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Isothermal diffusion and quasielastic light-scattering of macromolecular solutes at finite concentration

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The Brownian motion of a group of hydrodynamically interacting particles suspended in a low-Reynolds-number fluid is described by a generalization of the Einstein diffusion relation. The time integral of each velocity self- or cross-correlation function is found to be equal to one of the mobility tensors governing steady movement of the particles through the fluid. Using the approach of nonequilibrium thermodynamics and linear response theory, this result allows formulation of an expression for the concentration dependence of the Fick's law diffusion coefficient for mutual binary diffusion in terms of the mobility tensors. The relationship between the concentration dependence of the solute chemical potential, sedimentation rate and mutual diffusion coefficient D is proved. A distinction is made between D and the mean self-diffusion coefficient \bar{D} , for solute molecules, thus allowing interpretation of results obtained using different experimental techniques. An argument is presented which indicates that quasielastic light scattering is suitable for the determination of \bar{D} , and higher moments of the self-diffusion coefficient distribution for a monodisperse solution of macromolecules. The normalized second moment of this distribution is calculated for a solution of spherical macromolecules in terms of the solute volume fraction. It is concluded that quasielastic light scattering may be used to determine the value of a dimensionless parameter which is dependent solely on the shape of the hydrodynamic particle corresponding to a solvated macromolecule.

I. INTRODUCTION

The modeling of globular macromolecules in solution as rigid particles immersed in a fluid has allowed the construction of a consistent theory of transport phenomena and the interpretation of measurements in terms of the simple ideas of molecular size and shape. Theoretical and experimental aspects of macromolecular diffusion are of particular relevance. The result obtained by Einstein¹ relating the diffusion and frictional coefficients of a suspension of spherical particles has been widely applied but it has long been realized that it is an idealization referring to the limit of infinite dilution where particle-particle interactions can be neglected. More recently there has been interest in the possibility of giving a concise and accurate account of diffusion at finite concentration and it is one purpose of this work to contribute to this goal.

It has been the intention of several authors²⁻⁸ to describe the effect of hydrodynamic interactions on translational diffusion. This work has usually culminated in the presentation of an expression for the concentration dependence of the diffusion coefficient for spherical particles. Unfortunately, different formulations of the original problem have not always been related to one another and this has hindered meaningful comparison of the final results. Three elements are essential to any adequate account of diffusion at finite concentration. First, it must bear some relation to classical Brownian motion theory so that it can be interpreted microscopically. Second, it must be consistent with the established description of diffusion according to nonequilibrium thermodynamics so that it can be interpreted macroscopically. Third, it must make reference to parameters defined in fluid mechanics so that the effects of particle-particle hydrodynamic interactions can be conveniently expressed in terms of the size and shape of the solute molecules. With these demands in mind,

we shall now give a brief preview of the path to be followed here.

Using an extension of the argument of Batchelor,⁶ the Einstein diffusion relation is generalized to the case of a group of hydrodynamically interacting Brownian particles. The discovered equivalence between the relevant tensor mobilities and the time integral of velocity correlation functions allows results from linear response theory to be used to obtain a general expression for the concentration dependence of the mutual diffusion coefficient defined in nonequilibrium thermodynamics. Special attention is paid to the frames of reference in which flows may be measured and the result is interpreted in terms of the sedimentation rate of solute molecules. The applicability of classical Brownian motion theory to the description of solute diffusion as it is observed on different timescales is also discussed.

The second purpose of this work is to elucidate the relevance of quasielastic light scattering to the study of macromolecular diffusion. Results obtained using intensity fluctuation spectroscopy have been presented as data capable of distinguishing between the various expressions for the concentration dependence of the mutual diffusion coefficient.⁹ However, there has been no demonstration that this technique typically measures the correct parameter when a solution of finite concentration is used. The theory of solute diffusion presented in this work allows conclusions to be drawn regarding concentration effects. As is known,¹⁰ hydrodynamic interactions between solute molecules cause the initial decay of the first-order electric field correlation function of the scattered light to deviate from single exponential behavior even for a perfectly monodisperse solution. The magnitude of the first moment of the decay constant distribution is found to yield the mean self-diffusion coefficient of the solute molecules and the magnitude of the second moment of the distribution is calculated for

spherical molecules in terms of the solute volume fraction. Finally, the usefulness of quasielastic light-scattering measurements in relation to the problem of determining macromolecular size and shape is considered.

II. EINSTEIN DIFFUSION RELATION FOR HYDRODYNAMICALLY INTERACTING BROWNIAN PARTICLES

The probability distribution $P(\mathbf{x})$ of the position \mathbf{x} of a Brownian particle whose velocity is a stationary random function of time can be approximated by a Gaussian function provided \mathbf{x} is sampled over a timescale much longer than that required for the decay of velocity correlations. This may be understood as follows. The velocity \mathbf{v} of the center of mass of a Brownian particle which is initially at the origin satisfies the usual relation

$$\mathbf{x}(t) = \int_0^t \mathbf{v}(t') dt' . \quad (2.1)$$

If the range of integration of (2.1) is divided into a number of equal steps $\Delta t'$, then $\mathbf{x}(t)$ becomes the sum of a series of terms with equal means and equal variances. If the interval $\Delta t'$ is chosen to be sufficiently large, then adjacent terms in the series will appear to be uncorrelated and the central limit theorem can be applied to predict a limiting Gaussian form for $P(\mathbf{x})$. Such a probability distribution satisfies the diffusion equation

$$\frac{dP}{dt} = \nabla \cdot \mathbf{D} \cdot \nabla P , \quad (2.2)$$

where the tensor diffusion coefficient \mathbf{D} is the time integral of the velocity correlation function $\mathbf{R}(\tau)$:

$$\mathbf{D} = \int_0^\infty \mathbf{R}(\tau) d\tau \quad (2.3a)$$

$$= \int_0^\infty \langle \mathbf{v}(t) \mathbf{v}(t + \tau) \rangle d\tau \quad (2.3b)$$

and the angle brackets denote an ensemble average. Provided the integral in (2.3) is convergent, it attains its asymptotic value after a time of order τ_D known as the velocity relaxation time and given by

$$\tau_D = \int_0^\infty \frac{\text{tr}[\mathbf{R}(\tau)]}{\text{tr}[\mathbf{R}(0)]} d\tau , \quad (2.4)$$

where $\text{tr}(\mathbf{R})$ denotes the trace of \mathbf{R} . We note that the principle of equipartition of energy gives

$$\text{tr}[\mathbf{R}(0)] = \frac{3kT}{m} \quad (2.5)$$

for a particle of mass m in an equilibrium system, where k is Boltzmann's constant and T is the absolute temperature.

These familiar results describe the motion of a single Brownian particle which is free from interactions with other particles. However, under certain conditions they can be extended from 3 dimensions to $3N$ dimensions to describe the motion of an isolated group of N interacting, identical Brownian particles.⁶ If the velocities \mathbf{v}_i of the individual particles are themselves stationary random functions of time, then the joint velocity vector $\mathbf{V} \equiv (\mathbf{v}_1, \mathbf{v}_2, \dots, \mathbf{v}_N)$ will likewise be a stationary random function

of time provided that velocity cross correlations of the form $\langle \mathbf{v}_i(t) \mathbf{v}_j(t + \tau) \rangle$ decay on the same timescale as velocity self correlations. This will indeed be the case in situations where the individual particle velocities change as a result of random impulsive forces from molecular collisions. In such circumstances, the diffusion equation (2.2) will hold for the particles' joint probability distribution $P(\mathbf{X})$, where $\mathbf{X} \equiv (\mathbf{x}_1, \mathbf{x}_2, \dots, \mathbf{x}_N)$ represents the particles' configuration and

$$\nabla \equiv \left(\frac{\partial}{\partial \mathbf{x}_1}, \frac{\partial}{\partial \mathbf{x}_2}, \dots, \frac{\partial}{\partial \mathbf{x}_N} \right) .$$

The corresponding tensor coefficient \mathfrak{D} defined by

$$\mathfrak{D} = \int_0^\infty \langle \mathbf{V}(t) \mathbf{V}(t + \tau) \rangle d\tau \quad (2.6)$$

will have components which depend on cross correlations as well as self-correlations in the set $\{\mathbf{v}_i\}$ of individual particle velocities, and the relaxation time for \mathbf{V} will be $(m/3NkT)\text{tr}(\mathfrak{D})$.

According to the classical argument of Einstein,¹ the mobility of a Brownian particle may be related to its diffusion coefficient by considering a hypothetical equilibrium situation in which its movement under the action of an applied force \mathbf{F} is exactly opposed by the diffusive current $-\mathbf{D} \cdot \nabla P(\mathbf{x})$. The movement of a particle immersed in a low-Reynolds-number fluid conforms to the equation

$$\mathbf{v} = \mathbf{b} \cdot \mathbf{F} , \quad (2.7)$$

where the tensor mobility \mathbf{b} depends on the size and shape of the particle. If the force is derived from an externally applied potential field $\phi(\mathbf{x})$, then it is given by

$$\mathbf{F} = \nabla \phi(\mathbf{x}) \quad (2.8)$$

and the equilibrium probability density function for the particle position \mathbf{x} is given by the Boltzmann distribution

$$P(\mathbf{x}) = P_0 \exp[-\phi(\mathbf{x})/kT] , \quad (2.9)$$

where P_0 is a normalizing factor guaranteeing $\int P(\mathbf{x}) d\mathbf{x} = 1$. Under such circumstances, the flux-balance equation

$$P(\mathbf{x}) \mathbf{v} = P(\mathbf{x}) \mathbf{b} \cdot \nabla \phi(\mathbf{x}) = \mathbf{D} \cdot \nabla P(\mathbf{x}) \quad (2.10)$$

demand the relation

$$\mathbf{D} = kT \mathbf{b} \quad (2.11)$$

which can also be written

$$\int_0^\infty \langle \mathbf{v}(t) \mathbf{v}(t + \tau) \rangle d\tau = kT \mathbf{b} . \quad (2.12)$$

These results can also be extended to describe the motion of a system of N interacting identical particles alone in a fluid. Consider a hypothetical equilibrium situation in which the movement of the system through its $3N$ -dimensional configuration space under the action of forces $\mathbf{F}_1, \mathbf{F}_2, \dots, \mathbf{F}_N$ applied to respective particles is exactly opposed by the diffusive current $-\mathfrak{D} \cdot \nabla P(\mathbf{X})$. The movement of N hydrodynamically interacting particles immersed in a low-Reynolds-number fluid conforms to the equations¹¹

$$\mathbf{v}_i = \sum_{j=1}^N \mathbf{b}_{ij} \cdot \mathbf{F}_j \quad (2.13)$$

($i, j \in \{1, 2, \dots, N\}$; no summation convention implied) where the tensor mobilities \mathbf{b}_{ij} depend on the configuration of the N particles as well as the particle size and shape. The set of equations (2.13) may be summarized by

$$\mathbf{V} = \mathbf{b} \cdot (\mathbf{F}_1, \mathbf{F}_2, \dots, \mathbf{F}_N) \quad (2.14)$$

where \mathbf{b} corresponds to the matrix of mobility tensors $(\mathbf{b}_{ij})_{N \times N}$. If the forces on the particles are derived from an externally applied potential field $\Phi(\mathbf{X})$ and the mutual interaction potential $U(\mathbf{X})$ of the particles, then we may write

$$(\mathbf{F}_1, \mathbf{F}_2, \dots, \mathbf{F}_N) = \nabla[\Phi(\mathbf{X}) + U(\mathbf{X})] \quad (2.15)$$

and the equilibrium probability density function for the position \mathbf{X} of the system in configuration space is given by

$$P(\mathbf{X}) = P_0 \exp[-(\Phi(\mathbf{X}) + U(\mathbf{X}))/kT] \quad (2.16)$$

Under such circumstances, the flux-balance equation demands the relation

$$\mathfrak{D} = kT\mathbf{b} \quad (2.17)$$

from which it can be demonstrated that each of the mobilities \mathbf{b}_{ij} corresponds to the time integral of a particular velocity correlation function:

$$\int_0^\infty \langle \mathbf{v}_i(t) \mathbf{v}_j(t+\tau) \rangle d\tau = kT\mathbf{b}_{ij} \quad (2.18)$$

The meaning of this generalization of (2.11) and (2.12) must be explained.

Because the mobilities \mathbf{b}_{ij} defined in (2.13) depend on the configuration of the group of N particles, the correlation function in (2.18) is an ensemble average over all velocities \mathbf{v}_i and \mathbf{v}_j when the particles are in a fixed configuration. Other authors^{2,3,6} have assumed that the configuration \mathbf{X} of the particles is effectively constant during the time τ_D required for the integral in (2.18) to reach its asymptotic value, and in our later discussion of the data in Table I we shall find that this is a reasonable assumption in the case of macromolecular diffusion. It is apparent then that there is a value of \mathfrak{D} appropriate to every configuration \mathbf{X} and the value corresponding to a particular configuration describes the diffusion of the system through configuration space in the immediate vicinity of the point \mathbf{X} . We shall now consider how this result may be used in the description of the diffusion of molecular solutes.

III. THE FICK'S LAW DIFFUSION COEFFICIENT FOR HYDRODYNAMICALLY INTERACTING MOLECULES

In the linear thermodynamic theory of irreversible processes¹² the isothermal mutual binary diffusion of a solute (subscript s) and a solvent (subscript w) is described by the equations

$$\begin{aligned} -\mathbf{j}_s &= \Omega_{ss} \cdot \nabla \mu_s + \Omega_{sw} \cdot \nabla \mu_w \\ -\mathbf{j}_w &= \Omega_{ws} \cdot \nabla \mu_s + \Omega_{ww} \cdot \nabla \mu_w, \end{aligned} \quad (3.1)$$

where \mathbf{j}_p represents the mass flux of substance p per unit surface area in a given direction per unit time relative to the center of mass of the system and μ_p is the chemical potential per molecule of the substance p at a given point in the system. (It should be noted that other authors such as Fitts¹³ and Steele¹⁴ have expressed the chemical potential as a specific quantity (amount per gram).) For a mass-fixed frame of reference in mechanical equilibrium it can be shown¹⁵ that the four phenomenological coefficients Ω are related to one another through the equations

$$M_s \Omega_{ss} = -M_w \Omega_{sw} = -M_s \Omega_{ws} = M_w \Omega_{ww}, \quad (3.2)$$

where the M_p are molecular weights.

Expressions for the coefficients Ω are available from the linear response theory of nonequilibrium statistical mechanics¹⁴ in terms of momentum correlation functions. Given (3.2) we need specify only one coefficient:

$$\Omega_{ss} = \frac{1}{mkTV} \int_0^\infty \langle \mathbf{J}_s(t) \mathbf{J}_s(t+\tau) \rangle d\tau, \quad (3.3)$$

where m is the mass of a solute molecule,

$$\mathbf{J}_s = \int_V \sum_{i=1}^N \mathbf{p}_i \delta(\mathbf{x} - \mathbf{x}_i) d\mathbf{x} \quad (3.4)$$

is the average momentum flux of the solute and \mathbf{p}_i is the momentum of a particular solute molecule at a point \mathbf{x}_i in the system. The Dirac delta functions serve to localize N solute molecules in a volume element V , so that the value of the integral does not depend on the magnitude of V . Using the identity $\mathbf{p}_i = m\mathbf{v}_i$ where \mathbf{v}_i is the velocity of a particular molecule, after some simple rearranging (3.3) becomes

$$\Omega_{ss} = \frac{m}{kTV} \sum_{i=1}^N \sum_{j=1}^N \int_0^\infty \langle \mathbf{v}_i(t) \mathbf{v}_j(t+\tau) \rangle d\tau \quad (3.5)$$

and the angle brackets denote an ensemble average to be taken over all configurations of the molecules as well as the distribution of molecular velocities. If the movement of the solute molecules through the solvent can be described using the hydrodynamic equations (2.13), then the average over the distribution of molecular velocities can be taken first and the result (2.18) obtained previously can be substituted into (3.5) to give

$$\Omega_{ss} = \frac{m}{V} \sum_{i=1}^N \sum_{j=1}^N \langle \mathbf{b}_{ij} \rangle \quad (3.6)$$

where the angle brackets now indicate a configuration ensemble average. Making the assumption that all the solute molecules are identical and using the definition of the solute concentration $c \equiv (mN/V)$, we may follow Hertz¹⁶ and simplify Eq. (3.6) to give

$$\Omega_{ss} = c \sum_{j=1}^N \langle \mathbf{b}_{ij} \rangle \quad (3.7)$$

where the subscript i denotes a chosen reference molecule. This equation establishes the link we need between the results of linear response theory and fluid mechanics in order to adequately take account of the effect of hydrodynamic interactions on macromolecular diffusion.

It can be shown¹⁴ that in isotropic systems, the tensor coefficients Ω appearing in the phenomenological equations (3.1) are themselves isotropic and therefore each may be replaced by its eigenvalue $\Omega = \frac{1}{3} \text{tr}(\Omega)$. (The corresponding velocity correlation functions may be found by forming the scalar product of two vectors rather than the dyadic tensor). Using the Gibbs–Duhem equation and (3.2), the usual Fick's law expression for diffusive flow may be derived¹² from (3.1) if it is assumed that the chemical potential μ_s depends only on the solute concentration c . Thus

$$-j_s = D \nabla c, \quad (3.8)$$

where j_s is the flow of solute measured in the mass-fixed frame of reference and D is expressed in terms of the solute mass fraction x_s by the equation

$$D = \left(\frac{1}{1-x_s} \right) \left(\frac{\partial \mu_s}{\partial c} \right)_{T,P} \Omega_{ss}. \quad (3.9)$$

The differential of μ_s with respect to c at constant temperature and pressure depends on the solute activity coefficient and is most conveniently expressed as a virial expansion in the solute volume fraction ϕ . Equilibrium statistical mechanics can be used to calculate the coefficients of the various terms in the virial expansion, and it is found that if the only interaction potential between molecules is of the "excluded volume" type ($U(\mathbf{X})$ is infinite if any two molecules overlap, and zero otherwise) then the term of order ϕ in the expansion can be specified in terms of the ratio β_1 of the molecular self-covolume and the molecular volume¹⁷:

$$\left(\frac{\partial \mu_s}{\partial c} \right)_{T,P} = \left(\frac{\partial \mu_s}{\partial \phi} \right)_{T,P} \left(\frac{\partial \phi}{\partial c} \right)_{T,P} \quad (3.10a)$$

$$= \frac{kT}{c} (1-\phi) [1 + \beta_1 \phi + 2\beta_2 \phi^2 + \dots] \quad (3.10b)$$

For hard spheres, $\beta_1 = 8$ and its value is known for spheroids as a function of axial ratio.¹⁸ Combining (3.7), (3.9) and (3.10) we obtain

$$D = kT \left(\frac{1-\phi}{(1-x_s)} \right) [1 + \beta_1 \phi + 2\beta_2 \phi^2 + \dots] \sum_{j=1}^N \langle b_{ij} \rangle \quad (3.11)$$

where $b_{ij} = \frac{1}{3} \text{tr}(\mathbf{b}_{ij})$ and the angle brackets denote an equilibrium ensemble average over all configurations of the N molecules. In the limit of infinite dilution ($x_s, \phi = 0$) all the molecules can be considered to move independently of one another and so $\mathbf{b}_{ij} = 0$ for all $i \neq j$. A single configuration-independent mobility

$$b = \lim_{\phi \rightarrow 0} \left[\frac{1}{3} \text{tr}(\mathbf{b}_{ii}) \right] \quad (3.12)$$

suffices for the description of translational movements of freely rotating molecules and (3.11) reduces to the ideal Einstein result (2.11) allowing specification of the "molecular diffusion coefficient" D^0 where the superscript 0 is used to indicate the limit of infinite dilution. The value of b and hence D^0 may be calculated for spherical molecules using Stokes' law (with "stick" boundary conditions):

$$b = \frac{1}{6\pi\eta a}, \quad (3.13)$$

where η is the solvent viscosity and a is the molecular

radius. Analytical expressions are similarly available for the mobilities of spheroids as a function of axial ratio.¹⁹

An explicit expression for the concentration dependence of the Fick's law diffusion coefficient D may be obtained by evaluation of the sum $\sum_{j=1}^N \langle b_{ij} \rangle$ in (3.11). Performing this calculation is equivalent to obtaining an expression for the concentration dependence of the sedimentation rate of the solute. When all the solute molecules in a binary solution are subject to the same force \mathbf{F} due to gravity, the hydrodynamic equations (2.13) may be written

$$\mathbf{v}_i = \left(\sum_{j=1}^N \mathbf{b}_{ij} \right) \cdot \mathbf{F}. \quad (3.14)$$

When a single molecule is alone in the solvent fluid, (3.14) reduces to (2.7) and the molecule moves with velocity \mathbf{v}^0 where the superscript 0 is used to indicate that this is the sedimentation rate in the limit of infinite dilution. Evaluation of the ensemble average sedimentation rate $\langle \mathbf{v}_i \rangle$ of a random array of molecules is tantamount to evaluation of the isotropic tensor $\langle \sum_{j=1}^N \mathbf{b}_{ij} \rangle$ and this in turn can be identified with the sum $\sum_{j=1}^N \langle b_{ij} \rangle$. It is often convenient to determine the ensemble average sedimentation rate relative to the local center of volume (subscript V) and in this case a correction must be made in order to obtain the result relative to the local center of mass¹³:

$$\langle \mathbf{v}_i \rangle = \left(\frac{1-x_s}{1-\phi} \right) \langle \mathbf{v}_i \rangle_V \quad (3.15)$$

The quantity $\langle \mathbf{v}_i \rangle_V$ is conveniently expressed as a "virial" expansion in ϕ

$$\langle \mathbf{v}_i \rangle_V = \mathbf{v}^0 [1 - k_s \phi + \dots], \quad (3.16)$$

where the negative sign is employed to indicate the usual initial decrease in sedimentation rate as the solute volume fraction increases.²⁰ It is evident from (3.14) that the same expansion expresses $\langle \sum_{j=1}^N \mathbf{b}_{ij} \rangle$ in terms of the molecular mobility b since $\mathbf{v}^0 = b\mathbf{F}$. Thus we finally obtain an expression for $\sum_{j=1}^N \langle b_{ij} \rangle$ relative to the mass-fixed frame of reference:

$$\sum_{j=1}^N \langle b_{ij} \rangle = b \left(\frac{1-x_s}{1-\phi} \right) [1 - k_s \phi + \dots]. \quad (3.17)$$

This result may be used with (3.11) to write a general expression for the concentration dependence of the Fick's law diffusion coefficient:

$$D = D^0 [1 + (\beta_1 - k_s) \phi + O(\phi^2)]. \quad (3.18)$$

It is important to note that D is defined relative to the mass-fixed frame of reference whereas k_s expresses the decrease in the sedimentation rate measured relative to the volume-fixed frame of reference.²¹ For hard spheres with stick boundary conditions, the most recent calculation²² gives $k_s \approx 6.55$, leading to the result

$$D = D^0 [1 + 1.45 \phi + O(\phi^2)]. \quad (3.19)$$

This expression for the concentration dependence of the diffusion coefficient for spheres was first derived by Batchelor⁶ using an argument which required definition of an "effective thermodynamic force" and more recently by Felderhof⁸ who started with the Smoluchowski

equation. Our own derivation establishes the hitherto overlooked fact of vital concern for the experimentalist that (3.19) refers to the Fick's law diffusion coefficient defined in the mass-fixed frame of reference.

IV. DIFFUSION OF MACROMOLECULES AT FINITE CONCENTRATION

Fick's second law of diffusion is a direct consequence of his first law (3.8) and may be expressed

$$\frac{dc}{dt} = \nabla \cdot D \nabla c \quad (4.1)$$

It would seem that (4.1) and (2.2) are identical descriptions of diffusion in an isotropic binary system since the probability of finding a solute molecule at a point \mathbf{x} in a binary solution is proportional to the concentration $c(\mathbf{x})$ at that point. This suggests that the Fick's law diffusion coefficient D given in (3.9) and (3.11) is the time integral of the velocity self correlation function for a solute molecule in a solution of concentration c . However, as (3.5) and (3.9) show, D depends on velocity cross correlations as well as the self correlation, so the relationship between (4.1) and (2.2) requires explanation.

The derivation of (3.8) and hence (4.1) using linear response theory rests on the assumption of local equilibrium.¹⁴ This assumption requires that the timescale of changes in $\bar{N}(t)$, the time-average number of solute molecules with a small volume V surrounding a point \mathbf{x} , be much longer than the timescale of fluctuations in $N(t)$ which occur as a result of random molecular movements. The ergodic hypothesis then ensures that $\bar{N}(t)$ is also the equilibrium ensemble average of N , allowing definition of the intensive parameter c at a point \mathbf{x} in a macroscopic nonequilibrium system according to the identity $c(\mathbf{x}) \equiv (m \bar{N}(t)/V)$ where m is the mass of a solute molecule. It is to this ensemble average quantity $c(\mathbf{x})$ rather than the probability distribution $P(\mathbf{x}_i)$ of the position of a particular molecule that the phenomenological equations (3.8) and (4.1) refer. A mathematical representation of solute diffusion at infinite dilution may be achieved by considering the Brownian movements of noninteracting, identical, point particles in a random array. The diffusion equation (2.2) is satisfied for the probability distribution $P(\mathbf{x}_i)$ of each particle with an identical diffusion coefficient D^0 . Fick's laws, (3.8) and (4.1), which relate to $c(\mathbf{x})$, are also satisfied, and the same coefficient D^0 defined in terms of the particles' velocity self-correlation function obtains.

At finite concentration, the situation is much more complex. To simplify our discussion we shall consider the Brownian motion of macromolecules whose instantaneous movements are governed by the hydrodynamic equations (2.13) and whose configuration is effectively constant on a timescale of the order of the velocity relaxation time τ_D . The molecular radius provides an appropriate scale for the measurement of relative displacements which cause significant changes in the values of the mobilities \mathbf{b}_{ii} . If we observe the motion of a single reference macromolecule (subscript i) on a time-scale longer than τ_D but shorter than the time required for a significant displacement relative to its neighbors,

then we will find that the probability distribution $P(\mathbf{x}_i)$ of its center of mass will assume a limiting Gaussian form and $P(\mathbf{x}_i)$ will satisfy the diffusion equation (2.2) with the molecular self-diffusion coefficient defined by

$$\mathbf{D}_i = kT \mathbf{b}_{ii} \quad (4.2)$$

Because the tensor mobility \mathbf{b}_{ii} depends on the configuration \mathbf{X} of the molecules in the neighborhood of the reference molecule, \mathbf{D}_i will change slowly as the molecules undergo diffusive displacements relative to one another. Brownian motion provides the means whereby all configurations \mathbf{X} of a system of particles, and thus all values of \mathbf{D}_i , are realized with probabilities specified by the Boltzmann distribution (2.16). The ensemble average $\langle \mathbf{D}_i \rangle$ is an isotropic tensor whose scalar magnitude $D_s = \frac{1}{3} \text{tr}(\langle \mathbf{D}_i \rangle)$ may be called the "self-diffusion coefficient of the solute." In the case of uncharged, hard, spherical molecules, D_s may be calculated^{5,6,8} in terms of the solute volume fraction ϕ :

$$D_s = kT \langle b_{ii} \rangle \quad (4.3a)$$

$$= D^0 [1 - 1.83\phi + 0(\phi^2)] \quad (4.3b)$$

where D^0 is the diffusion coefficient in the limit of infinite dilution and applies to both (4.1) and (2.2). It is apparent that D_s is not appropriate to the description of macroscopic diffusive flow since it is calculated without reference to the effects of thermodynamic nonideality or cross correlations of molecular velocities.¹⁶ There has been a tendency in the literature⁵ to identify the solute self-diffusion coefficient D_s with the Fick's law coefficient D and it has thus been difficult to interpret measurements of the concentration dependence of the diffusion coefficient.

In the case of experiments which allow observation of the macroscopic flow of solute down a concentration gradient, it is the Fick's law coefficient which is determined. However, care must be taken in the use of (3.18) because flows are usually measured relative to a laboratory volume-fixed frame of reference. Conversion to the mass-fixed frame may be effected using (3.15) which requires

$$D = \frac{(1-x_s)}{(1-\phi)} D_V \quad (4.4)$$

where D_V is the coefficient appropriate to the expression of Fick's law (3.8) in the frame of reference of the laboratory,

$$-(\mathbf{j}_s)_V = D_V \nabla c \quad (4.5)$$

and $(\mathbf{j}_s)_V$ is the solute flow in the same reference frame.

In the case of experiments which rely on the technique of quasielastic light scattering, the correct interpretation of measurements pertaining to solutions of macromolecules at finite concentration is not obvious. The justification for the claim that this technique may be used to measure a diffusion coefficient is provided by Brownian motion theory and fluctuation theory. The apparent equivalence of (2.2) and (4.1) in the limit of infinite dilution has led to the conclusion that at finite concentrations the Fick's law diffusion coefficient defined in the laboratory frame of reference is appropriate to the description

of the Brownian motion of individual molecules and is thus determined using the light-scattering technique. However, as we shall see presently, Brownian motion theory indicates that it is the solute self-diffusion coefficient which may be determined, since laser light-scattering spectroscopy depends on the observation of molecular movements which occur on a relatively short timescale. The belief that it is the Fick's law coefficient which is obtained⁹ cannot be justified using the theory of concentration fluctuations and Onsager's regression hypothesis because the solute concentration $c(\mathbf{x})$ at a point \mathbf{x} in a binary solution is undefined on the timescale relevant to the observations.

The phase space operator corresponding to the solute mass density or concentration is defined by the equation

$$\rho_s(\mathbf{x}) = m \sum_{i=1}^N \delta(\mathbf{x} - \mathbf{x}_i), \quad (4.6)$$

where it is assumed that there are N solute molecules of mass m in a system of volume V . For a system in which equilibrium prevails, the concentration at a point is given by the definition

$$c(\mathbf{x}) = \langle \rho_s(\mathbf{x}) \rangle \quad (4.7a)$$

$$= \overline{\rho_s(\mathbf{x}, t)}, \quad (4.7b)$$

where the angle brackets denote an equilibrium ensemble average and the equivalence of (4.7a) and (4.7b) is ensured by the ergodic hypothesis. This definition of $c(\mathbf{x})$ identifies the concentration which is ordinarily measurable and which appears in Fick's laws of diffusion. Quasielastic light scattering on the other hand probes the temporal properties of $\rho_s(\mathbf{x}, t)$ and does not depend primarily on changes in the average value of this operator. The probability distribution for the position of a single molecule may be expressed in terms of one of the delta functions appearing in (4.6):

$$P(\mathbf{x}_i) = \langle \delta[\mathbf{x} - \mathbf{x}_i(t)] \rangle \quad (4.8)$$

As we have already seen, if the average is taken over an ensemble of systems which are initially in some configuration \mathbf{X} and the timescale of observation is suitably short, then $P(\mathbf{x}_i)$ satisfies (2.2) with the coefficient \mathbf{D}_i and we have a description of molecular self diffusion. However, if the angle brackets in (4.8) are taken to denote an equilibrium ensemble average (4.6) and (4.7a) may be used to obtain:

$$c(\mathbf{x}) = m \sum_{i=1}^N P(\mathbf{x}_i) \quad (4.9)$$

which, upon substitution into (4.1) gives

$$\sum_{i=1}^N \frac{dP(\mathbf{x}_i)}{dt} = \sum_{i=1}^N \nabla \cdot D \nabla P(\mathbf{x}_i). \quad (4.10)$$

If the diffusion of a solute is observed on a sufficiently long timescale, then the velocities of individual molecules are to a good approximation, independent stationary random functions of time. Thus, the probability distributions $P(\mathbf{x}_i)$ are independent Gaussian functions and (4.10) may be separated into N equations so that each of the $P(\mathbf{x}_i)$ satisfies (2.2) with an identical coefficient D . This describes what is commonly called "tracer diffusion," a phenomenon which may be observed

by measuring the macroscopic displacements of labeled solute molecules in a system of uniform chemical composition. The timescale for the observation of tracer diffusion must be longer than the time taken for a single molecule to attain a root-mean-square displacement of the order of \bar{r} , the mean separation of solute molecules. This corresponds to the time taken for the molecules in the neighborhood of a point \mathbf{x} to sample configurations representative of an equilibrium ensemble. It is also the timescale required for the decay of velocity self correlations which persist as the configuration of the solute molecules in the neighborhood of a reference molecule slowly changes. We assumed previously that these correlations decayed on the very much shorter timescale τ_D . This was a reasonable approximation which has provided an accurate description of short timescale molecular diffusion and even allowed us to calculate D , however it is too restrictive when we wish to achieve an understanding of tracer diffusion.

V. LASER LIGHT-SCATTERING OBSERVATIONS OF MACROMOLECULAR DIFFUSION

When laser light is incident on a solution of macromolecules, the measured scattered intensity falling on a fixed detector fluctuates with time due to the Brownian motion of the macromolecular scattering centers in the coherent electromagnetic field comprising the scattering volume. The quantity of primary experimental importance is the modulus of the temporal autocorrelation function $g(\mathbf{K}, \tau)$ of the scattered electric field. Here τ is the correlation delay time and the magnitude of the scattering vector \mathbf{K} is given by

$$K = (4\pi/\lambda)n \sin(\theta/2), \quad (5.1)$$

where λ is the wavelength of the light *in vacuo*, n is the refractive index of the solution and θ is the scattering angle. It may be readily shown⁹ that for light scattered by a volume containing N freely-rotating solute macromolecules which are spherically symmetric or small compared to λ or both, the modulus of the normalized electric field correlation function is given by

$$|g^{(1)}(\mathbf{K}, \tau)| = \frac{F_1(\mathbf{K}, \tau)}{F_1(\mathbf{K}, 0)}, \quad (5.2)$$

where $F_1(\mathbf{K}, \tau)$ is the correlation function of the K th spatial Fourier component of the phase space operator corresponding to the particle number density²³:

$$F_1(\mathbf{K}, \tau) = \frac{1}{N} \sum_{i=1}^N \sum_{j=1}^N \langle \exp[-i\mathbf{K} \cdot \mathbf{x}_i(0)] \exp[i\mathbf{K} \cdot \mathbf{x}_j(\tau)] \rangle \quad (5.3)$$

If the molecules are identical and noninteracting then¹⁰ $F_1(\mathbf{K}, 0) \approx 1$ and

$$F_1(\mathbf{K}, \tau) = N \exp(-\Gamma\tau) \quad (5.4)$$

where the decay constant Γ is given by

$$\Gamma = K^2 D^0 \quad (5.5)$$

and D^0 is the molecular diffusion coefficient.

In the derivation of (5.4) it is assumed that the probability distribution $P(\mathbf{x}_i)$ of each molecule's position is Gaussian and therefore satisfies (2.2) with the coefficient D^0 . If the particles are not all identical but the

TABLE I. Diffusion of macromolecules. These data refer to reported quasielastic light-scattering experiments using the enzyme lysozyme²⁵ and synthetic polystyrene spheres.²⁶ The magnitude of the molecular diffusion coefficient D^0 refers to a temperature of 293 K and the quoted coherence times assume use of laser light of wavelength 488 nm.

Parameter		Unit	Approximate value	
			lysozyme	polystyrene spheres
Particle mass	m	g	2.4×10^{-20}	6.9×10^{-17}
Particle radius	R	nm	1.8	25.0
Diffusion coefficient	D^0	cm^2s^{-1}	1.0×10^{-6}	8.6×10^{-8}
Velocity relaxation time	τ_D	s	2.0×10^{-13}	1.5×10^{-10}
Displacement time	τ_I	s	1.6×10^{-8}	3.6×10^{-5}
Coherence time	τ_c	s	1.7×10^{-5}	2.0×10^{-4}
Concentration	c	g l^{-1}	20.0	1.0
Mean particle separation	\bar{r}	nm	11.0	230.0

probability distributions of their individual positions are independent Gaussian functions, then (5.4) can be generalized to give

$$F_1(K, \tau) = N \int f(\Gamma) \exp(-\Gamma\tau) d\Gamma, \quad (5.6)$$

where $f(\Gamma)$ is the normalized distribution of decay constants corresponding to the distribution of molecular diffusion coefficients D_i^0 . The method of cumulants discussed by Koppel²⁴ may be used to obtain information about $f(\Gamma)$ from $F_1(K, \tau)$ and this method of data analysis has found application where quasielastic light-scattering measurements have been made using polydisperse samples. We shall discuss the application of (5.6) to the laser light-scattering observation of monodisperse samples in which the diffusion coefficients of the individual macromolecules depend on their configuration relative to their neighbors in the solution.

Suppose that a quasielastic light-scattering experiment using a dilute macromolecular solution is conducted in which $|g^{(1)}(K, \tau)|$ is determined for a range of values of τ which are much longer than the velocity relaxation time τ_D , but shorter than the time required for the molecules' configuration X to change to such an extent that any of the tensor mobilities b_{ij} governing the motion of single molecules is significantly altered. It is evident from the data in Table I that for macromolecules covering a broad range of sizes, the time τ_I required for the molecules to attain a root-mean-square diffusive displacement equal to their radius is much greater than τ_D , and thus the proposed timescale for τ is well defined. It is also evident that the determination of $|g^{(1)}(K, \tau)|$ for the specified range of values of τ is a practical possibility at least in the case of relatively large macromolecules, since the coherence time

$$\tau_c = 1/\Gamma \quad (5.7)$$

which indicates the observational timescale, may be

chosen to be of the same order as τ_I and then the desired information is contained in the initial decay of $|g^{(1)}(K, \tau)|$.²⁷ Under such circumstances, $F_1(K, \tau)$ is a sum of exponentials corresponding to a distribution of decay constants

$$\Gamma_i = K \cdot D_i \cdot K, \quad (5.8)$$

where D_i is the self-diffusion coefficient for a given molecule and is defined by (4.2) and (2.18). Because the velocity v_i of any molecule is, to a good approximation, a stationary random function of time on the time-scale of observation, the probability distribution of the molecule's position $P(x_i)$ is Gaussian and satisfies (2.2) with the coefficient D_i . However, because $kTb_{ij} \neq 0$ in general, the distribution functions $P(x_i)$ and $P(x_j)$ for different molecules are not independent. We shall now show that correlations between the positions x_i and x_j of distinct particles make no direct contribution to $F_1(K, \tau)$ in the cases of current interest.

The spatial Fourier transform of $F_1(K, \tau)$ is the van Hove space-time correlation function

$$G(x, t) = \frac{1}{(2\pi)^3} \int \exp(-iK \cdot x) F_1(K, \tau) dK \quad (5.9a)$$

$$= \frac{1}{N} \sum_{i=1}^N \sum_{j=1}^N \langle \delta[x + x_j(0) - x_i(t)] \rangle \quad (5.9b)$$

Consider the contribution to $G(x, t)$ due to the Brownian motion of a single solute macromolecule (subscript i):

$$G_i(x, t) = \frac{1}{N} \sum_{j=1}^N \langle \delta[x + [x_j(0) - x_i(0)] - [x_i(t) - x_i(0)] \rangle \rangle \quad (5.10)$$

The only time dependent term in the delta function in (5.10) is $x_i(t) - x_i(0)$. Thus, each term of the sum (5.10) defining $G_i(x, t)$ is a Gaussian function satisfying (2.2) with a diffusion coefficient D_i . Fourier inversion of (5.9) then gives the desired result:

$$F_1(K, \tau) = \sum_{i=1}^N \int \exp(iK \cdot x) G_i(x, t) dx \quad (5.11a)$$

$$= \sum_{i=1}^N \exp(-\Gamma_i \tau) \quad (5.11b)$$

which is equivalent to (5.6).

It is now evident that if the method of cumulants²⁴ is used to analyze the initial decay of $|g^{(1)}(K, \tau)|$ for the laser light scattered by a dilute solution of macromolecules, then the solute self-diffusion coefficient D_s will be determined. Thus, in the case of uncharged, spherical macromolecules, we expect to observe concentration dependence as described by (4.3b) whereas mass-flow and tracer methods are anticipated to give results described by (3.19) and (4.4).²⁸

Because the initial decay of the first order electric field correlation function for a solution of macromolecules is determined by a distribution $f(\Gamma)$ of decay constants, it will deviate from single exponential form even when the scattering sample is a dilute, monodisperse solution. The normalized variance q of the decay constant distribution is defined by

$$q = \frac{1}{\bar{\Gamma}^2} \int f(\Gamma) (\Gamma - \bar{\Gamma})^2 d\Gamma \quad (5.12a)$$

$$= \frac{1}{N\Gamma^2} \sum_{i=1}^N (\Gamma_i - \bar{\Gamma})^2 \quad (5.12b)$$

where $\Gamma = \langle \mathbf{K} \cdot \mathbf{D}_i \cdot \mathbf{K} \rangle = K^2 D_s$. In the case of a dilute suspension of uncharged spheres, it is possible to calculate q in terms of the solute volume fraction ϕ by evaluating $\langle (\mathbf{K} \cdot \mathbf{D}_i \cdot \mathbf{K} - K^2 D_s)^2 \rangle$. Details of this calculation are given in Appendix A where the following result is obtained:

$$q = 0.046\phi + O(\phi^2). \quad (5.13)$$

As expected, $q=0$ in the limit of infinite dilution. When it is considered that (5.13) applies in the limit of small ϕ and that measurements of q typically have an absolute error of ± 0.01 , it appears that the deviation of the initial decay of $|g^{(1)}(\mathbf{K}, \tau)|$ from single exponential behavior will prove to be experimentally insignificant for hydrodynamically interacting, uncharged spheres.

The conclusions we have reached in this section are in general agreement with those of Ackerson,¹⁰ however our analysis indicates that the concentration dependence of the diffusion coefficient determined by quasielastic light-scattering cannot be specified solely in terms of the static structure factor $F_1(\mathbf{K}, 0)$. Equation (4.6) of Ackerson¹⁰ may be used to make minor corrections to our (4.3b) and (5.13) so that the \mathbf{K} -dependence of $F_1(\mathbf{K}, 0)$ is taken into account.

VI. DISCUSSION

The main predictive thrust of this work is directed toward different determinations of the concentration dependence of the diffusion coefficient for suspensions of hard spheres in a low-Reynolds-number fluid. Whether an experimental system can be found which adequately approximates to these theoretical requirements and thus allows for an empirical test of (3.19) and (4.3b) is an open question. Although molecular dynamic simulation experiments²⁹ have demonstrated a very close resemblance between the interaction of a solute molecule with surrounding solvent molecules and the interaction of a rigid particle with a fluid continuum, the differences between these two model systems may prove to be of vital importance in relation to the interpretation of proposed experiments. Our neglect of possible electrostatic and dispersion forces between the solute molecules is also worthy of attention, but the predictions can be readily modified to take account of these in specific cases provided any change in the potential energy of a given molecule on the timescale τ_D is small compared to kT .²⁷ Satisfaction of this condition is necessary to guarantee the validity of the results of Sec. II.

The characterization of solution transport processes (diffusion, sedimentation, viscosity) is an established way of obtaining information about the tertiary structure of biological macromolecules *in vitro*. Methods for determining the size and shape of model hydrodynamic particles corresponding to macromolecular solutes have been described by Scheraga and Mandelkern,³⁰ Ogston³¹ and Jeffrey, Nichol, Turner, and Winzor.³² Because the hydrodynamic particles comprise the solute macromolecules and any bound solvent, they generally occupy a vol-

ume fraction ϕ' of the solution which is greater than the solute volume fraction ϕ .³³ It is thus necessary to eliminate the ratio ϕ'/ϕ by measurement of two independent parameters before a quantity which depends only on the shape of the hydrodynamic particle can be specified. Choice of a model shape then allows estimation of ϕ'/ϕ by back calculation. Assuming that the molecular weight M of the solute is known and its partial specific volume \bar{v} has been determined at a number of concentrations and found to be constant, the most easily measurable parameters are the molecular diffusion coefficient D^0 , the molecular sedimentation coefficient s^0 and the intrinsic viscosity $[\eta]$.³⁰ Other useful parameters are the first order coefficient k_s for the concentration dependence of the sedimentation coefficient³¹ and the second virial coefficient β_1 .³²

The result (3.18) for the first order concentration dependence of the Fick's law diffusion coefficient D determined in the mass-fixed frame of reference,

$$k_D = \beta_1 - k_s \quad (6.1)$$

proves that the redundancy in the independent measurement of the parameters M , D^0 and s^0 apparent in the Svedberg equation, carries over to the measurement of the concentration dependence of the chemical potential, diffusion coefficient and sedimentation coefficient defined in nonequilibrium thermodynamics. However because the self-diffusion coefficient D_s is not identical to D except in the limit of infinite dilution, determination of the first order coefficient k_D' for the concentration dependence of D_s allows access to a further independent variable. Furthermore, a simple argument³⁰⁻³² indicates that the dimensionless quantity

$$\xi = \frac{\eta}{kT} \left(\frac{162\pi^2}{N^0} \right)^{1/3} (M\bar{v}k_D')^{1/3} D^0 \quad (6.2)$$

depends only on the shape of the macromolecular hydrodynamic particle. Here, η is the solvent viscosity and N^0 is Avogadro's number. It will be possible to assess the potential usefulness of this quantity, which depends on the technique of quasielastic light scattering for its measurement, when the concentration dependence of D_s has been calculated for particles of a variety of non-spherical geometries.

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APPENDIX A. NORMALISED VARIANCE OF THE MOLECULAR SELF-DIFFUSION COEFFICIENT FOR A DILUTE SUSPENSION OF HARD SPHERES

When a large number of molecules is distributed more or less randomly in space and they occupy a small fraction ϕ of the available volume, the effect of weak intermolecular interactions on the thermodynamic and transport properties of the system can often be conveniently

expressed in the manner of a "virial expansion" in powers of ϕ . The magnitude of terms of order ϕ^N in the expansion may be calculated by considering the interactions within groups of $N+1$ molecules in the system. Thus, the concentration dependence of D and D_s for spheres as given by (3.19) and (4.3b) is calculated by considering hydrodynamic interactions between pairs of spheres. Here, we shall consider the proof of (5.13) which gives the first term in the virial expansion for the normalized variance q of the molecular self-diffusion coefficient distribution for a dilute solution of uncharged, hard, spherical molecules.

Of primary importance in the calculation is the distribution function which expresses the probability of finding another molecule at a distance between r and $r+dr$ from a reference molecule. In the case of a dilute solution of rigid spherical molecules whose only interaction is of the excluded-volume type, this distribution function is given correct to order ϕ by the formula

$$\left. \begin{aligned} p(r)dr &= 4\pi r^2 n dr & r \geq 2a \\ &= 3\phi \alpha^2 d\alpha & \alpha \geq 2 \\ p(r) &= 0 & r < 2a \end{aligned} \right\} \quad (A1)$$

where n is the molecular number density, a is the radius of the spheres and $\alpha = (r/a)$. Other interaction potentials $U(X)$ may be taken into account by modification of (A1.1) using the Boltzmann distribution (2.16) with $\phi(X)=0$.

When two equal spheres of radius a with centers at \mathbf{x}_1 and \mathbf{x}_2 are acted on by separate forces \mathbf{F}_1 and \mathbf{F}_2 and are otherwise alone in a fluid which is at rest at infinity, provided there is zero applied torque on each sphere¹¹ and the inertia forces on both the fluid and spheres are negligible, the velocities \mathbf{v}_1 and \mathbf{v}_2 of the two spheres are given by the Eqs. (2.13):

$$\mathbf{v}_1 = \mathbf{b}_{11} \cdot \mathbf{F}_1 + \mathbf{b}_{12} \cdot \mathbf{F}_2 \quad (A2a)$$

$$\mathbf{v}_2 = \mathbf{b}_{21} \cdot \mathbf{F}_1 + \mathbf{b}_{22} \cdot \mathbf{F}_2 \quad (A2b)$$

and the mobility tensors \mathbf{b}_{ij} depend on the separation vector $\mathbf{r} = \mathbf{x}_2 - \mathbf{x}_1$ through relations of the form:

$$\mathbf{b}_{ij}(\mathbf{r}) = \frac{1}{6\pi\eta a} \left\{ A_{ij}(\mathbf{r}) \frac{\mathbf{r}\mathbf{r}}{r^2} + B_{ij}(\mathbf{r}) \left(\mathbf{I} - \frac{\mathbf{r}\mathbf{r}}{r^2} \right) \right\} \quad (A3)$$

($i, j \in \{1, 2\}$; no summation convention implied) where η is the viscosity of the fluid, \mathbf{I} is the unit isotropic tensor and we have assumed that stick boundary conditions obtain. The asymptotic ($r \gg a$ or $\alpha \gg 1$) forms of $A_{ij}(\mathbf{r})$ and $B_{ij}(\mathbf{r})$ are given by

$$A_{ij}(\mathbf{r}) = 1 - \frac{15}{4\alpha^4} + O(\alpha^{-6}) \quad (A4a)$$

$$B_{ij}(\mathbf{r}) = 1 + O(\alpha^{-6}) \quad (A4b)$$

and the values of these coefficients have been accurately calculated for a few values of α of order unity. The relevant data have been assembled by Batchelor.⁶

It is evident from (A3) and (A4) that the self-mobility tensor \mathbf{b}_{ii} for a sphere deviates significantly from its infinite dilution value of $(1/6\pi\eta a)\mathbf{I}$ only when there is another sphere within a range of a few radii. For a dilute suspension, the probability of such an event is of

order ϕ , and the probability that a third sphere is close to any pair of close neighbors is of order ϕ^2 , which is much smaller. It is thus possible to evaluate the ensemble average of suitable functions of \mathbf{b}_{ii} correct to order ϕ by considering an isolated pair of spheres whose relative positions are governed by the distribution function (A1). The molecular self-diffusion coefficient \mathbf{D}_i defined by (4.2) and (2.18) is proportional to \mathbf{b}_{ii} and quasi-elastic light scattering is sensitive to the derived scalar Γ given in (5.8).

The quantity q defined in (5.12) may be calculated by evaluating $\langle (\mathbf{K} \cdot (\mathbf{D}_i - D_s \mathbf{I}) \cdot \mathbf{K})^2 \rangle$. If the coordinate system is fixed so that the x axis is in the direction of \mathbf{K} , then the above expression reduces to $K^4 \langle (D_i^{xx} - D_s)^2 \rangle$ where D_i^{xx} is the xx element of \mathbf{D}_i . The problem is further simplified by the fact that the required quantity may be calculated correct to order ϕ by evaluating $K^4 \langle (D_i^{xx} - D^0)^2 \rangle$. We then have:

$$q = \frac{\langle (D_i^{xx} - D^0)^2 \rangle}{(D^0)^2} + O(\phi^2) \quad (A5)$$

If the separation vector \mathbf{r} between a reference sphere and a nearby neighbor has spherical coordinates (r, θ, ψ) in the chosen frame of reference, then

$$D_i^{xx}(\mathbf{r}) = \frac{kT}{6\pi\eta a} [A_{ii}(\mathbf{r}) \cos^2 \theta + B_{ii}(\mathbf{r}) \sin^2 \theta] \quad (A6a)$$

$$= D^0 [(A_{ii}(\mathbf{r}) - 1) \cos^2 \theta + (B_{ii}(\mathbf{r}) - 1) \sin^2 \theta + 1] \quad (A6b)$$

Oriental averaging of $(D_i^{xx} - D^0)^2$ may be performed by giving each direction a weighting $(1/4\pi) \sin \theta d\theta d\psi$ within the ranges $0 < \theta \leq \pi$ and $0 < \psi \leq 2\pi$. The quantity obtained after this step appears in the final expression for q :

$$q = \int_{2a}^{\infty} \frac{1}{15} [3(A_{ii}(\mathbf{r}) - 1)^2 + 8(B_{ii}(\mathbf{r}) - 1)^2 + 4(A_{ii}(\mathbf{r}) - 1)(B_{ii}(\mathbf{r}) - 1)] p(r) dr + O(\phi^2) \quad (A7)$$

Evaluation of this integral using (A1), (A4) and the data provided by Batchelor⁶ gives (5.13).

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