus far we have only discussed the heterodyne experiment and its associated spectrum. What about the homodyne experiment? Under the same conditions that led to the derivation of eq 5, Schaefer and Berne[11] have found that the homodyne correlation function is proportional to

$$g_2(k,t) = \langle N\rangle^2[1 + |F_s(k,t)|^2] + \langle\delta N(0)\delta N(t)\rangle \qquad (18)$$

where $\delta N(t) \equiv N(t) - \langle N\rangle$ is the deviation of the number of particles from the average number of particles in the scattering region V. The correlation function $\langle\delta N(0)\delta N(t)\rangle$ is of order $\langle N\rangle$ and can be ignored if $\langle N\rangle$ is large because then the first term, which is of order $\langle N\rangle^2$, dominates. In this eventuality $g_2(k,t)$ is entirely determined by $F_s(k,t)$, and no new information is found. Nevertheless, in applications involving bacteria or large polymers, the concentration is sufficiently small that it is possible[12] to measure $\langle\delta N(0)\delta N(t)\rangle$. This is accomplished by focusing the incident laser beam down to a small

(9) For a review see V. A. Bloomfield and J. A. Bentasat, *Macromolecules*, 4, 609 (1971), and references cited, particularly work by B. J. Berne, H. L. Frisch, L. Blum, W. Salsburg, and coworkers.

(10) R. Nossal and S. H. Chen, *J. Phys. (Paris)*, 33, ci-171 (1971).
(11) D. Schaefer and B. J. Berne, *Phys. Rev. Lett.*, 28, 475 (1972).
(12) D. Schaefer, *Science*, in press.

diameter. The scattering volume then decreases, and the number of scattering centers in the scattering volume decreases. Thus, by focusing down the incident laser beam Schaefer and Berne have measured $\langle \delta N(0)\delta N(t)\rangle$.

Why are these considerations important? For the simple reason that $\langle \delta N(0)\delta N(t)\rangle$ decays on a time scale determined by the time it takes a microorganism to swim across the region V. Since this region is large compared to the average swimming path length of the microorganism, the microorganism reverses its direction several times before leaving V. This allows[12] extraction of the mean free swimming path length of the bacteria from an analysis of $\langle \delta N(0)\delta N(t)\rangle$. This property cannot be determined from $F_s(k,t)$. Thus, in one experiment we are able to get speed distributions and path lengths.

Another application of this result is to the study of turbulent flow: $\langle \delta N(0)\delta N(t)\rangle$ decays by virtue of the large molecules being swept through the scattering volume. Thus a study of the decay of $\langle \delta N(0)\delta N(t)\rangle$ should reveal something about the velocity field in turbulent flow.

Fluorescence Correlation Spectroscopy

An interesting application of occupation number fluctuations has recently been carried out by Webb, *et al.*[13] In this application the sample consists of an equilibrium solution in which A is in dynamic equilibrium with B.

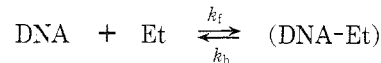$$A \underset{k_b}{\overset{k_f}{\rightleftharpoons}} B$$

The incident laser light is chosen such that it can be absorbed by either A or B or by both A and B. In the experiment *only B can fluoresce,* that is

$$B^* \longrightarrow B + h\nu$$

The fluorescent light is collected in a square-law detector at right angles to the incident beam after first passing through a narrow slit. The experiment is so arranged that the laser is continuously on. Thus, at any time t, the fluorescence intensity, I_f, that is measured at the detector is proportional to the instantaneous number of molecules in state B located in the volume, V, defined by the incident and fluorescing beam. This number fluctuates in time by virtue of either diffusion of B molecules out of and into this volume or by chemical interconversion of B to A (or *vice versa*). The experiment is analyzed by feeding the output of the detector into an autocorrelator which computes the time-correlation function of the fluorescence intensity fluctuations, $\langle \delta I_f(0)\delta I_f(t)\rangle$ or, equivalently, the autocorrelation function of the number of B molecules in the volume V. For the unimolecular reaction

$$\langle \delta I_f(0)\delta I_f(t)\rangle \propto \frac{C_B}{C_A + C_B}[C_B + C_A e^{-(k_f + k_b)t}]$$

where C_A and C_B are the equilibrium concentrations of A and B and $1/\tau = k_f + k_b$ is the kinetic rate constant. Here it is assumed that diffusion is slow compared to the reaction rate. This provides a novel method for the determination of rate coefficients. Actually Webb, *et al.*, studied the intercolation reaction of ethydium ions into DNA. Ethydium fluoresces only when it is in the intercolated state. This reaction is of a different form than above. Nevertheless, the same kind of analysis gives $\langle \delta I_f(0)\delta I_f(t)\rangle$ in

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

terms of the reaction rates.

Although these experiments are not light-scattering experiments, they share much in common with light-scattering experiments in that they probe concentration fluctuations. We expect many future applications of these ideas to chemical problems.

Other Applications

Due to limitations of space we can only hint at other applications of light scattering.

The polarizability structure factor $S(k)$ that appears in eq 10 is sensitive to the radius of the sphere. Thus, if there are breathing modes that involve substantial changes in the radius, $S(k)$ should be replaced by a time-dependent factor $S(k,t)$ which varies in time because of the intramolecular motions. An analysis of $S(k,t)$ would then give the frequencies and relaxation times of the internal modes.[6] Although this has never been seen in spherical polymers, it has been reported for nonspherical molecules. A theory of light scattering from nonspherical molecules has been derived.[6] The final results are similar to eq 10 but contain widths that depend not only on translational diffusion coefficients but also on rotational diffusion coefficients, thereby permitting the molecular reorientations to be probed. Moreover, the polarizability structure factors contain anisotropic polarizability components. Bending and stretching modes of the molecules will contribute to changes in these structure factors and thereby can be probed by light scattering. As an example, muscle filaments have recently been studied by this method.[14]

We have ignored a whole class of light-scattering experiments on pure fluids and solvents. Light scattering has been used to probe collective modes in solids, liquids, and gases.[1] It has been particularly useful in the study of critical phenomena and as a probe of the kinetic theory of gases.[1]

We have discussed only a few applications of laser light scattering in the hope of conveying to the reader the idea that laser light scattering is an important and useful tool for chemistry.

(13) D. Magde, E. Elson, and W. W. Webb, *Phys. Rev. Lett.*, **29**, 705 (1972).
(14) S. Ishiwata and S. Fujime, *J. Mol. Biol.*, **68**, 511 (1972).