Single-Particle Tracking: Brownian Dynamics of Viscoelastic Materials

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ABSTRACT   A unifying theoretical framework for analyzing stochastic data from single-particle tracking (SPT) in viscoelastic materials is presented. A generalization of the bead-spring model for linear polymers is developed from a molecular point of view and from the standpoint of phenomenological linear viscoelasticity. The hydrodynamic interaction in the former is identified as the dashpots in the latter. In elementary terms, the intimate correspondence between time-correlation of the fluctuation measurements and transient relaxation kinetics after perturbation is discussed, and the central role of the fluctuation-dissipation relation is emphasized. The work presented here provides a bridge between the microscopic and the macroscopic views of linear viscoelastic biological materials, and is applicable to membrane protein diffusion, linear DNA chain dynamics, and mechanics of intracellular cytoskeletal networks.

INTRODUCTION

Recently developed microrheology based on tracking the movements of individual Brownian particles provides a novel approach for studying viscoelastic materials and biological tissues at a subcellular and molecular level (Gittes et al., 1997; Mason et al., 1997). The methodology belongs to a class of optical techniques that quantitatively follow the movement of small noninvasive optical markers with high spatial resolution (less than nanometers), known as single-particle tracking (SPT) among many other names (Geerts et al., 1987; Gelles et al., 1988; Gross and Webb, 1988; Qian et al., 1991b; Amblard et al., 1996; Peters et al., 1998; Qian and Elson, 1999; also see a review by Saxton and Jacobson, 1997, on its wide applications to cell membrane dynamics). Interpreting the quantitative yet stochastic data demands a theoretical framework. Two parallel approaches, a molecular and a phenomenological, are possible. In this paper I develop, on the one hand, a molecular approach based on a natural extension of the simple discrete polymer theory, which treats a polymer gel as beads connected by springs in an aqueous solution with hydrodynamic interactions (Doi and Edwards, 1986). The phenomenological treatment of linear viscoelasticity in mechanics, on the other hand, is based on the concept of memory functions known as creep and relaxation (Fung, 1965; Ferry, 1980). Combining these two approaches, I show that the creep function in the phenomenological theory can be derived in terms of the molecular model. Consequently, the stochastic movement of a particle in a viscoelastic material can be analyzed either as a Brownian motion of one particle in an N-particle system with spatially correlated white noise (N-dimensional Langevin equation), or a single particle with a non-white noise (generalized Langevin equation) where the noises represent the random collisions between the beads and the solvent molecules (Brownian forces). Based on this approach, the present work provides a unifying theory for SPT, demonstrates a correspondence between the time-correlation of the novel fluctuation measurements and the traditional relaxation kinetics after perturbation (Onsager's hypothesis of linear irreversibility), establishes an equivalence between the Onsager's hypothesis and the fluctuation-dissipation relation, and develops a bridge between the microscopic and the macroscopic views of linear viscoelasticity. The approach in this work is applicable to SPT in viscous fluids with and without drift (Qian et al., 1991b), SPT of single polymer chains (Qian and Elson, 1999), and SPT in polymer gel networks.

The paper is organized as follows. In the next two sections we develop our main analyses based on the molecular approach and the phenomenological approach, respectively. It will be shown that fluctuation-dissipation relation leads to Onsager's hypothesis, and vice versa. The fourth section deals with SPT measurements in viscoelastic liquids (complex fluids) in which the Brownian motion is nonstationary. Hence, mean-square displacement rather than the time correlation function has to be introduced. The last section provides a comprehensive discussion on SPT, its theories, applications, relations with other work, and its mathematical foundation.

MOLECULAR VISCOELASTICITY: SPRINGS AND DASHPOTS

The simplest molecular theory of viscoelasticity assumes that the microscopic dynamics of a polymer network is described by a system of spherical Brownian particles in an aqueous solution, connected by springs and interacting with each other via hydrodynamic interactions (Doi and Edwards, 1986). Let the element k_{ij} of a matrix $\mathbf{K}$ be the stiffness constant of the spring connecting beads i and j, and h_{ij} of a matrix $\mathbf{H}$ be the hydrodynamic interaction between beads i and j. $\mathbf{H}$ is known as the mobility matrix. The Newton's equation for overdamped motion of a system of

particles in viscous solution, therefore, is

$$\frac{d\mathbf{X}}{dt} = \mathbf{H}(-\mathbf{KX} + \mathbf{\Sigma F}) \tag{1}$$

where $\mathbf{X} = (x_1, x_2, \ldots, x_N)^T$ are the coordinates of beads 1, 2, ..., N. $\mathbf{F} = (\mathbf{f}_1, \mathbf{f}_2, \ldots, \mathbf{f}_N)^T$ are white noise like random forces satisfying $\langle \mathbf{f}_i(t)\mathbf{f}_j(t') \rangle = \delta_{ij}\delta(t' - t)$. $\mathbf{\Sigma\Sigma}^T$ is a covariance matrix, to be discussed later, characterizing the spatial correlation between the random forces. The random forces represent the incessant random collisions between the beads and the solvent molecules. A self-contained brief discussion of random forcing in Brownian dynamics can be found in Klapper and Qian (1998). For more general discussions see Fox (1978) and Kubo et al. (1978).

In general symmetric matrices $\mathbf{K}$ and $\mathbf{H}$ are functions of $\mathbf{X}$, and Eq. 1 is nonlinear. For linear springs, $\mathbf{K}$ is a constant matrix, and in the case of a single linear polymer molecule, the $\mathbf{K}$ is tridiagonal. Zimm has given a simplified, approximate $\mathbf{H}$ which is also independent of $\mathbf{X}$. This treatment of $\mathbf{H}$ led to the well-known theory for non-free-draining polymer dynamics (Zimm, 1956). In general, the matrices are full for polymer networks. Linearized Eq. 1 with constant $\mathbf{K}$ and $\mathbf{H}$ corresponds to linear viscoelasticity, as we shall show.

Rewrite Eq. 1 into

$$\mathbf{H}^{-1}\frac{d\mathbf{X}}{dt} = -\mathbf{KX} + \mathbf{\Sigma F} \tag{2}$$

we see that this is the equation for a set of springs and dashpots (Fung, 1965; Ferry, 1980). The element c_{ij} of matrix $\mathbf{H}^{-1}$ now is the frictional coefficient of the dashpot connecting points x_i and x_j. The hydrodynamic interaction in the molecular theory, therefore, is directly related to the dashpot in the traditional mechanical models.

The nontrivial issue concerning Eq. 1 is the covariance matrix $\mathbf{\Sigma\Sigma}^T$, which has to be specifically related to $\mathbf{H}$. This relation, known as the fluctuation-dissipation relation, reflects that the Brownian forces and the frictions are two different manifestations of a single physical entity: the solvent. The larger the hydrodynamic interaction between beads i and j, the stronger the correlation between the random forces acting upon them. Equilibrium thermodynamics requires the fluctuation-dissipation relation as (Fox, 1978; Klapper and Qian, 1998):

$$\mathbf{\Sigma\Sigma}^T = 2k_B T\mathbf{H}^{-1}, \tag{3}$$

and furthermore

$$\langle x_i(t)x_j(t') \rangle = \tfrac{1}{2}(e^{-|t'-t|\mathbf{HK}}\mathbf{K}^{-1}\mathbf{\Sigma\Sigma}^T\mathbf{H})_{ij}$$

$$= k_B T(e^{-|t'-t|\mathbf{HK}}\mathbf{K}^{-1})_{ij}, \tag{4}$$

where $\mathbf{X}(t)$ is a 3N-dimensional Gaussian-Markovian (Ornstein-Uhlenbeck) process (Wang and Uhlenbeck, 1945; van Kampen, 1997).

In a real SPT experiment, one usually only measures the Brownian motion of one of the N beads in the above system (Qian and Elson, 1999). Without loss of generality, let's denote the position of the optical marker as x_N; also, drop the vector notations for x and $\mathbf{f}$ in the remainder of the paper to avoid cluttering. If one applies an external force $f_N(t)$ *at* x_N, then the solution of Eq. 1 yields the transient response of $x_N(t)$:

$$x_N(t) = \int_0^t (e^{-(t-s)\mathbf{HK}}\mathbf{H})_{NN}f_N(s)ds$$

$$= \int_0^t c(t-s)\frac{df_N}{dt}(s)ds, \tag{5}$$

where the second equality is due to integration by parts, and we have

$$c(t) = c(\infty) - (e^{-t\mathbf{HK}}\mathbf{K}^{-1})_{NN}, \quad (t \geq 0). \tag{6}$$

$c(t)$ is known as the creep function in linear viscoelasticity (Fung, 1965). It consists of N exponential decays. $1/c(\infty)$ is the equilibrium spring constant (see below).

We are now in a position to demonstrate an important relationship in nonequilibrium statistical physics (Callen, 1965; Kubo, 1986). According to Onsager's hypothesis (1931), a thermal system's nonstationary response to an external perturbation (Eq. 6) is linearly related to the stationary thermal fluctuations spontaneously produced in the system in the absence of external perturbation. Note that the latter is given in Eq. 4 for our polymer network:

$$\langle x_N(0)x_N(t) \rangle = k_B T(e^{-t\mathbf{HK}}\mathbf{K}^{-1})_{NN}, \quad (t \geq 0). \tag{7}$$

Hence, there is indeed a linear relation between the fluctuation correlation in Eq. 7 and the creep function in Eq. 6. This relation can be justified in a more general setting with a microscopic theory for irreversible thermodynamics using a projection operator (Zwanzig, 1961; Fox, 1978; Kubo et al., 1978), but the above analysis provides a simple and elementary illustration in the context of linear viscoelasticity. It is shown that the fluctuation-dissipation relation (Eq. 3) leads to the validation of Onsager's hypothesis.

MECHANICAL VISCOELASTICITY: CREEP AND RELAXATION FUNCTIONS

The SPT analysis from the phenomenological linear viscoelasticity will now be developed (Fung, 1965; Ferry, 1980). According to this theory, the linear force-displacement relation with memory can be expressed as

$$F(t) = \int_0^t k(t-\tau)\frac{dx}{dt}(\tau)d\tau \tag{8}$$

or its equivalence in the inverse form

$$x(t) = \int_0^t c(t - \tau) \frac{dF}{dt}(\tau)d\tau \qquad (9)$$

where F is force and x is displacement. Function $k(t)$ is called the relaxation function and $c(t)$ is called the creep function. They are characteristic functions of a linear viscoelastic material. It is easy to verify that the Fourier transforms of $k(t)$ and $c(t)$ are directly related by:

$$\omega^2 \tilde{k}(\omega)\tilde{c}(\omega) = -1. \qquad (10)$$

In viscoelasticity, $i\omega\tilde{k}(\omega)$ is also known as the complex modulus (Ferry, 1980; Fung, 1965).

For a simple system of one spring and one dashpot in parallel (The Voigt solid), $k(t) = \xi\delta(t) + \mu h(t)$, where μ and ξ are spring constant and frictional coefficient, respectively, $h(t)$ is the unit-step function, $\delta(t)$ is the unit-impulse function, or Dirac-delta function, and $dh(t)/dt = \delta(t)$. In this case, Eq. 8 becomes

$$F(t) = \xi \frac{dx}{dt} + \mu x. \qquad (11)$$

Balancing the viscoelastic force in Eq. 11 with a Brownian random force $f(t)$, we have a Langevin-like linear stochastic differential equation:

$$\xi \frac{dx}{dt} = -\mu x + f(t). \qquad (12)$$

In general, we have the following linear stochastic equation for particle movement in viscoelastic material:

$$\int_0^t k(t - \tau) \frac{dx}{dt}(\tau)d\tau = f(t), \qquad (13)$$

or equivalently (see Eq. 9):

$$x(t) = \int_0^t c(t - \tau) \frac{df}{dt}(\tau)d\tau. \qquad (14)$$

As in Eq. 1, the nontrivial issue here is the random force $f(t)$. Because of the memory in a viscoelastic material, the stationary $f(t)$ is no longer a white noise in general, but rather should satisfy a particular condition in order to ensure Onsager's hypothesis (e.g., Eqs. 6 and 7):

$$\langle x(t_0)x(t_0 + t) \rangle = A(c(\infty) - c(|t|)), \qquad (15)$$

where $A = [k_B T/k_\infty(c(\infty) - c(0))]$ since $\langle x^2 \rangle = k_B T/k_\infty$; k_B is the Boltzmann constant and T is the temperature. For any linear viscoelastic material, the long-time behavior is like a single spring k_∞; hence the thermal equilibrium distribution

for $x(t)$ is the Boltzmann distribution:

$$P_{eq}(x) = \sqrt{\frac{k_\infty}{2\pi k_B T}} e^{-k_\infty x^2/2k_B T}. \qquad (16)$$

If $k_\infty = 0$, then the system is a liquid and the P_{eq} is a uniform distribution over the size of the entire system.

Fourier-transforming Eq. 13, we have

$$\tilde{x}(\omega) = \frac{i\tilde{f}(\omega)}{\omega\tilde{k}(\omega)}.$$

The power spectra of the two processes $x(t)$ and $f(t)$ are related to each other by

$$I_x(\omega) = \frac{I_f(\omega)}{\omega^2 |\tilde{k}(\omega)|^2}. \qquad (17)$$

When the spectrum $I_f(\omega)$ for the random force is given, Eq. 17 yields $I_x(\omega)$, from which the correlation function $\langle x(0)x(t) \rangle$ is obtained by the Wiener-Khintchin theorem. Fourier-transforming Eq. 15, noting $c(t)$ is defined only for $t > 0$, and using Eq. 10, we have

$$I_x(\omega) = -2A \, \text{Re}[\tilde{c}(\omega)] = 2A \frac{\text{Re}[\tilde{k}^*(\omega)]}{\omega^2 |\tilde{k}(\omega)|^2}, \quad (\omega \neq 0).$$

Comparing this with Eq. 17 we have

$$I_f(\omega) = 2A \, \text{Re}[\tilde{k}^*(\omega)], \quad (\omega \neq 0). \qquad (18)$$

This means the particular condition for the random force $f(t)$ is

$$\langle f(t)f(s) \rangle = 2A(k(s - t) - k_\infty), \quad (s \geq t). \qquad (19)$$

The left-hand side is the fluctuation of Brownian force, and the right-hand side is the dissipation in relaxation kinetics: Eq. 19 is the fluctuation-dissipation relation in an alternative form. Therefore, it is shown that Onsager's hypothesis (Eq. 15) leads to the fluctuation-dissipation relation (Eq. 19).

Combining the analyses in the above two sections, we have reached the pleasing duality that fluctuations in displacement $x(t)$ are linearly related to the creep function; fluctuations in force $f(t)$ are linearly related to the relaxation function; and Onsager's hypothesis is a sufficient and necessary condition for the fluctuation-dissipation relation.

VISCOELASTIC LIQUIDS AND MEAN-SQUARE DISPLACEMENT

In a real SPT measurement, it is important to determine whether the viscoelastic material is a solid or a liquid. For a viscoelastic liquid (complex fluid), the stochastic displacement $x(t)$ will not reach stationarity until it covers the entire system. In practical laboratory terms, $x(t)$ is not stationary. Hence, instead of using correlation function $\langle x(0)x(t) \rangle$ one uses mean-square displacement (MSD) $\langle x^2(t) \rangle$

for quantitatively characterizing a Brownian movement (Qian et al., 1991b). For a viscoelastic solid, the MSD will quickly plateau, similar to that of a diffusion in a harmonic well. The plateau value represents the long-time equilibrium elasticity (k_∞). However, if the material is a liquid, then the MSD will increases without bound, similar to that of a free diffusion. Mathematically speaking, if $k_\infty = 0$, then it is a viscoelastic liquid, and if $k_\infty > 0$, then it is a viscoelastic solid. Of course, making this distinction in the laboratory depends crucially on the time scale of measurements.

The MSD can be calculated from Eq. 15:

$$\langle x^2(t) \rangle = 2\langle x^2 \rangle - 2\langle x(t)x(0) \rangle = \frac{2k_{\mathrm{B}}T}{k_\infty} \frac{c(t) - c(0)}{c(\infty) - c(0)}. \quad (20)$$

If the material is viscoelastic liquid, then $c(\infty) = 1/k_\infty \rightarrow \infty$, and

$$\langle x^2(t) \rangle = 2k_{\mathrm{B}}T(c(t) - c(0)).$$

In terms of the Fourier transforms

$$\langle \widetilde{x^2} \rangle (\omega) = 2k_{\mathrm{B}}T\tilde{c}(\omega) = -\frac{2k_{\mathrm{B}}T}{\omega^2 \tilde{k}(\omega)}, \quad (\omega \neq 0). \quad (21)$$

Now let's use the Maxwell viscoelastic liquid as an example. This model has one spring and one dashpot in series:

$$c(t) = \left(\frac{1}{\mu} + \frac{1}{\xi} \right)h(t).$$

Therefore,

$$\langle x^2(t) \rangle = 2k_{\mathrm{B}}Tt/\xi,$$

as expected. Eq. 21 should be compared with Eq. 4 of Mason and Weitz (1995). See below for more discussion.

DISCUSSION

SPT: a new approach to cellular and macromolecular mechanics

Carrying out precise measurements on Brownian particles with quantitative analyses has become one of the major approaches in cellular and molecular biophysics. Fundamentally different from relaxation kinetics, this approach measures spontaneous thermal fluctuations without introducing major external perturbations into the systems being investigated. Other well-established methods in this family are dynamic light scattering (DLS), fluorescence correlation spectroscopy (FCS), and single membrane channel conductance recording (Sakmann and Neher, 1983). Quantitative analysis of fluctuation measurements relies on a statistical treatment of stochastic data in terms of correlation functions (or power spectra) and mean-square displacement. As illustrated in this paper, a correlation function is directly related to the linear relaxation kinetics usually obtained after a perturbation (Onsager's hypothesis).

Using spontaneous thermal fluctuation to study polymer gel-like viscoelastic material started with DLS (Tanaka et al., 1973). Later, thermal fluctuation of inert particles embedded in actin gels was studied using DLS (Schmidt et al., 1989) and FCS (Qian et al., 1992). With the superior spatial and temporal resolution of SPT with photodiode detection (Gittes et al., 1997; Mason et al., 1997), the methodology is now applicable to viscoelastic material at the subcellular and macromolecular level.

With recent advancement in spatial and temporal resolution to subnanometer and millisecond, SPT is now applicable to studies of macromolecules (Qian and Elson, 1999) and intracellular components such as cytoskeletal networks (Elson, 1988). Such quantitative measurements pave the way for the development of macromolecular mechanics, cellular mechanics, and tissue engineering. This method also complements the exciting development in measuring molecular and cellular forces by atomic force microscopy (AFM; Fernandez, 1997; Qian and Shapiro, 1999).

SPT: its applications to biological systems

In the past decade, SPT has been widely used as a quantitative tool in studying membrane protein movement on the surface of living cells (Saxton and Jacobson, 1997). Through these studies, a molecular view of the organization of the cellular membrane skeleton has emerged (Kusumi and Sako, 1996). The quantitative analyses of these experiments are mainly based on a free diffusion in two dimensions (Qian et al., 1991b). For cell surface receptors undergoing active transport and restricted motion in addition to Brownian motion, a systematic drift and a spatial limitation have been introduced. On the spatial scale of microns, the motion of membrane proteins on the cell surface can be represented by a particle moving in Maxwell liquid. With respect to the molecular structures, the dashpot is related to the dynamics of cytoskeletal filaments, while the spring is related to the interaction between the membrane proteins and the cytoskeleton.

One novel application of SPT to probing DNA dynamics on the level of a single molecule was carried out by Finzi and Gelles (1995). This application has opened the possibility of studying DNA conformational dynamics without perturbation. Qian and Elson (1999) have shown that, in the framework of viscoelasticity, DNA polymer represents a simple Voigt solid. The significance of this application is that it advances the SPT methodology further into the microscopic molecular scale, and provides a new approach for studying polymer dynamics in aqueous solution. The Voigt solid, which is equivalent to a dumbbell model with only a single relaxation time ξ/μ, is the simplest model for DNA polymers. A more realistic model of DNA is a set of springs in series and corresponding dashpots in parallel (Qian and Elson, 1999).

SPT has also been applied to study the material properties of F-actin gel (Gittes et al., 1997) and intracellular matrices (Mason et al., 1997). These biological materials are complex; hence, a new quantitative analysis beyond simple liquid and solid is needed. The objective of the present work is to provide a theoretical basis for the experimental approached based on SPT. It points out the important conceptual difference between the fluctuation measurements and the traditional relaxation measurements, and illustrates the deep relation between these two types of experimental measurements (Onsager's hypothesis). It also unifies the standard treatments based on macroscopic viscoelasticity and the microscopic Brownian motion.

SPT: its theoretical bases

The current repertoire of models for analyzing stochastic data from SPT is still limited. It includes diffusion with and without drift, in a free space or in confinement (Qian et al., 1991b; Saxton, 1995), and in complex fluids (Mason and Weitz, 1995), and Brownian motion of optical markers attached to a simple spring and a single chain polymer (Qian and Elson, 1999). For studies of viscoelastic material such as a polymer gel (Gittes et al., 1997; Mason et al., 1997) and biological systems such as the cytoskeleton (Elson, 1988), we need to extend the SPT analysis to a more general formalism applicable to linear viscoelastic solids and liquids (Fung, 1965; Ferry, 1980). Here, rather than a particular model, a framework for constructing linear viscoelastic models that are to be specified in terms of the matrices $\mathbf{K}$ and $\mathbf{H}$ have been developed. Several classes of models deserve further detailed analysis: two- and three-dimensional networks with uniform spring constant, and systems with random spring constants (for a very recent work, see Denneman et al., 1999). Theoretical studies of these models are directly connected to modern statistical mechanics of membranes and many-body problems in semidilute polymer solutions.

The phenomenological equations 8 and 9 for linear viscoelasticity are special cases of the general linear response theory (Kubo et al., 1978) in which the Fourier transform of the creep function, $\tilde{c}(\omega)$, is called complex admittance. If we rewrite the relaxation function $k(t)$ as

$$k(t) = \xi_0 \delta(t) + k_\infty h(t) + k_1(t),$$

then $k_1(t)$ is bounded at $t = 0$ and asymptotically approaching 0 at $+\infty$:

$$k_1(0) < +\infty, \qquad k_1(+\infty) = 0.$$

The ξ_0 represents the short-time viscosity and k_∞ represents the long-time elasticity. We then have Eq. 13 in the form:

$$\xi_0 \frac{dx}{dt} + k_\infty x(t) + \int_0^t k_1(t - \tau) \frac{dx}{dt}(\tau) d\tau = f(t) \qquad (22)$$

which is a more familiar form of generalized Langevin equation in nonequilibrium statistical mechanics (Fox, 1978; Kubo et al., 1978).

So far we have only discussed the over-damped particle motion in which we have neglected the effect of inertia. If this condition is not met, though this is rare in aqueous solution (Webb et al., 1977), then instead of Eq. 22 one starts with:

$$m \frac{d^2x}{dt^2} + \xi_0 \frac{dx}{dt} + k_\infty x + \int_0^t k_1(t - \tau) \frac{dx}{dt}(\tau) d\tau = f(t) \qquad (23)$$

where m is the mass of the moving particle. This second-order stochastic differential equation (23) can be analyzed following Kubo (1978). In particular for fluids with $k_\infty = 0$, Eq. 23 can be first solved in terms of $dx/dt = v$ and MSD for the nonstationary $x(t)$ can then be obtained from the velocity correlation function $\langle v(t)v(t') \rangle$ (Mason and Weitz, 1995).

It is important to point out that the theoretical approach we present here is not limited only to model stationary fluctuation measurements. Nonstationary solutions of Eqs. 1 and 23 also form the basis for modeling transient kinetics and force measurements by AFM (Shapiro and Qian, 1997, 1998; Qian and Shapiro, 1999). More importantly, the discussion in the present paper provides a basis for correlating these two complementary types of measurements.

One of the insights generated from the present analysis is the nature of hysteresis. It is well known that kinetics with memory functions such as Eq. 8 gives rise to hysteresis. The present analysis clearly points out that such phenomena are directly related to the internal dynamics of the molecular systems, i.e., $x_1, x_2, \ldots, x_{N-1}$ in Eq. 1. This point has also been made in connection with titin elasticity (Qian and Shapiro, 1999; Qian and Bustamante, manuscript in preparation). The present analysis puts the statement in a much broader context.

Finally, note that treating macromolecules in terms of viscoelasticity is, of course, not new. Many biophysicists in the past have developed such a point of view (see Mizraji et al., 1987, and extensive references cited). With the possibilities of quantitatively measuring forces and movements of single molecules in laboratories, these theories will soon find their increasing roles in macromolecular mechanics.

SPT in complex fluids

Mason and Weitz (1995) have developed a theory for SPT in complex fluids. Their approach is deceptively similar to ours, but with several important differences. First, it is important to realize that the complex modulus $i\omega \tilde{k}(\omega)$ from SPT is, in general, a function of the size of the optical maker, while the complex viscosity $\tilde{\eta}(\omega)$ is an intrinsic properties of a viscoelastic material that should be independent of it. The key element in the theory of Mason and

Weitz is to assume the optical marker as a particle in a continuous fluid follows Stokes law, from which the viscosity of the fluid is obtained: $\tilde{\eta}(\omega) = \tilde{k}(\omega)/6\pi a$, where a is the radius of the spherical optical marker. The approach is analogous to obtaining the viscosity of a liquid by measuring the frictional coefficient of a probing particle. In this approach, the Stokes particle is sufficiently large and its has no hydrodynamic effect on the fluids. The approach presented here, however, assumes that the optical marker is a member of the N-particle system in an aqueous solution, and the hydrodynamic interaction can be introduced into the **H** matrix. With the increasing frictional coefficient between x_N and the rest of the particles, this model should approach theirs. A similar situation for the SPT of single linear polymer molecules has been studied. It has been shown that for a small enough optical marker, its dynamics is independent of the marker size (Qian and Elson, 1999). This is easy to understand because only the slow rate processes dominate the kinetics. Therefore, the present theory can be applied to a wide range of marker sizes. A second difference is that the present framework also can be applied equally well to viscoelastic solids and liquids.

Other work on fluctuations of viscoelastic materials

A brief discussion of earlier theories on fluctuations of viscoelastic material is now presented. All models are developed based on stochastic differential equations in which the deterministic mechanical equations (Newton's equation and its overdamped form for a set of particles, or elastic equations for continuous material) are augmented by Brownian forces. Tanaka et al. (1973) treated the viscoelastic gel as a continuous elastic body with simple drag from the solvent. Barkley and Zimm (1979) treated the bending of a single DNA molecule as a rigid continuous rod in the context of fluorescence depolarization. Amblard et al. (1996) treated an actin network as a system of bendable rods, and Maggs (1998) has presented a detailed analysis of dynamic measurements with respect to the size of the optical marker in a system of semidilute polymers. Qian (submitted for publication) has developed a model for particle tetered polymers, either flexible or rigid but bendable, based on continuous approximations of the chain molecules.

SPT and some mathematical issues on reversibility

Equation 12 defines a stochastic process $x(t)$. It is well known that when the random force $f(t)$ is a white noise with an infinitely short relaxation time the $x(t)$ is Markovian and Gaussian, known as the Ornstein-Uhlenbeck process (Wang and Uhlenbeck, 1945). Equation 12 is a special case of Eq. 13, which in general defines a non-Markovian Gaussian

process when the $f(t)$ itself is Gaussian and stationary with a finite relaxation time (Fox, 1978). A Gaussian process has all its joint distribution functions (i.e., singlet, doublet, and s-multiplet, $s = 1, 2, 3, \ldots$) being Gaussian, and one usually takes this fact as the defining property of a Gaussian process. Such a process is fully specified by its expectation and second moment (van Kampen, 1997), and thus a one-dimensional stationary Gaussian process is always time reversible with its s-multiplet distribution satisfying (Weiss, 1975; Tong, 1990):

$$P[x_0 0, x_1 t_1, x_2 t_2, \ldots, x_s t_s]$$

$$= P[x_s 0, x_{s-1}(t_s - t_{s-1}),$$

$$x_{s-2}(t_s - t_{s-2}), \ldots, x_0 t_s]. \quad (24)$$

The time reversibility is a necessary condition for a stochastic process at thermal equilibrium (Qian et al., 1991a; Qian, 1998). Analysis given in the current paper, however, indicates that the reversibility is not sufficient for the thermal equilibrium. Rather, an augmented condition, the Onsager's hypothesis, has to be introduced. This raises a mathematical question about the nature of a reversible, non-Markovian Gaussian process that does not satisfy the hypothesis.

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