Table V
Parameters of the Systems

System	T_1* (deg)	c_1	τ^2	$10^3\,\nu^2$	$c_1\tau^2$	$c_1\nu^2$
Polystyrene-benzene	4685	1.23	0.122	0[a]	0.150	0[a]
Polystyrene-toluene[b]	4979	1.57	0.095	0[a]	0.150	0[a]
Polystyrene-methyl ethyl ketone	4546	1.76	0.136	0[a]	0.240	0[a]
Polystyrene-cyclopentane	4494	0.706	0.142	31.2	0.100	0.022
Polystyrene-cyclohexane[b]	4720	1.01	0.119	15.9	0.120	0.016
Polystyrene-methylcyclohexane[b]	4870	1.14	0.105	15.8	0.120	0.018

[a] Assumed value. [b] From ref 7.

Assuming the geometric mean rule for the contact energy

$$\tau \simeq \delta + \lambda$$
$$\nu^2 \simeq \delta^2/4 + 9\rho^2 \qquad (9)$$

The τ and ν^2 are correlated through the δ parameter. In mixtures of polymer and solvent for which the ν^2 parameter is assumed to be zero, the τ parameter directly reflects the structural factor. Values of pairs of $c_1\nu^2$ and $c_1\tau^2$ used to fit the theoretical curves to the experimental points in Figures 4 and 5 are given in Table V. Values obtained from the previous work[7] are also included. One-third of the external degrees of freedom of the solvent expressed by c_1 seems to be the controlling factor for the χ_1(crit) through $c_1\tau^2$ irrespective of τ^2 in the six polystyrene solutions. The value of c_1 is largest in the polystyrene-methyl ethyl ketone system and smallest in the polystyrene-cyclopentane system in these polystyrene solutions. The value of the ν^2 parameter for polystyrene in cyclohexane is almost equal to the value in methylcyclohexane and is about one half the value in cyclopentane.

In this work we conclude that the phase separation behavior of the nonpolar polymer solutions and also the solvent effects on the ucst and lcst are adequately described by Patterson-Delmas theory of corresponding states and the Flory model with the Flory-Huggins critical value of χ_1. The polystyrene-cyclopentane system with the ucst and lcst in a convenient temperature range is one of the most suitable ones for studying the excluded-volume effect in dilute polymer solutions.

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Inelastic Light Scattering Study of Macromolecular Reaction Kinetics. II. The Association Reaction $mA + nB \rightleftarrows pC$

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ABSTRACT: We present a study of the effect of chemical reactions on the spectrum of light scattered from macromolecular solutions. We employ the general formalism developed in paper I of this series using the matrix eigenvalue technique of Salsburg and coworkers and considering only diffusion and chemical reaction as processes perturbing local equilibrium. This paper considers the general reaction $mA + nB \rightleftarrows pC$, focusing on the special case where at least two of the diffusion coefficients of reactants and products are different, while their polarizabilities are identical. Numerical calculations have been made using plausible values of diffusion coefficients and reaction rates for macromolecular solutions for two special cases, $A + B \rightleftarrows C$ and $A + 144B \rightleftarrows C$. These indicate that macromolecular assembly reactions should measurably perturb inelastic light scattering spectra of polydisperse solutions due to differences in diffusion coefficients alone, providing a means of determining both equilibrium constants and kinetic rate constants for the reaction under investigation. Particularly favorable systems are those in which both of the reactants are small while the product is large. For the reaction $A + B \rightleftarrows C$, a system in which one of the reactants is large, while both the other reactant and the product are small, constitutes another favorable combination. And for the reaction $A + 144B \rightleftarrows C$, a large reactant combining with 144 smaller reactants to form a relatively small product would be a favorable system. In any case the ratio of the reciprocal reaction half-life to the spectral half-width due to diffusion should be ≥ 1.

A major theme in biophysical chemistry over the past few years has been the development of new methods for the study of structure and macromolecular assembly of subunit aggregates such as viruses, multimeric proteins, and enzyme-substrate complexes.[1-4] Inelastic light scattering has proven to be an accurate and rapid tool for the determination of diffusion coefficients of macromolecules, thereby yielding valuable information on biopolymer size

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and shape. A review of this technique and some of its applications has been written by Chu.[5] It has also been shown theoretically and, in a few cases, experimentally, that inelastic light scattering may provide a feasible approach to the determination of equilibrium and kinetic parameters for fast reactions. Reference 6 cites most of the previous papers on this topic. Since then, experimental papers have appeared on the association reactions of the muscle proteins F-actin[7] and myosin.[8]

An advantage of the inelastic light scattering technique is that the scattering arises from spontaneous fluctuations in solution refractive index, without the imposition of external perturbations; and that the small relative size of these fluctuations permits linearization of the kinetic rate equations in the manner of the rapid perturbation techniques pioneered by Eigen and coworkers.[9]

In a recent paper[6] (which will subsequently be referred to as I) we developed the theory and examined the feasibility of studying the kinetics of two simple types of macromolecular reactions—reversible isomerization and reversible dimerization—by inelastic light scattering. It is the purpose of the present article to extend this treatment to the more general reversible assembly reaction $m\mathrm{A} + n\mathrm{B} \rightleftarrows p\mathrm{C}$. The general equations are derived incorporating differences in both diffusion coefficients and polarizabilities of reactants and products. However, as in I, we concentrate for purposes of calculation on the situation most likely to be important in practice, in which all polarizabilities (or refractive index increments) are identical, and in which the various species differ only in their diffusion coefficients. The matrix eigenvalue technique of Blum and Salsburg[10] is used to develop a general formalism for the calculation of inelastic light scattering spectra as sums of Lorentzian line shapes characterized by intensities and half-widths related to diffusion and reaction parameters. We have simplified the Blum and Salsburg treatment by considering diffusion and reaction as the only important dissipative processes, neglecting viscous relaxation and heat conduction which are unlikely to contribute significantly to the scattering from macromolecular solutions.

In the next section of this paper, we apply the general formalism for the calculation of inelastic light scattering spectra, as developed in I, to the association reaction $m\mathrm{A} + n\mathrm{B} \rightleftarrows p\mathrm{C}$. When m, n, and p are 1, and A and B are identical, numerical calculations confirm the results obtained in paper I for the dimerization reaction $2\mathrm{A} \rightleftarrows \mathrm{A}_2$. For $m = 0$ and $p = 1$ the theoretical equations overlap the equations derived by McQueen and Hermans for a micellization reaction.[11] Section III discusses the feasibility and limitations of the proposed approach, considering specifically the bimolecular association reaction $\mathrm{A} + \mathrm{B} \rightleftarrows \mathrm{C}$ and the assembly reaction $\mathrm{A} + 144\mathrm{B} \rightleftarrows \mathrm{C}$. Consideration of this latter reaction is motivated by the potential applicability to biomolecular polymerization reactions such as the growth of bacteriophage tail cores,[12] a reaction which will be discussed in greater detail in section II.

I. The Reaction $m\mathrm{A} + n\mathrm{B} \rightleftarrows p\mathrm{C}$

The general equations pertaining to the spectrum of light scattered from a reacting system were derived in part I. We restate the basic result here for convenient reference.

The intensity $I(\mathbf{K},\omega)$ of light scattered in direction $\mathbf{K}$ with angular frequency shifted by ω from that of the irradiating light is given by a sum of Lorentzians with half-widths of half-height of $-\mu_m$ and intensities B_m

$$I(\mathbf{K},\omega) = -\frac{\Sigma B_m \mu_m}{\omega^2 + \mu_m^2} \tag{1}$$

μ_m is the mth eigenvalue of the concentration fluctuation matrix $\mathbf{M}$ whose elements are

$$M_{ij} = -K^2 D_i \delta_{ij} + T_{ij} \tag{2}$$

δ_{ij} is the Kronecker delta, D_i is the diffusion coefficient of the ith species, and T_{ij} is the ijth element of the reaction matrix (given explicitly below for the reaction $m\mathrm{A} + n\mathrm{B} \rightleftarrows p\mathrm{C}$) which specifies the rate at which a fluctuation in the concentration of species i changes due to a fluctuation in the concentration of species j and $\mathbf{K}$ is the scattering vector whose magnitude is

$$K = \frac{4\pi n}{\lambda_0} \sin \frac{\theta}{2} \tag{3}$$

As a particularly important case of the preceding formalism, we consider the reversible assembly of A and B to form C with forward and backward rate constants k_f and k_b and an equilibrium constant

$$K_{eq} = \frac{(\bar{n}_C)^p}{(\bar{n}_B)^n(\bar{n}_A)^m} \tag{4}$$

The $\mathbf{M}$ matrix for the case is given by eq 5, where $\bar{n}_i$ is the equilibrium molar concentration of component i, M_i refers to the molecular weight of component i (i = A, B, and C).

$$\mathbf{M} = \begin{bmatrix} -K^2D_A - k_f m^2 \bar{n}_A^{m-1}\bar{n}_B^{n} & -k_f mn \frac{M_A}{M_B}\bar{n}_A^{m}\bar{n}_B^{n-1} & k_b mp \frac{M_A}{M_C}\bar{n}_C^{p-1} \\ -k_f mn \frac{M_B}{M_A}\bar{n}_A^{m-1}\bar{n}_B^{n} & -K^2D_B - k_f n^2 \bar{n}_A^{m}\bar{n}_B^{n-1} & k_b np \frac{M_B}{M_C}\bar{n}_C^{p-1} \\ k_f pm \frac{M_C}{M_A}\bar{n}_A^{m-1}\bar{n}_B^{n} & k_f np \frac{M_C}{M_B}\bar{n}_A^{m}\bar{n}_B^{n-1} & -K^2D_C - k_b p^2 \bar{n}_C^{p-1} \end{bmatrix} \tag{5}$$

The eigenvalue equation $|\mathbf{M} - \mu\mathbf{I}| = 0$ yields a cubic equation in μ which can be solved for the eigenvalues μ_1, μ_2, and μ_3, each of which represents the half-width of one of the Lorentzians making up the inelastic light scattering spectrum. The integrated intensities of the component Lorentzians, B_m, and the half-widths of the resulting spectra can be solved for numerically. The spectral half-width, which would equal K^2D for a nonreacting solution of a monodisperse spherical polymer, becomes in the general case a function of the reaction rate and the relative amounts of the various species. As such it is a useful parameter for summarizing the effect of chemical reactions on the observed light scattering spectrum.

Results for the limiting cases in which the reaction rate is very much larger, or very much smaller, than the diffu-

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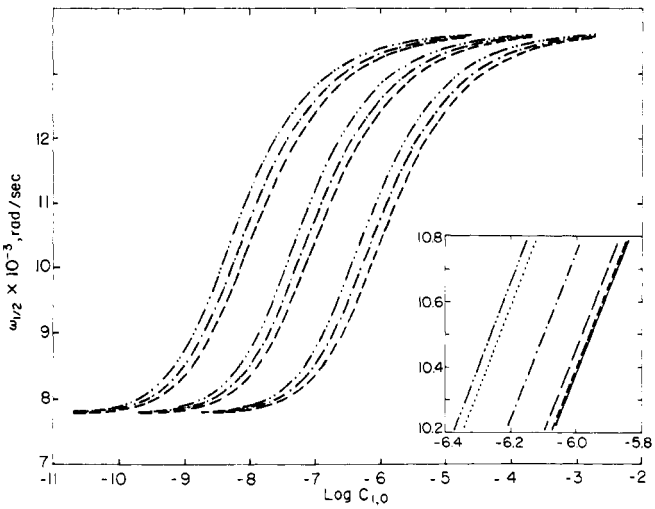

Figure 1. Spectral half-width $\omega_{1/2}$ computed as a function of initial concentration $C_{1,0}$ of component 1 for a hypothetical reaction between polynucleotide phosphorylase ($M_1 = 2 \times 10^5$, $D_1 = 4.8 \times 10^{-7}$ cm^2/sec) and polyribonucleotide ($M_2 = 1.65 \times 10^5$, $D_2 = 1.6 \times 10^{-7}$ cm^2/sec) to give complex with $M_3 = 3.65 \times 10^5$, $D_3 = 3.9 \times 10^{-7}$ cm^2/sec. From right to left, the three groups of sigmoidal curves correspond to $K_{eq} = 10^6$, 10^7, and 10^8. Inset is an enlargement of the central region of the curve for $K_{eq} = 10^6$. Lines correspond to values of reaction half-life τ^{-1} (sec^{-1}): ——, $\tau^{-1} = 0$; - - -, 10^2; ——, 10^3; -·-·-, 10^4; ···, 10^5; -···-, 10^6. calculations assume a scattering angle of 90° and $C_{2,0} = C_{1,0}$.

sion rate can be obtained by perturbation expansion of the above equations. For very slow reactions $K^2D_i \gg \tau^{-1}$, where the chemical relaxation time τ is a function of the rate constants and equilibrium concentrations. In this case the eigenvalue approach those of the nonreacting mixture: $\mu_1 \rightarrow -K^2D_A$, $\mu_2 \rightarrow -K^2D_B$, $\mu_3 \rightarrow -K^2D_C$. For very rapid reactions, in which $K^2D_i \ll \tau^{-1}$, $\mu_1 \rightarrow \tau^{-1}$, while μ_2 and $\mu_3 \rightarrow 0$. However, in this case B_1 also becomes very small, so that the polarized light scattering spectrum to be expected from a very rapidly reacting system is a single low, broad Lorentzian with half-width τ^{-1}.

II. Feasibility Calculations and Discussion

A major aim of this paper is to delineate those experimental systems in which inelastic light scattering may be a feasible technique for measuring macromolecular reaction kinetics, given realistic limits of experimental precision. It is evident from eq 5 that, in order to determine the unknown rate constants k_f and k_b, one must first determine the diffusion coefficients of the various species, and their equilibrium concentrations, from prior measurements. The diffusion coefficients can, of course, be determined by inelastic light scattering measurements on the pure components. This technique can also be used to measure the equilibrium constant for the reaction, as shown in the exemplary study of myosin dimerization by Herbert and Carlson.[8]

It is interesting to note that, at least in some cases, it may be possible to obtain a "titration curve" of the spectral half-width to determine the equilibrium constant of the reaction. This can be shown for a hypothetical reaction between the enzyme polynucleotide phosphorylase ($M = 200{,}000$,[13] $D = 4.8 \times 10^{-7}$ cm^2/sec) and a single-stranded polyribonucleotide chain ($M = 165{,}000$, $D = 1.6 \times 10^{-7}$ cm^2/sec)[14] to give a complex with $M = 365{,}000$ and a diffusion coefficient of 3.9×10^{-7} cm^2/sec, corresponding to a partial wrapping of the polynucleotide chain around the enzyme.[15] In Figure 1 the spectral half-width is plotted *vs.* the log of the initial reactant weight concentration $C_{1,0}$ (taken as equal to $C_{2,0}$ for simplicity). We see that the reaction rate, for a plausible range of values of τ^{-1},[16] does not blur the separation of these curves into groups characterized by a particular value of $K_{eq}(=k_f/k_b)$. Each group of curves has an inflection point at log $C_{1,0} \approx pK_{eq}$. (In other cases, the reaction may cause overlapping of curves corresponding to different values of K_{eq}.)

In order to compute the realm of feasibility of determining macromolecular association kinetics by inelastic light scattering, it is economical to work in terms of reduced variables, since the important factors are the ratios of diffusion to reaction rates and of the diffusion coefficients of A, B, and C to one another. Therefore we write the eigenvalue equation corresponding to eq 5 as

$$\begin{vmatrix} -1 - rm^2A - \mu' & -r\frac{n^2}{\beta}B & rp^2\left(\frac{1}{1+\beta}\right)C \\ -rm^2\beta A & -b - rn^2B - \mu' & rp^2\left(\frac{\beta}{1+\beta}\right)C \\ rm^2(1+\beta)A & rn^2\left(\frac{1+\beta}{\beta}\right)B & -c - rp^2C - \mu' \end{vmatrix} = 0 \quad (6)$$

where

$$r = k_b/K^2D_A \quad (7)$$

$$b = D_B/D_A \quad (8a)$$

$$c = D_C/D_A \quad (8b)$$

$$\beta = \frac{nM_B}{mM_A} = \frac{n}{m}\left(\frac{D_A}{D_B}\right)^3 \qquad \left(\beta + 1 = \frac{pM_C}{mM_A}\right) \quad (9)$$

$$A = \bar{n}_C{}^p/\bar{n}_A$$

$$B = \bar{n}_C{}^p/\bar{n}_B \quad (10)$$

$$C = \bar{n}_C{}^{p-1}$$

The eigenvalues μ' obtained from eq 6 are related to the desired eigenvalues of eq 5 by

$$\mu_i' = \mu_i/K^2D_A \quad (11a)$$

while the intensities calculated using the reduced eigenvalues are related to the true intensities by

$$B_m' = B_m/M_A{}^2 \quad (11b)$$

The feasibility calculations were performed for two assembly reactions A + B $\rightleftarrows$ C and A + 144B $\rightleftarrows$ C.

For these two cases we have plotted contours (Figures 2a-d and 3a-c) in the log D_B/D_A − log D_C/D_A plane outside of which the halfwidth of the spectrum of the reacting system is calculated to differ by more than 5% from the expected half-width for a nonreactive system ($\tau^{-1} = 0$) of the same equilibrium composition. The various subfigures are for values of r from 10^{-2} to 1 or 10^1; higher values of r give plots that are virtually indistinguishable from that for $r = 10$.

It will be observed from the sequence of plots in Figures 2 and 3 that as the rate of reaction increases relative to the diffusion rate (*i.e.*, as r increases), the reaction kinet-

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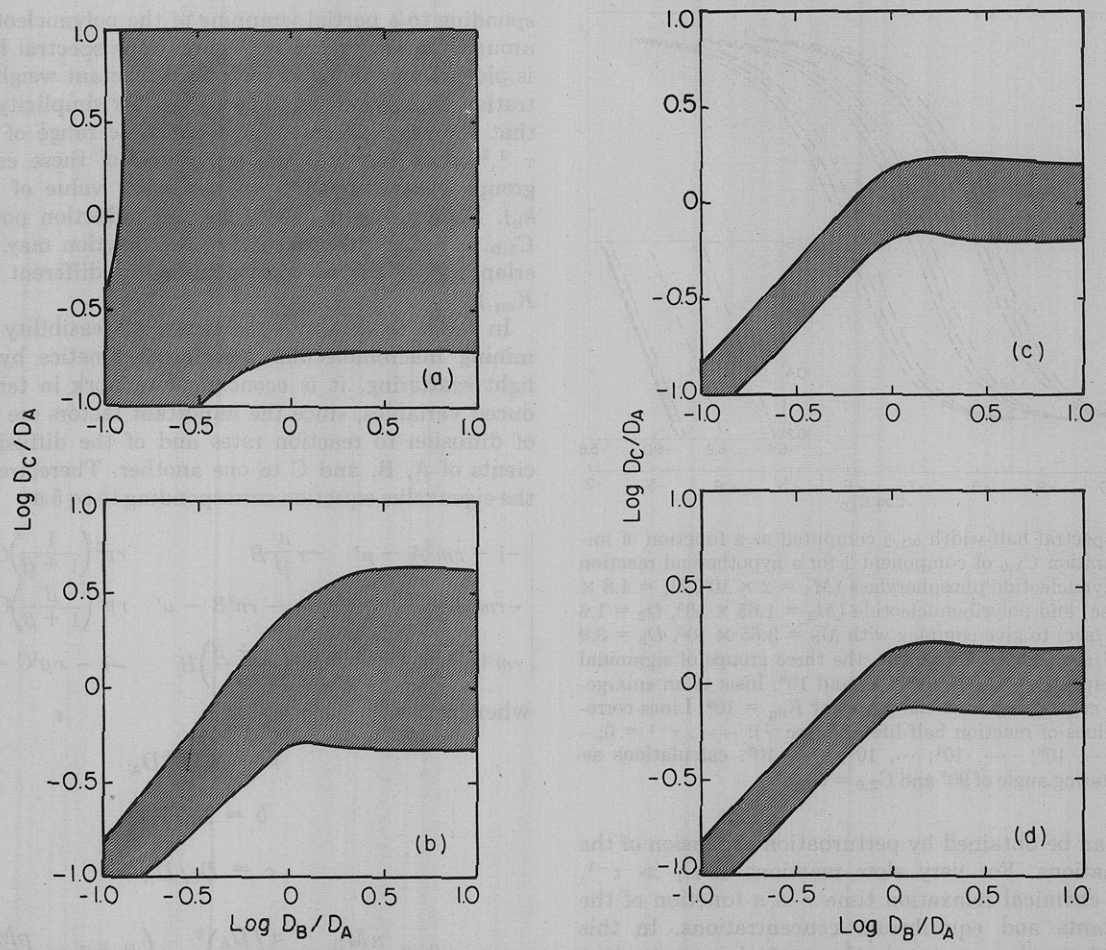

Figure 2. Nonshaded regions correspond to values of the diffusion coefficient ratios D_B/D_A and D_C/D_A for which the spectrum of a reacting mixture (A + B $\rightleftarrows$ C) has a half-width differing by more than 5% from the expected half-width of the nonreacting system. Calculations assume $\beta = M_B/M_A = (D_A/D_B)^3$, and $f_R = 0.382$; $k_b/K^2D_A = 10^{-2}$ (a), 10^{-1} (b), 1 (c), 10 (d).

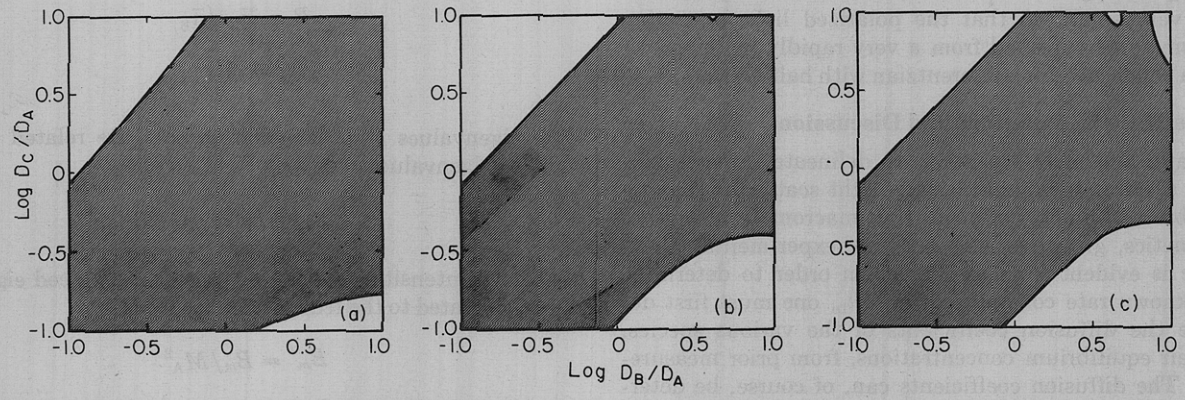

Figure 3. Same as Figure 2. Reaction: A + 144B $\rightleftarrows$ C, $\beta = 144M_B/M_A = 144(D_A/D_B)^3$, $f_R = 0.5$; $k_b/K^2D_A = 10^{-2}$ (a), 10^{-1} (b), 1 (c).

ics will be detectable (as a change in spectral width) with progressively less disparate values of the diffusion coefficients of the various species. Nevertheless, it is important to note that three regions remain resolutely resistant to a change of spectral width accompanying even very rapid reaction. These are the regions ($D_B/D_A \approx 1$, $D_C/D_A \approx 1$), ($D_B/D_A > 1$, $D_C/D_A \approx 1$), and ($D_A > D_B \approx D_C$).

The first of these regions corresponds to the circumstance that both of the reactants and the product have nearly equal diffusion coefficients (are of similar hydrodynamic radius). In this case it is intuitively obvious that reaction can be detected by inelastic light scattering only if the reactants and products have markedly different polarizabilities. (Polarizabilities only affect the intensities of the component Lorentzians but do enter in the half-width of the sum spectrum since $\omega_{1/2}$ is a function of both the μ_i's and B_i's.) This is the case analyzed by several other groups. (See refs 3, 4, and 9–13 in part I.) The second region corresponds to B being rather small, while A and C are of similar size. This would be exemplified by detergent molecules (B) adding to a large micelle (A) to form an only slightly larger micelle (C); or by protein monomers (B) combining with an already large helical protein aggregate (A) to form one only slightly larger (C), as in the polymerization of tobacco mosaic virus or bacterial flagella. The third region corresponds to the situation where B and C are of similar size and are larger than A, and is therefore the same as the second region for a reaction of type A + B $\rightleftarrows$ C, with A and B interchanged.

Reaction A + B $\rightleftarrows$ C. Calculations for this reaction

were carried out assuming that the system reaches equilibrium after 38.2% of the reactants have reacted. This percentage corresponds to $f = K_{eq}n_{A,O} = 1$, where $n_{A,O}$ is the initial molar concentration of A. The initial molar concentrations of A and B are assumed to be the same and β is defined as $M_B/M_A = (D_A/D_B)^3$, corresponding to the interaction of spherical macromolecules of constant density. Other plausible assumptions such as $\beta = (D_A/D_B)^2$ give similar results.

It appears from the results that a type of associating system that would be well suited to analysis by inelastic light scattering is one well along the negative log (D_B/D_A) axis, or in the upper left quandrant, of Figure 2. This is a system in which, in the extreme instance, one of the reactants is large, while both the other reactant and the product are small. Such a reaction might be the combination of a small globular protein with a highly swollen flexible coil polymer to produce a complex in which the flexible coil is wrapped around the protein, as in the polynucleotide phosphorylase–RNA system cited earlier.

Another type of suitable system is that found in the lower right quadrant of Figure 2. This is exemplified by one in which one of the reactants is substantially smaller than the other, while the product is larger than either. A reaction of this type might be the interaction of a small denaturing molecule with a compact globular protein to form a swollen, denatured protein chain.

Reaction A + 144B ⇄ C. The allowed region in the lower right quadrant of Figure 2 suggests that a promising system in which to observe reaction by inelastic light scattering is one in which a number of small molecules add to a "base" or "nucleus" of intermediate size to form a large product. Several systems of this type are known in molecular biology. Prominent among these is the polymerization of tail core protein subunits on the preexisting base plate in the morphogenesis of bacteriophage T4.[12] It has been shown by optical and X-ray diffraction[17] that there are 144 subunits in the core, arranged in 24 annuli each containing 6 subunits. Although these subunits may well add to the base plate sequentially rather than all at once, it is valid[18] to treat the kinetics as a concerted addition so long as there is a negligible concentration of intermediates. Therefore we have made calculations for the case A + 144B ⇄ C. Results obtained here should be qualitatively valid whenever the stoichiometric coefficient of B is large, regardless of its exact value.

Calculations in this case were carried out assuming that equilibrium is reached when f_R, the reacted fraction of A, is 0.5. The initial molar concentrations of A and B are related by $144n_{A,O} = n_{B,O}$. We also assumed that $\beta = 144M_B/M_A = 144(D_A/D_B)^3$, resulting in the same implications as in the above case.

For this type of assembly reaction the 5% contours in the log (D_B/D_A) − log (D_C/D_A) plane have the same general characteristics as the A + B ⇄ C case (Figure 3). There are two main regions of suitable systems: the lower right quadrant will accommodate systems in which a reactant (A) combines with n smaller reactants (B) to form a large product (C) (*e.g.*, core polymerizing on a base plate during the assembly of T-even phages,[12] dye intercalation into a superhelical DNA,[19] and pH-dependent unfolding of proteins). The upper left quadrant refers to a reacting system in which n large reactants (B) bind one smaller reactant (A) to form an even smaller product (C). It is difficult to think of an example of this type of behavior on the molecular level. But the far upper right corner denotes another feasible region exemplified by a system in which a reactant (A) combines with n smaller reactants (B) to form a product (C), small compared to (A). The reaction of n ions with a highly expanded polyelectrolyte of opposite charge to form a compact neutral polymeric species is of this type.

The results for both the above studied cases are independent of the equilibrium constant for the reaction. Dependence on the equilibrium constant enters the equations when $p \neq 1$.

The use of a 5% difference contour line in Figure 2 to indicate systems suitable for kinetic study is rather optimistic, since it requires that the diffusion coefficients and equilibrium concentrations of the various species be known to 1% or better. This is somewhat better precision (by a factor of 2–5) than is currently obtained in most inelastic scattering work, but should not be outside the range of possibility in the next few years as instrumental techniques are improved. The qualitative shape of the contours in Figure 2 will not be changed markedly if the limits of precision are expanded to more realistic 10 or 25%, though of course, in these cases the diffusion coefficient ratios needed for detection of reaction kinetics would also become more extreme. Even at the 5% discrimination level, a reaction between two spherical monomers of equal size ($D_B/D_A = 1$) to form a dumbbell-shaped dimer ($D_C/D_A = 3/4$) is just at the limit of detectability for $k_b/K^2D_A > 1$. Since $K^2D \cong 10^3$ sec^{-1} for typical biopolymers, Figure 2 indicates that unimolecular rate constants, k_b, must be at least of this magnitude for kinetic studies to be feasible. The detectability of reactions is improved by working at lower scattering angles, where K^2 is minimized.

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