Dynamical Behaviour of Structured Macromolecular Solutions

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DYNAMICAL BEHAVIOUR OF STRUCTURED MACROMOLECULAR SOLUTIONS

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A large number of experimental results have shown that macromolelcules in solution give rise to ordered (thyxotropic) structures, a noticeable role being played by a dynamic correlation. In the present paper we develop a semi-phenomenological model that can be solved analytically. The model is able to describe in a quantitative way both structural and dynamical properties of a thyxotropic solution.

A careful experimental investigation is also carried out and the experimental results are compared to the theoretical prediction. In such a way the relevant parameters implied in the structure can be evaluated. Some implications concerning energy exchanges between proteins and the thermal bath are discussed.

KEY WORDS: Macromolecular solutions, enzymatic catalysis.

1 INTRODUCTION

As a deeper understanding of the biological activity of macromolecules is gained, so it is becoming clear that the role played by collective properties of macromolecular solution requires a full investigation. It is in fact well known that in a solution the macromolecules no longer behave as randomly distributed particles. On the contrary there is a well defined tendency to a self-organization that gives rise to long-ranged structures.

As a consequence the energy exchanges between the macromolecules and the surrounding medium cannot be treated in terms of the usual "thermal bath"; the macromolecules and the solvent being involved together in a collective structure.

In the past years the collective properties of macromolecular solutions has been extensively investigated by our group, using a number of widely different experimental techniques.

The results obtained from viscosity[1,2], acoustic[3], and light scattering[3,4] measurements indicate a rather general behavior that seems to apply to different kinds of macromolecules like lysozyme, B.S.A., haemoglobin and DNA. As a general rule, macromolecules in aqueous solution tend firstly to cluster, giving rise to regions in which the concentration is very high. Successively the clusters organize themself in a

long-range spatial structure, that turns out to be quite stable and, in fact, behave like a thixotropic structure being able to give macroscopic mechanical effects, like a non zero shear stress at zero shear rate, as showed in rheological measurements[1].

The latter process requires a much longer time than the former one. The spatial structure builds up gradually in the course of the time and becomes fully developed only after many hours or even days.

The existence of solid-like structures in a solution has been reported elsewhere[5]. Purely repulsive potential can give rise to a structure (Wigner-crystallization)[6] whose properties, however, are mainly determined by boundary conditions. A two-minima potential (Landau potential)[7] can give rise, as well, to an ordered structure.

In our case the origin of the structure seems to be statistical in character, rather than due to some specific interparticles interaction, and behave as a collective property of the system.

In fact if one considers the whole spatial spectrum of the fluctuations of concentration (i.e. the structure factor $S(\mathbf{k})$), our preceding results seems to indicate that there are some Fourier components characterized by an anomalously long relaxation time. As a consequence such components will dominate the structure factor, giving rise to some sort of order. Therefore a rather direct correlation would exist between static and dynamic properties in a macromolecular solution.

Although the static structural properties can be considered as firmly established from the above mentioned experimental results, the same do not hold as far as the detailed dynamical behaviour is implied.

It is well known that in the case of freely diffusing object (Brownian particles), the autocorrelation function of scattered light, $C(\tau)$ behave like a single exponential

$$C(\tau) = Ae^{-\Gamma t}$$

where the time-constant Γ is related to the diffusion coefficient.

$$\Gamma = 2Dk^2$$

where k is the exchanged wave vector, and the diffusion coefficient D is a k-independent quantity.

In our preceding results, however, the correlation function cannot be fitted with a single exponential law. In addition, if an "effective" diffusion coefficient D_{eff} is calculated as the derivative at the origin of the autocorrelation function[8]

$$D_{\text{eff}} = \frac{1}{k^2} \left(\frac{dC(\tau)}{d\tau} \right)_{\tau \to 0}$$

it turns out that such a quantity is a function of the exchanged wave-vector k. Actually its inverse, D_{eff}^{-1}, well reproduces the behaviour of the structure factor $S(k)$, according to the semi-phenomenological equation[8]

$$D_{\text{eff}} = \frac{D_0}{S(k)}$$

being D_0 a constant. In any case, the values of D_{eff} are much lower than the literature values for the diffusion coefficient of the given macromolecule (at infinite dilution).

In order to perform a detailed experimental analysis of the dynamical properties of our solution, i.e. to get an experimental evaluation of the dynamical structure factor $S(k, t)$ two main points are to be taken into account.

Firstly, the preceding results indicate that the dynamical behaviour of a structured solution encompasses a wide range of time. A typical autocorrelation function could be fitted with the sum of at least two exponential, whose time-constants differs for more than an order of magnitude[9]. In the present work we overcomed such a difficulty by using an expecially designed single-clipped digital correlator, as described in Section 4.

The second point arise because of the need to compare results obtained in different experiments performed at various exchanged wave vector k. Obviously the temporal behaviour of the correlation function is not affected by the over all instrumental efficiency, but the same does not happen as far as the absolute values of the scattered intensity are concerned. There are some unavoidable, although very small, misaligne-ment and some spurious light entering the photomultiplier that changes in a unpredictable way from experiment to experiment.

We overcomed such a difficulty by finding an "internal standard" in our samples, as described in Section 4, that allows a careful normalization of data taken at different scatering angle.

In such a way we are able to perform an experimental investigation, in which the autocorrelation functions of the scattered light are obtained in a very large scale of time (from 200 ns to ~ 0.1 sec.) and for different exchanged wave vector k. In the same time we construct a theoretical model for a structured solution, that can be solved analitically and allows the calculation of both the static structure factor $S(k)$ and the dynamical autocorrelation function $\langle S(k, t)S(k, t + \tau)\rangle$.

The comparison between the calculated behaviour and the experimental results gives support to the validity of our model, and allows a better understanding of the interconnection between dynamics and structure.

In Sections 2 and 3 the theoretical model is developed, Section 4 is devoted to the description of the experimental set-up and to the comparison of the obtained result with the model, while in Section 5 we report some concluding remarks.

2 THEORETICAL MODEL: GENERAL DESCRIPTION AND STATIC STRUCTURE FACTOR

As pointed out in Ref. 4, the scattered intensity at a given exchanged wave vector k is given by

$$I(\mathbf{k}) = \int e^{ik\xi}C(\xi)d\xi \tag{1}$$

where

$$C(\xi) = \int \bar{\rho}(x)\bar{\rho}(x + \xi)dx \tag{2}$$

is the spatial autocorrelation function of the "planar" density (concentration)

$$\bar{\rho}(x) = \int \rho(x, y, z) dy \, dz \tag{3}$$

being the x axis parallel to the exchanged wave vector $\mathbf{k}$. Therefore, because of the Wiener–Kintchine theorem, the scattered intensity, Eq. (1), reproduce the power spectrum of the fluctuation of density (concentration) along the direction spanned by $\mathbf{k}$, i.e. the "structure factor" $S(\mathbf{k})$.

As a consequence we can treat our model in a one-dimensional way. In the sequel we call "cluster" a localized positive fluctuation of the (planar) density $\bar{\rho}(x)$, living for a long enough time to give a well defined "form factor" in the scattered intensity.

We will be concerned with the spatial arrangement of clusters along the x axis, together with their dynamical behaviour.

The existence of clusters, spatially distributed in a rather regular way has been evidentiated by preceding measurements, as discussed in Section 1.

We indicate with $R_i(t)$ the center-of-mass position of a cluster, at the time t. Then the density distribution will be the convolution of the "shape" of a cluster (the form factor) with an array of delta-functions $\delta(x - R_i)$. Our calculation is mainly concerned with the structure factor, its product with a form factor being quite trivial.

Therefore we write

$$\bar{\rho}(x, t) = \sum_n \delta(r - R_n(t)) \tag{4}$$

The Fourier transform of the spatial autocorrelation function, i.e. the time-dependent structure factor, will be given by

$$S(k, t) = \sum_{n, m} \iint \delta(x - R_n(t)) \delta(x + \xi - R_m(t)) e^{ik\xi} dx \, d\xi$$

$$= \sum_{n, m} e^{ik(R_n(t) - R_m(t))} \tag{5}$$

Such a quantity is proportional to the instantaneous value of the scattered intensity, so that the experimental time autocorrelation function will be proportional to

$$\langle S(k, 0) S(k, t) \rangle = \left\langle \sum_{n, m} e^{ik(R_n(0) - R_m(0))} \sum_{p, q} e^{-ik(R_p(t) - R_q(t))} \right\rangle \tag{6}$$

Now, as far as our model is concerned, we hypotize that the center-or-mass position of a cluster can be written as:

$$R_n(t) = nL + \sum_{i=1}^{n} \varepsilon_i(t) \tag{7}$$

where L is a fixed parameter, while $\varepsilon_i(t)$ are stochastic variables describing the displacement from the distance L between two nearest neighbours.

In other words we assume that, given a cluster at R_i, there is a "preferred distance" L for the position of the next cluster, the fluctuation of the actual distance being described by the stochastic variable ε_i.

In such a way we are concerned with an order that is gradually lossed at increasing distances.

As far as the time evolution of the stochastic variables ε_i are concerned, we use a Langevin-like equation. Let us consider a small time interval τ, that plays the role of a mean collision time. Then:

$$\varepsilon_i(t + \tau) = \varepsilon_i(t)e^{-\alpha\tau} + \delta_i \tag{8}$$

In Eq. (8) the first term on the RHS describes a systematic evolution that tends to reestablish the "preferred" distance L, while δ_i is a random variable distributed according to a Gaussian law:

$$p(\delta_i) = \frac{1}{\sqrt{\pi\mu^2}} e^{-\delta_i^2/\mu^2} \tag{9}$$

From a physical point of view, the parameter α in Eq. (8) would be concerned with some restoring force, while the parameter μ in Eq. (9) plays the role of an "effective" temperature.

Starting from an arbitrary initial values of the displacement, $\varepsilon_i(0)$, after a time interval $t = \nu\tau$ (ν being an integer), the displacement $\varepsilon_i(t)$ will be given, according to Eq. (8), by:

$$\varepsilon_i(t) = \varepsilon_i(0)e^{-\nu\alpha\tau} + \sum_{j=1}^{\nu} \delta_{ij}e^{-(\nu-j)\alpha\tau} \tag{10}$$

where δ_{ij} is the random displacement of the ith cluster, taking place at the times $j\tau$, the δ_{ij} are supposed to be independent from j (Markovian process).

Obviously after a long enough time, the memory of the initial displacement is lost, so that the equilibrium distibution of the stochastic variables ε_i will be given solely by the sum in Eq. (10):

$$(\varepsilon_i(t))_{t \gg \alpha^{-1}} = \sum_{j=1}^{\nu} \delta_{ij}e^{-(\nu-j)\alpha\tau} = \sum_{\lambda=0}^{\nu-1} \delta_{ij}e^{-\lambda\alpha\tau} \tag{10'}$$

being $\lambda = \nu - j$. We are therefore concerned with a sum of independent stochastic variables. As a consequence the ε_i are distributed according to a Gaussian law, and the mean square value σ will be given by:

$$\sigma^2_{(t)} = \mu^2 \sum_{\lambda=0}^{\nu-1} e^{-2\lambda\alpha\tau} = \mu^2 \frac{1 - e^{-2\nu\alpha\tau}}{1 - e^{-2\alpha\tau}} \tag{11}$$

that for $t \to \infty$ ($\nu \to \infty$) reduces to

$$\sigma^2 = \frac{\mu^2}{1 - e^{-2\alpha\tau}} \tag{11'}$$

Notice that if we let $\alpha \to 0$ in Eq. (11) then σ^2 becomes a linearly increasing function of time ($\sigma^2 = \mu^2\nu$), and corresponds to a purely diffusive (brownian) motion; the diffusion coefficient being given by μ^2/τ.

On the contrary if $\alpha \to \infty$ (i.e. any displacement is cancelled before the next occurs), then $\sigma^2 = \mu^2 = $ const. and we are left with non-diffusing objects, that move in a "thermal cloud" around a fixed initial position.

A structured solution is in an intermediate condition, the parameter α measuring the tendency to the order.

In order to evaluate the structure factor $S(k)$ at equilibrium, we can write Eq. (5) as follows

$$\langle S(k) \rangle = \sum_{m,n} \langle e^{ik(R_n - R_m)} \rangle = \sum_{m,n} e^{ik(n-m)L} \langle e^{ik\Delta_{nm}} \rangle$$

being

$$\Delta_{nm} = \sum_{i=m+1}^{n} \varepsilon_i$$

The latter quantity is the sum of $(n - m)$ independent stochastic variables, whose rms is given by Eq. (11'). As a consequence the probability distribution for Δ_{nm} turns out to be:

$$p(\Delta_{nm}) = \frac{1}{\sqrt{\pi |n - m| \sigma^2}} e^{-(\Delta_{nm}^2 / |n-m| \sigma^2)}$$

and the mean value furnishes:

$$\langle e^{ik\Delta_{nm}} \rangle = e^{-(k^2 \sigma^2 / 4)|n-m|} \tag{12}$$

Therefore the structure factor will be given by:

$$\langle S(k) \rangle = N \sum_s \exp\left(iksL - \frac{k^2 \sigma^2 |s|}{4} \right)$$

being $s = (n - m)$ and N the total number of clusters.

Now, because of the eveness of the terms implied in the summation, the latter reduces to twice the sum of the real part, taken for $S \geq 0$. In addition the damping terms allows to take $S \to +\infty$, so that one is concerned with an absolutely convergent geometrical series. We obtain, apart from a normalization factor

$$\langle S(k) \rangle = \frac{1 - e^{-(k^2 \sigma^2 / 4)} \cos kL}{1 - 2e^{-(k^2 \sigma^2 / 4)} \cos kL + e^{-(k^2 \sigma^2 / 2)}} \tag{13}$$

The behaviour of such a function is plotted in Figure 1. It can be shown that the structure factor is characterized by peaks corresponding to $kL = 2n\pi$, whose height tends to infinity as $\sigma \to 0$ (perfectly ordered array of clusters). On the contrary for large values of σ the structure factor tends to unity (unstructured "perfect" fluid).

As mentioned before a thixotropically structured solution is in an intermediate condition and will show a more or less long-ranged order according to the presence of more or less pronounced peaks in the structure factor.
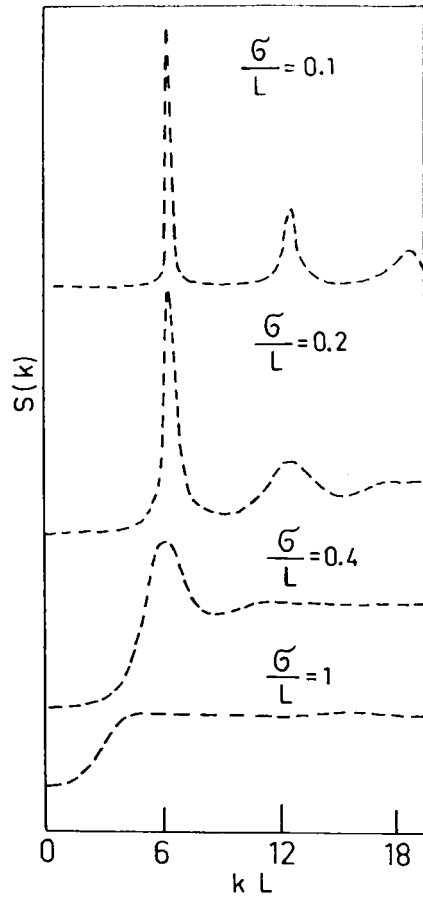
Figure 1   Structure factors in partially ordered systems. The ratio σ/L measures the disorder.

3   DYNAMICAL BEHAVIOUR: THE AUTOCORRELATION FUNCTION.

According to our model, the autocorrelation function (Eq. 6) can be written as

$$\langle S(k, 0)S(k, t)\rangle = \sum_{nmpq} e^{ik(m-n)L}e^{-ik(p-q)L}\langle e^{ik\Delta_{nm}(0)}e^{-ik\Delta_{pq}(t)}\rangle \qquad (14)$$

where, accordingly to the time evolution law (Eq. 10):

$$\Delta_{qp}(t) = \Delta_{qp}(0)e^{-v\alpha\tau} + \sum_{\lambda=0}^{v-1} \Delta_{qp,\lambda}^{*}e^{-\alpha\tau}$$

being

$$\Delta_{qp,\lambda}^{*} = \sum_{i=p+1}^{q} \delta_{i,\lambda}$$

Now, if we suppose that the random displacements $\delta_{i,\lambda}$ are uncorrelated with the initial position, the mean in Eq. (14) can be written as:

$$\langle e^{ik\Delta_{nm}(0)}e^{-ik\Delta_{pq}(t)} \rangle$$

$$= \langle \exp(ik\Delta_{nm}(0)) \cdot \exp(-ik\Delta_{qp}(0)e^{-v\alpha\tau}) \rangle \left\langle \exp\left(-ik \sum_{\lambda=0}^{v-1} \Delta_{qp,\lambda}^* e^{-\lambda\alpha\tau} \right) \right\rangle \quad (15)$$

As mentioned before, $\Delta_{p,q}^*$ is a Gaussian variable with r.m.s. $= |p-q|\mu^2$, so that we can write

$$\left\langle \exp\left(-ik \sum_{\lambda=0}^{v-1} \Delta_{qp,\lambda}^* e^{-\lambda\alpha\tau} \right) \right\rangle = \exp\left(\frac{-k^2|p-q|\mu^2}{4} \sum_{\lambda=0}^{v-1} e^{-2\lambda\alpha\tau} \right)$$

$$= \exp\left(\frac{-k^2|p-q|\mu^2}{4} \frac{1-e^{-2v\alpha\tau}}{1-e^{-2\alpha\tau}} \right)$$

$$= \exp\left(\frac{-k^2|p-q|\sigma^2}{4} (1-e^{-2v\alpha\tau}) \right)$$

Notice that for $t \to \infty$ ($v \to \infty$), the second factor in the first mean of Eq. (15) disappear. In such a case, taking into account Eq. (12), one obtain

$$\langle e^{ik\Delta_{nm}(0)}e^{ik\Delta_{pq}(t)} \rangle_{t\to\infty} = e^{-(k^2\sigma^2/4)|m-n|}e^{-(k^2\sigma^2/4)|p-q|}.$$

In such a case the sum in Eq. (14) factorize, and we get:

$$\langle S(k,0)S(k,t) \rangle_{t\to\infty} = \langle S(k) \rangle^2$$

i.e. the correct asymptotic value of the autocorrelation function. In the same way one find:

$$\langle S(k,0)S(k,t) \rangle_{t\to 0} = \langle S(k)^2 \rangle$$

In order to evaluate the time-behaviour of the autocorrelation function, we have to calculate the first mean in Eq. (15).

Now such a mean cannot be factorized unless the two intervals mn and pq are disjoint. In such a case the factorization of the mean leads to the results:

$$\langle e^{ik\Delta_{nm}(0)}e^{-ik\Delta_{qp}(t)} \rangle = \left(\exp\left(-\frac{k^2\sigma^2}{4}|m-n| \right) \exp\left(-\frac{k^2\sigma^2}{4}|p-q|e^{-2v\alpha\tau} \right) \right.$$

$$\left. \exp\left(-\frac{k^2\sigma^2}{4}|p-q|(1-e^{-2v\alpha\tau}) \right) \right)$$

$$= \exp\left(-\frac{k^2\sigma^2}{4}|m-n| \right) \exp\left(-\frac{k^2\sigma^2}{4}|p-q| \right)$$

$$\text{if: } mn \cap pq = \varnothing$$

and we obtain the same kind of contribution as those appearing in $\langle S(k) \rangle^2$. In other words the only variable contribution to the autocorrelation function comes from those terms, in Eq. (14), for which a non zero intersection exists between the two

intervals mn and pq, as e.g. for the sequence $n < q < m < p$: The intersection $(m - q)$ furnishes a time dependent correlation between the two stochastic variables Δ_{nm} and Δ_{pq}, that prevents the factorization.

Let us put:

$$q - n = s$$
$$p - m = s'$$
$$m - q = \xi$$

the three intervals above being disjoint.

Now we notice that any other permutation of indices that left unchanged the absolute values of s, s' and ξ amount or to an exchange of role between $(s + \xi)$ and $(s' + \xi)$, that do not affect the result, or to a change of signum of $(s + \xi)$ or $(s' + \xi)$ or both.

As a consequence the variable part of the autocorrelation function can be written as:

$$\langle S(k, 0)S(k, t)\rangle_{\mathrm{VAR}} = \sum_{s, s', \xi} e^{\pm ik(s + \xi)L}e^{\pm ik(s' + \xi)L}\langle e^{\pm ik(\Delta_s(0) + \Delta_\xi(0))}e^{\pm(\Delta_{s'}(t) + \Delta_\xi(t))}\rangle \quad (16)$$

the average being now factorizable as follows:

$$\langle e^{\pm ik\Delta_s(0)}\rangle = \exp\left(\frac{-k^2\sigma^2}{4} s\right)$$

$$\langle e^{\pm ik\Delta_{s'}(t)}\rangle = \langle e^{\pm ik\Delta_{s'}(0)e - p}\rangle e^{-(k^2\sigma^2/4)s'(1 - e^{-2v\alpha\tau})}$$

$$= e^{-(k^2\sigma^2/4)s'e^{-2v\alpha\tau}}e^{-(k^2\sigma^2/4)s'(1 - e^{-2v\alpha\tau})} = e^{-(k^2\sigma^2/4)s'}$$

Analogously:

$$\langle e^{\pm ik(\Delta_\xi(0) + \Delta_\xi(t))}\rangle = e^{-(k^2\sigma^2/2)\xi(1 + e^{-v\alpha\tau})}$$

$$\langle e^{\pm ik(\Delta_\xi(0) - \Delta_\xi(t))}\rangle = e^{-(k^2\sigma^2/2)\xi(1 - e^{-v\alpha\tau})}$$

Calling:

$$z = \sum_s e^{iksL}e^{-(k^2\sigma^2/4)s} = \frac{1}{1 - e^{ikL}e^{-(k^2\sigma^2/4)}}$$

Equation 16 becomes (apart from a constant factor):

$$\langle S(k, 0)S(k, t)\rangle = R_e z^2 \sum_\xi e^{2ik\xi L}e^{-(k^2\sigma^2/2)\xi(1 + e^{-v\alpha\tau})} + zz^\lambda + \sum_\xi e^{-(k^2\sigma^2/2)\xi(1 - e^{-v\alpha\tau})} \quad (17)$$

We notice the presence of the two time-dependent terms give by

$$(1 + e^{-v\alpha\tau}) \quad \text{and} \quad (1 - e^{-v\alpha\tau}).$$

Actually in the correlation function (Eq. (16)), we are concerned with terms of the kind $\cos(\Delta_\xi(0)) \cos(\Delta_\xi(t)) = \cos(\Delta_\xi(0) + \Delta_\xi(t)) + \cos(\Delta_\xi(0) - \Delta_\xi(t))$. Now the stochastic variable $\Delta_\xi(0) + \Delta_\xi(t)$ changes in the course of time from twice the stochastic variable $\Delta_\xi(0)$, with a rms $4\sigma^2\xi$, to the sum of two uncorrelated variables, with a rms $2\sigma^2\xi$. On

the contrary the stochastic variable $\Delta_\zeta(0) - \Delta_\zeta(t)$ is characterized by a rms that increases from 0 to $2\sigma^2\zeta$.

As a consequence the first one gives a contribution that increases in the course of time, while the second one gives a decreasing contribution.

The increasing contribution, however, is weighted by terms of the kind $\cos 2k\zeta L$: it is just such a circumstance that, originates in the autocorrelated function a time-behaviour that turns out to be k-dependent (apart from the trivial k^2 dependence), accordingly to the structural properties of the system.

The explicit calculation of Eq. (17), although cumbersome can be carried out, and furnishes:

$$\langle S(k,0)S(k,t)\rangle = f(x, \vartheta) = \frac{A^2 - B^2}{D_1^2 \cdot D_2} - \frac{E \cos\left[2x + \arccos\dfrac{2AB}{D_1}\right]}{D_1 \cdot D_2} + \frac{C}{D_1 D_3} \quad (18)$$

where

$$A = (1 - e^{-(\gamma^2 x^2/4)}\cos x) \qquad D_1 = (1 - 2e^{-(\gamma^2 x^2/2)}\cos x + e^{-(\gamma^2 x^2/2)})$$

$$B = e^{-(\gamma^2 x^2/4)}\sin x \qquad D_2 = (1 - 2e^{-(\gamma^2 x^2/2)(2-\vartheta)}\cos x + e^{-\gamma^2\omega^2(2-\vartheta)})$$

$$C = 1 - e^{-(\gamma^2 x^2/2)N\vartheta} \qquad D_3 = (1 - e^{-(\gamma^2 x^2/2)\vartheta})$$

$$E = e^{-(\gamma^2 x^2/2)(2-\vartheta)}$$

being $x = kL$; $\gamma = \sigma/L$ and $\vartheta = 1 - e^{-v\alpha\tau}$. The parameter N appearing in the expression of C arise as an upper limit for the last summation in Eq. (17), that otherwise will diverge as $t \to 0$ ($v \to 0$), (self-correlation of an *infinite* number of segments).

As far as the first sum in Eq. (17) is concerned, the truncation of the sum will give rise to rapidly oscillating terms of the kind $\cos 2NkL$, that can be neglected because of the finite angular resolution of any actual experiment.

In a sense N plays the role of a correlation length: regions, in the system, spaced apart more than NL will behave as uncorrelated subsystem. Then both structural and dynamical properties will be determined solely by the properties of a single region, the total number of region contained in the scattering volume giving rise simply to an over-all intensity factor.

4   EXPERIMENTAL MEASUREMENTS. PROCEDURE AND RESULTS.

As mentioned in Section 1 a detailed experimental investigation of the dynamical structure factor, $S(k, \omega)$ requires the measurments of the autocorrelation function of the scattered intensity over a wide range of time. In addition, results obtained for different exchanged wave vector **k** must be properly normalized before a comparison can be made.

The former requirement is fulfilled by means of a specially designed digital autocorrelator, working in the single-clipping model.

The intensity autocorrelation function is performed as the product $\langle n_s(0)n_c(-r\tau)\rangle$. Here $n_s(0)$ is the number of photons recorded in a given time-interval τ_{min}, at time zero, while $n_c(-r\tau)$ is the clipped signal recorded at the preceding time $r\tau$, where r is an integer that labels the channels and τ is the time-delay introduced at each channel. In the single-clipping mode, n_c is equal to zero if no photons has been detected in the given time interval τ_{min}, and is equal to 1 elsewhere.

A general description of the autocorrelation technique can be found in Ref. (10).

In our case, the correlation function is performed in 88 channels, divided in 11 groups of 8. The delay time between two successive channels is doubled at each group, so that, while in the first group two channel are spaced in time by a given interval, say τ_{min}, the spacing in the last group is $2^{10}\tau_{min}$. Such a configuration allows the detection of the autocorrelation function on an enormous scale of time, the delay between the first and the last channel being $16376\ \tau_{min}$. In such a way not only both short and long time behaviour can be showed simultaneously, but also the uncorrelated part of the signal can be easily detected. We recall that the subtraction of the uncorrelated signal is of paramount importance for the analysis of the correlation.

Special care is taken in order to avoid the spurious effect due to after-pulse in the photomultiplier, and to the statistical loss of those pulses that fall just at the switching time of the clock[10]. The latter troublesome is overcomed by using a de-randomization circuitry that sincronizes the incoming pulses with the main clock of the correlator.

In our correlator we can select for τ_{min} a value as small as 200 ns. Test analysis performed with static scatterers shows a perfectly flat correlation function. Also the ratio between clipped and unclipped signals perfectly agree with a theoretical calculation based on the hypothesis of a Gaussian statistic for the number of photon detected in a given time interval. As a final test we perform the correlation of the light emitted by an artificial source (LED), randomly modulated. The statistical properties of the source are exactly reproduced by the autocorrelation function.

In Figure 2 the experimental arrangement is shown.

The optical apparatus is mounted on a two arms interferometer. The angle ϑ between the two arms can be adjusted at any wanted value. We use an He–Ne 5 mW laser, the intensity of the beam being controlled by means of a variable attenuator F.

A small pin-hole, P_1 allows a first rough selection of the angle ϑ, mainly with the purpose of avoiding spurious light collection. The lens L_1 provides a real image of the optical cell containing the sample, onto a plane S_1, where a slit selects the scattering volume.

The lens L_2 gives onto its focal plane S_2 the spatial Fouier transform of the scattered light intensity, so that the very small pin-hole P_2 allows the final selection of the scattering wave-vector $\mathbf{k}$.

The lens L_3 collects the selected light toward the single-photon counting photomultiplier PM, the output pulses being sent to the correlator.

As far as the sample is concerned, we use a 10% by weight Lyozyme solution. The solution is prepared by using high purity materials, purchased from Miles Lab. Inc. No buffer is used, so that the pH of the solution has the standard value (pH $= \sim 5$). The optical cell (S) is filled and the solution is filtered "in situ" by means of a peristaltic pump (PP) that forces the fluid though a $0.2\ \mu$ millipore filter, (MF) in a
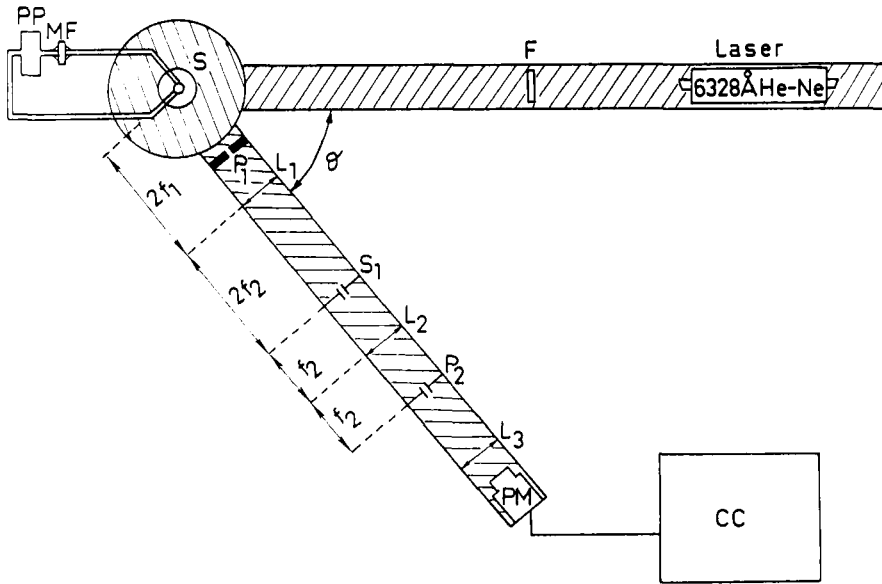
Figure 2   Experimental set-up. For a detailed description, see text.

closed circuit. (see Figure 2). The scattering amplitude is periodically recorded, and the filtering procedure continued until the scattering efficiency reaches a steady value.

Experimental measurements are performed 24 hours after the end of the filtering, in order to allow the structure to build up completely, according to the results of previous measurements.

Actually we also performed measurements in order to monitor the building-up of the structure in the course of the time, the obtained results being in agreement with the preceeding ones. All the measurements are performed at constant (room) temperature ($T = 25°C$).

With the previously described procedure, for each scattering wave vector, we record two autocorrelation functions. In the first one the minimum time interval is $\tau_{min} = 0.8 \mu s$, while in the second one is $\tau_{min} = 200$ ns. The large delay from the first and last channel implied in the first correlation function (~ 13 ms) allows a very careful evaluation of the D.C. terms, that is to be compared with the calculated value $\langle n_s \rangle \langle n_c \rangle$.

On the contrary, the short time behaviour, is better seen in the second autocorrelation function. A tipical result is shown in Figure 3. It can be seen that a rapidly-decaying sharp contribution takes place in the initial part of the curve. Such a contribution can be easily estracted from the main (comparatively slow) decay that infact behave like a constant on such a short time scale.

It turns out that the fast contribution can be fitted to a great accuracy by a single exponential law, whose time constant Γ behave like:
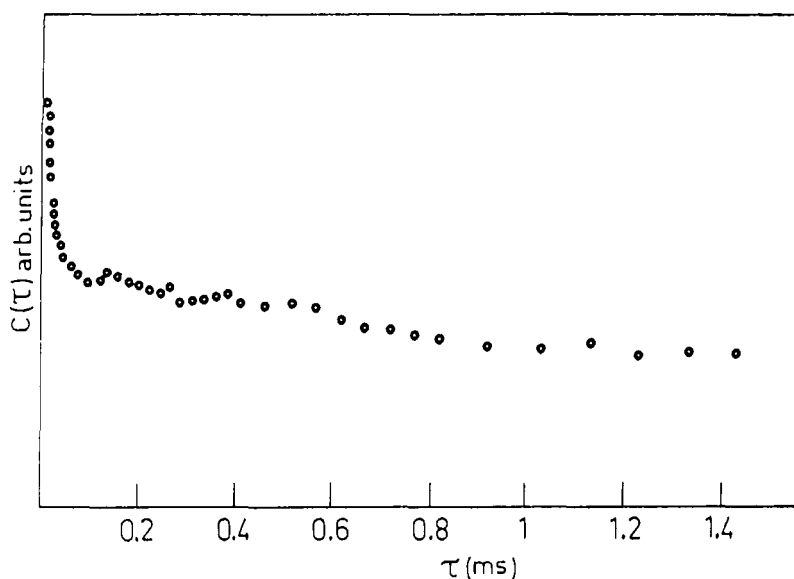
$$\Gamma = Dk^2$$

Figure 3 Intensity autocorrelation function.

where D turns out to be independent from the scattering vector k, and is equal to 10^{-6} cm^2/sec. The latter figure is just the diffusion coefficient of the lysozyme molecules given in the literature (at infinite dilution). Such a circumstance, together with the independence of D from k, suggests that the observed fast contribution is due to the presence of freely diffusing molecules, not implied in the structure. As a consequence, due to the smallness of the molecules (~ 30 Å of diameter) compared to the wavelength of light (6328 Å), also the amplitude of the fast contribution must be independent from k. Therefore such a contribution can be used as an "internal standard" in order to properly normalize measurements taken at different scattering wave-vector k.

In such a way we obtain (apart from a constant factor) the true amplitude of the variable part of the intensity autocorrelation as a function of k.

Such a quantity is to be compared with the theoretical prediction, $f(x, 0) - f(x, \infty)$, given by Eq. (18), that turns out to be strictly connected to the structure factor $S(k)$ (Eq. (13)).

In order to make such a comparison we need some hypothesis concerning the form factor $F(k)$ of the clusters. Although the detailed behaviour of the form factor depends on the actual shape of the scatterer, in general $F(k)$ is a decreasing function of k, whose width is determined by the linear size of the scatterer. For the sake of simplicity we assume that in a cluster the concentration decreases exponentially starting from a maximum value attained at the center of mass. As a consequence the form factor will behave like a lorentzian, $F(k) \sim (k^2 + a^2)^{-1}$.

We notice, however, that the relevant features in our fit are the poisition of the peaks, that crucially determined the "preferred distance" L (mean intercluster spacing).
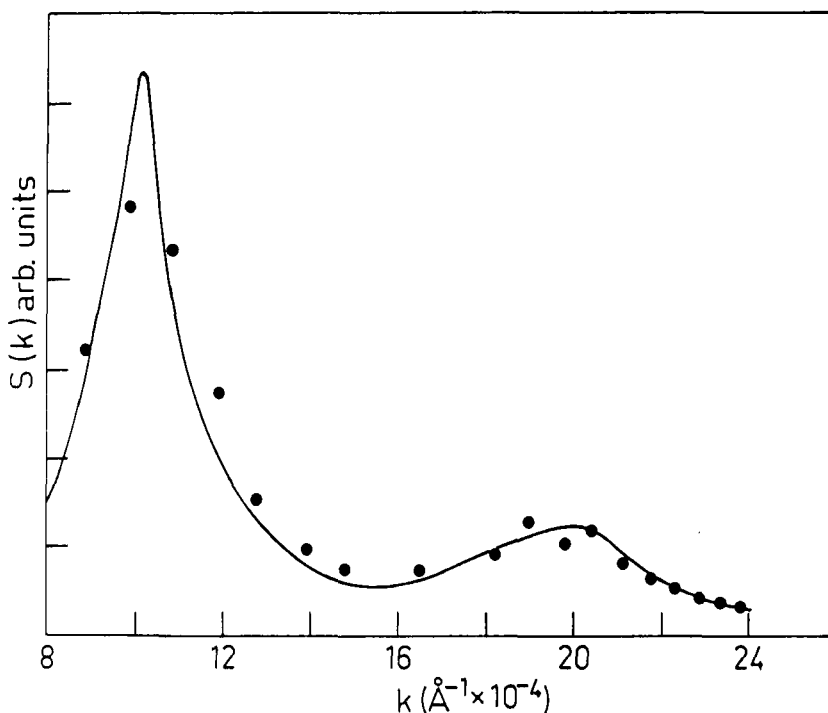
Figure 4   Structure factor in a 10% Lysozyme solution. Dots are experimental points.

Such a fit is shown in Figure 4, and furnishes the following values for the implied parameters: intercluster spacing $L = 6160$ Å; $\sigma = 1500$ Å. In addition we found a value of 350 Å for a.

The next step is the fitting of the experimental autocorrelation functions with Eq. (28).

A typical result is shown in Figure 5. It can be shown that Eq. (18) describe the correct time-behaviour of the autocorrelation function on the entire scale of time. Actually a function like that of Figure 5 could be fitted by the sum of two exponential. This is a rather common procedure, usually employed in phenomena showing both short and long time behaviours, that we used in preceeding works.[2,4,9] However our present model shows in itself such a peculiarity, so that an unique (although rather complicated) expression is able to give account to the full time evaluation of the autocorrelation function.

We would stress that the values of the implied parameters (i.e. L/σ, α and N) turn out to be nearly the same for the various function taken at different k, to a 10% accuracy.

Taking into account that different measurements refer to different (although equally prepared) samples, such a circumstance, in our opinion, gives a strong support to the usefulness of our model.

To obtain value are: $L = 5700 \pm 500$, $\sigma = 600 \pm 50$ Å; $\alpha = 166 \pm 10 \sec^{-1}$ and $N = 13 \pm 1$.
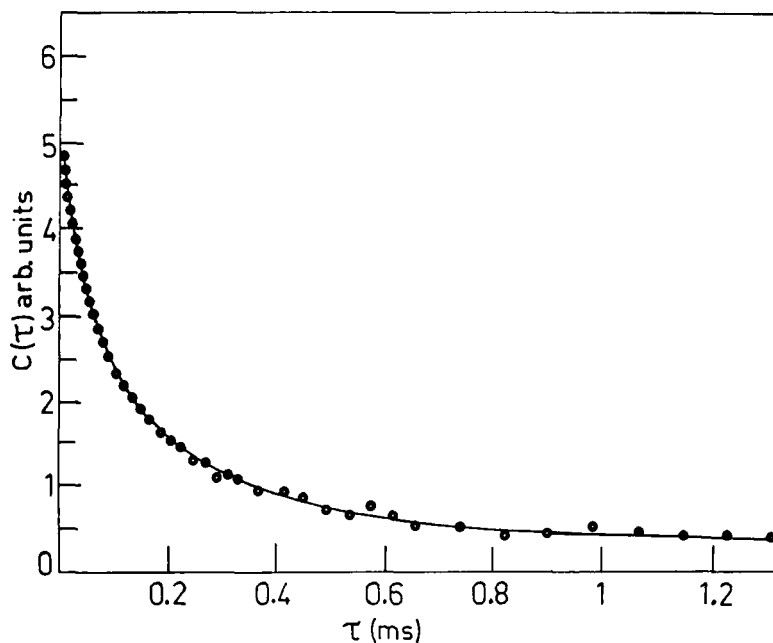
Figure 5   Autocorrelation function fitted the models equation.

Notice that, according to the obtained value of σ the ordering between clusters is practically lost after ~ 10 intercluster spacing L, in agreement with the value obtained for N. Such an internal consistency supports the role of correlation length attributed to N,

The comparison with data obtained in the first fit (the structure factor), shows that while the two fits give the same value of L, the same circumstance do not hold as far as σ is concerned. It seems that the system is more disordered from a statistical point of view than from a dynamical one. Actually in our model all the disorder comes from the dynamics of the system. The experimental results seems to indicate that, in addition, also some configurational disorder takes place.

5 CONCLUDING REMARKS

The characteristic properties of a macromolecular solution, i.e. the building up of a thyxotropic structure and the peculiar hydrodynamical behaviour, can be understood in terms of a stochastic phenomenological model.

In the model, a preferred distance L exists between nearest neighbours. However, starting from any point in the system, the order is increasingly lossed at increasing distancies. The tendency to order is introduced as some sort of restoring force, while stochastic displacements (random forces) give rise to disorder. The latters, however, obey to a spatial correlation requirement: the actual displacement of a cluster from its initial position is, in fact, the sum of the displacements of the preceding clusters. In a

sense any displacement moves the network of the ordered equilibrium positions around any given space-point. The experimental comparison of the model shows that such a correlation is of finite range, being lossed after about ten intercluster spacing. There is therefore an indication for a jerarchy of structures. Macromolecules gives rise to clusters, i.e. regions in which a strong correlation of velocities must exist in order to maintain the size and shape of the cluster for a long enough time to allow the detection of a form factor. In turns the same sort of correlation seems to exist, at a larger scale, between clusters.

The dynamical behaviour of the present model gives rise to an autocorrelation function that decays faster at short time, but shows a very long tail. If one treats approximately the initial behaviour of the autocorrelation function in terms of an exponential decay, the diffusion coeficient will be given by $\sigma^2\alpha/2$. According to the value founded for σ, we obtain a value between 2×10^{-8} and 2×10^{-9} cm^2/sec, that is the correct order of magnitude founded in preceding works.[9] However our formalism allows the description of both short and long time behaviour. A description in terms of a superposition of different processes giving rise to a sum of exponential decays is therefore unnecessary.

In addition the k-dependence of the dynamical behaviour of the system is self-contained in our model, unlike the usual assumption of a k-dependent diffusion coefficient, $D_{eff} = D_0/S(k)$. The k-dependence arise, in our model, from the competition of terms of the kind $\exp(1 - e^{-\alpha t})$ and $\exp(1 + e^{-\alpha t})$ whose weight is determined by the structure factor $S(k)$: in a sense our model evidentiate the common assumption of "long lived components" in the spectrum of fluctuations, that give rise to spatial order.

Biophysical implication of the results presented in the present paper are quite evident: in a macromolecular solution a dynamical correlation between aggregates of correlated macromolecules extends up to semi-macroscopic distancies ($\sim 6\mu$). In such a system we believe that energy exchange between macromolecules and solvent can hardly be treated by the customarily assumed thermodynamic interaction between a system and its thermal bath. In a sense in our model tendency to a spatial order utilizes the thermal disorder to give rise to a correlated behaviour.[11]

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