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STUDY OF DEXTRAN SOLUTIONS BY QUASIELASTIC LIGHT-SCATTERING*

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ABSTRACT

The autocorrelation function of the intensity fluctuation of light scattered from dextran solutions, together with the angular dependence of the integrated intensity of the scattered light, has been measured by means of single-photon counting. The data were analyzed by considering the molecular-weight distributions of the samples obtained by gel-permeation chromatography. The molecular-weight dependence of the diffusion coefficient, and the relation between the radii of gyration and the hydrodynamic radii thus obtained, proved that dextran molecules behave as random coils in aqueous solution. The molecular-weight-distribution curve measured by gel-permeation chromatography has been demonstrated to be useful in the analysis of light-scattering experiments for polydisperse, macromolecular solutions.

INTRODUCTION

Quasielastic light-scattering is a useful technique for the study of hydrodynamic properties of macromolecules in solution[1,2]. By investigating the autocorrelation functions of the scattered light from a macromolecular solution, and analyzing them by a suitable program, the translational-diffusion coefficient of the macromolecule can be obtained in a reasonably short time. The angular dependence of the intensity of the scattered light provides additional information about the size and conformation of macromolecules[3]. In the case of polysaccharide solutions, however, light-scattering experiments meet difficulty in the analysis of data, because the polydisperse nature of polysaccharides makes interpretation of the data an intricate process.

We have employed gel-permeation, chromatographic data for polysaccharide samples in order to aid in the interpretation of light-scattering data, and have obtained reasonable results, and we now present the results of a study of solutions of dextran, a typical polysaccharide consisting of D-glucopyranose polymerized mainly by α-(1→6) linkages.

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

EXPERIMENTAL

Commercial samples of dextran (T40, T70, T250, and T500, from Pharmacia Fine Chemicals) from *Leuconostoc mesenteroides* strain B512 were dissolved in 0.12M borate buffer, pH 10, to a concentration of 0.1–1.0%. A sample (ST500) having a relatively low polydispersity was fractionated several times from T500 by preparative, gel-permeation chromatography, using Sepharose CL-4B gel (Pharmacia Fine Chemicals), and 0.12M borate buffer, pH 10, as the eluant. The gel-permeation study was conducted by one of the authors (K.S.) and his colleagues[4].

After clarification by centrifuging at 10,000*g* for 1 h at room temperature, sample solutions were carefully placed in the light-scattering cell. The single-clipped, photon correlator used in the present study was essentially that described by Chen *et al.*[5]. The detailed features of our equipment, and its operational procedure will be reported[6]. All of the light-scattering measurements were conducted at 20°.

DATA ANALYSES

1. Autocorrelation functions

The measured photocount correlation function has the form

$$g^{(2)}(\tau) = B(1 + \beta|g^{(1)}(\tau)|^2), \tag{1}$$

where τ is the correlation-delay time; B, a measured background; β, a beat efficiency, and treated as an unknown parameter in the fitting procedure; and $g^{(1)}(\tau)$ is the normalized, first-order autocorrelation function of the scattered-light electric-field, which contains information about the hydrodynamic behavior of macromolecules in a solution. If the size of sample macromolecules is monodisperse and sufficiently small compared with the wavelength of the light,

$$|g^{(1)}(\tau)| = \exp(-\Gamma\tau), \tag{2}$$

with $\Gamma = Dk^2$, (3)

where D is the translational-diffusion coefficient of the macromolecule, and $k = 4\pi n \sin(\theta/2)/\lambda$ is the magnitude of the scattering vector at a scattering angle θ for an incident wavelength λ (= 632.8 nm in the present study) through a solution of refractive index n.

The polydispersity of the sample produces some deviation of the autocorrelation function from a single, exponential-decay curve. In this case, the autocorrelation function can be analyzed by the method of cumulant expansion[7]:

$$|g^{(1)}(\tau)| = \exp\left(-\bar{\Gamma}\tau + \frac{\mu_2}{2!}\tau^2 - \frac{\mu_3}{3!}\tau^3 + \ldots\right), \tag{4}$$

with $\bar{\Gamma} = \int_0^\infty \Gamma \cdot G(\Gamma)\mathrm{d}\Gamma$, (5)

$$\mu_i = \int_0^\infty (\Gamma - \bar{\Gamma})^i \cdot G(\Gamma)d\Gamma, \tag{6}$$

where $G(\Gamma)$ is a normalized, distribution function of the relaxation-rate Γ's.

Instead of *Eq. 3*, an "apparent" diffusion coefficient is introduced in the polydisperse system as:

$$D_{app} = \bar{\Gamma}/k^2. \tag{7}$$

For a polydisperse solution of sufficiently small macromolecules compared with λ, which is the case for the dextrans studied here, the "apparent" diffusion coefficient is a so-called "z-average" diffusion coefficient:

$$\langle D \rangle_z = \frac{\Sigma C_i M_i D_i}{\Sigma C_i M_i}, \tag{8}$$

where C_i, M_i, and D_i are the weight concentration, the molecular weight, and the diffusion coefficient, respectively, of species i. In the same way, an index of polydispersity is introduced as:

$$\rho = \frac{\mu_2}{\bar{\Gamma}^2} = \frac{\langle D^2 \rangle_z - \langle D \rangle_z^2}{\langle D \rangle_z^2}. \tag{9}$$

2. Gel-permeation data

We analyzed the light-scattering data quantitatively, taking the molecular-weight distribution as measured by gel-permeation chromatography into consideration. In the latter experiment, the relation between the molecular weight M and the elution volume v is written as:

$$M = a \cdot \exp(-bv), \tag{10}$$

where a and b are characteristic constants for each column used in the gel-permeation study. The experimental procedure for the determination of a and b will be described in detail[4]. The characteristic, molecular constants are obtained as follows:

$$\langle D \rangle_z = \frac{\Sigma P_i M_i D_i}{\Sigma P_i M_i}, \tag{11}$$

$$\rho = \frac{\Sigma P_i M_i D_i^2 \cdot \Sigma P_i M_i}{(\Sigma P_i M_i D_i)^2} - 1, \tag{12}$$

where P_i is the optical density (which is proportional to the weight concentration) of the i-th elution volume v_i, and $M_i = a \cdot \exp(-bv_i)$. If we assume the general relationship between the diffusion coefficient and the molecular weight of the polymer molecule,

$$D = K_D M^{-\alpha}, \tag{13}$$

with K_D being a proportionality constant, *Eqs. 11* and *12* can be written as:

$$\langle D \rangle_z = K_D \frac{\Sigma P_i M_i^{1-\alpha}}{\Sigma P_i M_i}, \tag{14}$$

$$\rho = \frac{\Sigma P_i M_i^{1-2\alpha} \cdot \Sigma P_i M_i}{(\Sigma P_i M_i^{1-\alpha})^2} - 1. \tag{15}$$

By introducing a "diffusion-average" molecular weight, M_D, defined as:

$$\langle D \rangle_z = K_D M_D^{-\alpha}, \tag{16}$$

we get

$$M_D = M_w \frac{\Sigma P_i \cdot (\Sigma P_i M_i)^{(1-\alpha)/\alpha}}{(\Sigma P_i M_i^{1-\alpha})^{1/\alpha}}, \tag{17}$$

where M_w is a weight-average, molecular weight.

Gel-permeation data enable us to compute M_D for various values of α by the use of *Eq. 17*, and we can determine the values of α and K_D to obtain the best fit between the investigated $\langle D \rangle_z$ and the calculated M_D for *Eq. 16*.

3. Characteristic dimensions of macromolecules

The radius of an equivalent, hydrodynamic sphere, *i.e.*, the hydrodynamic radius, of a macromolecule is defined as:

$$R_e = \frac{k_B T}{6\pi\eta D}. \tag{18}$$

It is considered to be proportional to its radius of gyration[3]:

$$R_e = \xi R_G. \tag{19}$$

The proportionality constant ξ is dependent on the molecular conformation, and $\xi = 3\sqrt{\pi}/8$ $(= 0.665)$ in the case of an ideal, random coil.

In the light-scattering experiment for a polydisperse, macromolecular solution, one of the observed characteristic dimensions of macromolecules is from $\langle D \rangle_z$:

$$\langle R_e^{-1} \rangle^{-1} = \frac{k_B T}{6\pi\eta \langle D \rangle_z} = \frac{\Sigma C_i M_i}{\Sigma C_i M_i (R_e)_i^{-1}}, \tag{20}$$

and the other is from the angular dependence of integrated intensity of the scattered light:

$$\langle R_G^2 \rangle_z^{1/2} = \left(\frac{\Sigma C_i M_i (R_G^2)_i}{\Sigma C_i M_i} \right)^{1/2}, \tag{21}$$

where $(R_e)_i$ and $(R_G)_i$ are the hydrodynamic radius and the radius of gyration of the i-th species.

Therefore, for the calculation of ξ from light-scattering data, the polydispersity correction is indispensable; that is, ξ should be calculated by

$$\xi = \left(\frac{\langle R_e^2 \rangle_z^{1/2}}{\langle R_e^{-1} \rangle_z^{-1}} \right) \cdot \frac{\langle R_e^{-1} \rangle_z^{-1}}{\langle R_G^2 \rangle_z^{1/2}} \tag{22}$$

Experimental evaluation of ξ has great importance in the study of macromolecules, but such an evaluation has not so far been successful for polydisperse, macromolecular solutions, because of this complicated effect of polydispersity.

Now that, from gel-permeation study, we know the molecular-weight distributions of samples, the polydispersity correction-factor $C_\xi = \langle R_G^2 \rangle_z^{1/2} \cdot \langle R_e^{-1} \rangle_z$ can be calculated with relation to *Eq. 13* as:

$$C_\xi = \frac{(\Sigma P_i M_i^{1+2\alpha})^{1/2} \cdot (\Sigma P_i M_i^{1-\alpha})}{(\Sigma P_i M_i)^{3/2}}, \tag{23}$$

with the value of α determined for *Eq. 16* (see the preceding section). Thus, we can evaluate ξ by *Eq. 22* from the two characteristic dimensions observed in the light-scattering experiment.

RESULTS AND DISCUSSION

Typical, semi-logarithmic plots of the autocorrelation function of the light scattered from a dextran solution are shown in Fig. 1. As expected from the polydisperse nature of samples (the gel-permeation chromatograms are shown in Fig. 2), the autocorrelation curve deviates from a single-exponential decay. The apparent diffusion coefficients calculated from Eq. *7* by the force-fitting of measured autocorrelation functions to *Eq. 4* does not depend on either the scattering angle or the sampling interval $\Delta\tau$, as several data in Fig. 3 show.

Angular dependence of the integrated intensity of the scattered light was investigated simultaneously (see Fig. 4). For low-molecular-weight samples T40 and T70, we could not find any notable dependence. Experimental results are summarized in Table I.

The best-fit relation between $\langle D \rangle_z$ and M_D was found to be $\langle D \rangle_z = 7.96 \times 10^{-5}\ M_D^{-0.485}\ \mathrm{cm^2.s^{-1}}$. The double logarithmic plot of $\langle D \rangle_z$ *versus* molecular weight is shown in Fig. 5. If we take the "equivalent hydrodynamic-sphere model"[3,8], our result is consistent with the molecular-weight dependence of intrinsic viscosity of dextrans, which was measured by Granath[9].

Values of ρ are calculated for $\alpha = 0.485$ *via Eq. 12*, and the results are listed in Table II. When these values are compared with the investigated ρ-values in Table I, agreement between calculated and investigated values is excellent for samples having relatively broad molecular-weight distribution (T250 and T500). For samples having relatively narrow, molecular-weight distribution (T40, T70, ST500), however, investigated values are greater than the calculated values. This is probably due to the

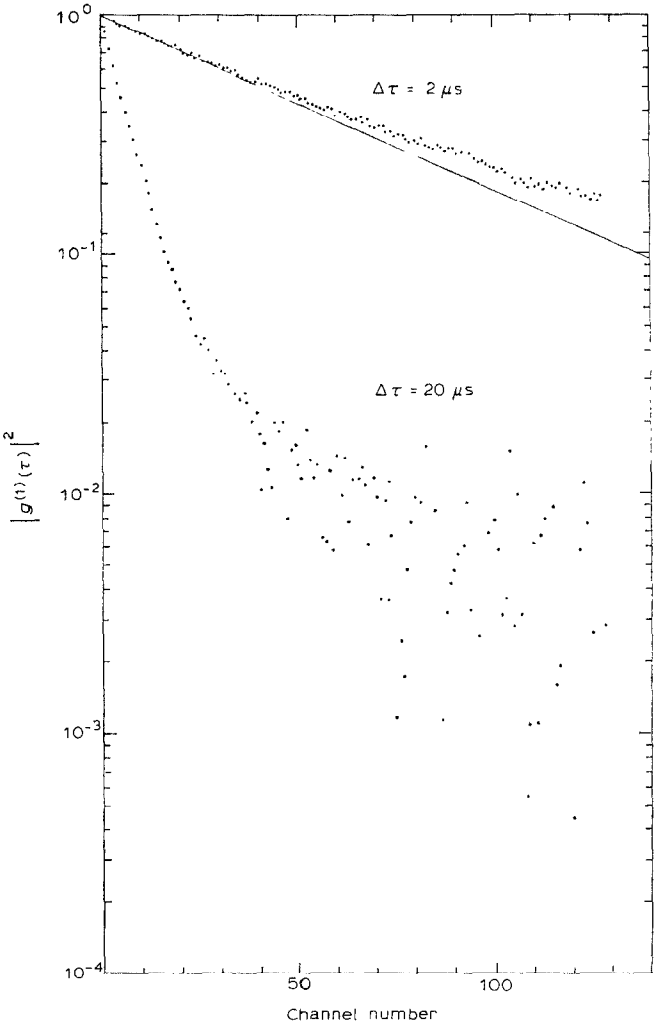

Fig. 1. Semi-logarithmic plots of observed autocorrelation functions *versus* correlation delay time τ (= channel number × sampling interval $\Delta\tau$) for 0.1% solution of dextran T500. $\theta = 90°$, $k^2 = 3.50 \times 10^{10}$ cm^{-2}. [The solid line indicates the initial slope, $2\bar{\Gamma} = 2\langle D\rangle_z k^2$.]

TABLE I

SUMMARY OF EXPERIMENTAL RESULTS

Sample	M_W[a] *(10⁴)*	*Gel permeation*		*Light scattering*			
		M_W *(10⁴)*	M_W/M_N	$\langle D\rangle_z$ *(10⁻⁷cm².s⁻¹)*	ρ	$\langle R_e^{-1}\rangle_z^{-1}$ *(nm)*	$\langle R_G^2\rangle_z^{1/2}$ *(nm)*
T40	4.00	4.27	1.39	4.22 ±0.20	0.19 ±0.06	5.06 ±0.26	—
T70	6.44	5.85	1.46	3.71 ±0.24	0.19 ±0.08	5.76 ±0.40	—
T250	24.0	25.3	2.31	1.90 ±0.09	0.23 ±0.06	11.0 ±1.1	21.5 ±5.5
T500	51.1	53.4	3.14	1.20 ±0.07	0.29 ±0.10	17.0 ±1.0	31.0 ±3.0
ST500	—	66.0	1.52	1.03 ±0.04	0.17 ±0.04	20.7 ±0.9	34.0 ±4.0

[a] M_W provided by Pharmacia Fine Chemicals, together with dextran samples.

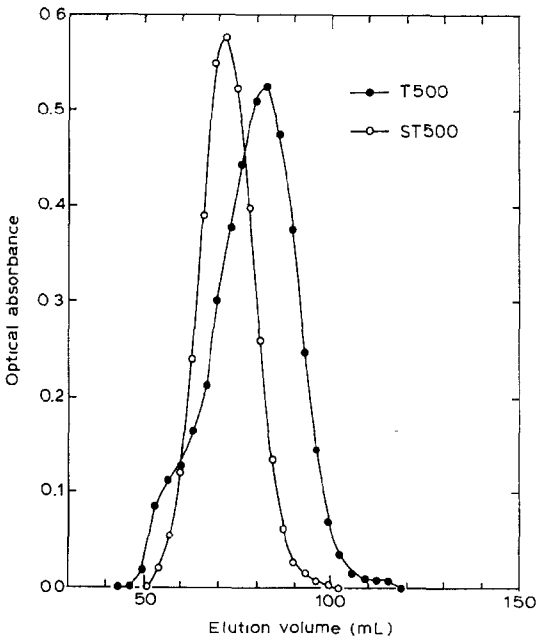

Fig. 2. Gel-permeation chromatograms of dextran solutions.

presence of a very small proportion either of aggregates or of extraordinarily large molecules. Such particles are considered to bring a serious effect into the index of polydispersity on the sample of narrow molecular-weight distribution. At any rate, it is confirmed by light-scattering experiments that T40, T70, and ST500 have nearly the same width of molecular-weight distributions, and that T250 and T500 have broader distributions, which is a result consistent with the gel-permeation study.

Polydispersity correction for the hydrodynamic radius and radius of gyration was conducted *via Eq. 23*, with $\alpha = 0.485$, and the calculated values of C_ξ and ξ

TABLE II

RESULTS OF NUMERICAL CALCULATIONS FROM GEL-PERMEATION DATA, WITH $\alpha = 0.485$

Sample	M_D/M_W	ρ	C_ξ	ξ
T40	1.09	0.08	1.12	
T70	1.10	0.09	1.14	
T250	1.26	0.24	1.37	0.70
T500	1.33	0.30	1.39	0.80
ST500	1.11	0.10	1.14	0.69

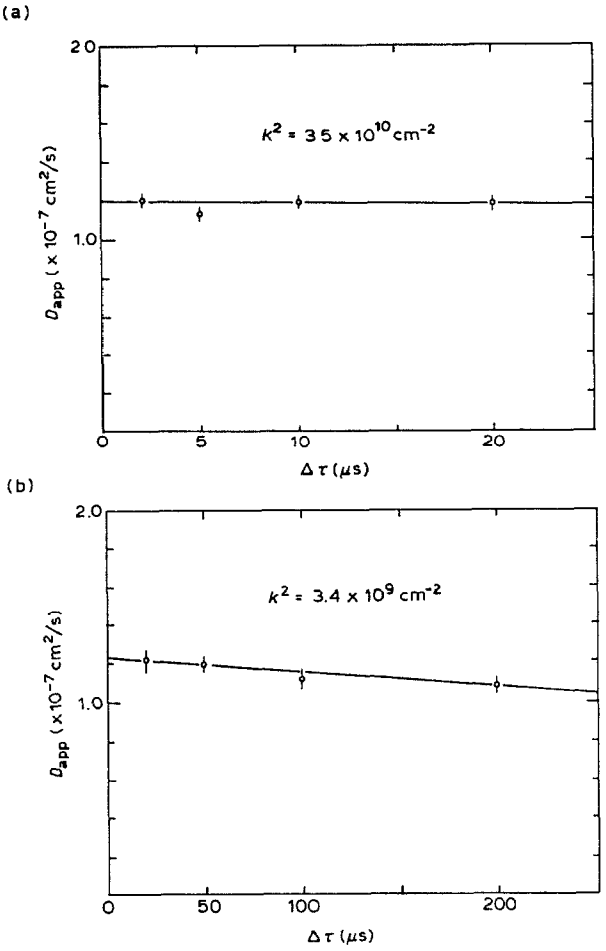

Fig. 3. Dependence of the apparent diffusion coefficient on sampling interval $\Delta\tau$ and scattering angles; 0.1% solution of dextran T500. [(a) $\theta = 90°$, (b) $\theta = 25.5°$.]

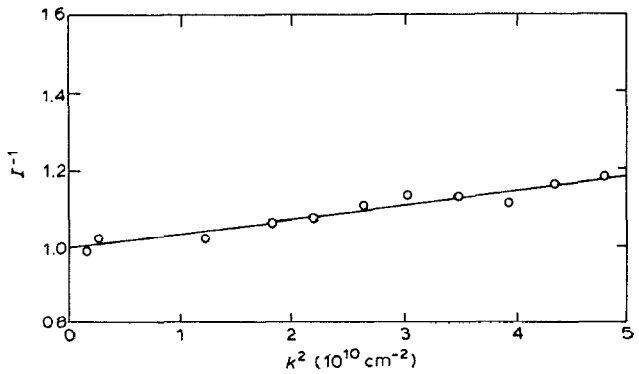

Fig. 4. A plot of reciprocal, relative scattering-intensity *versus* k^2. [0.1% solution of dextran T500.]

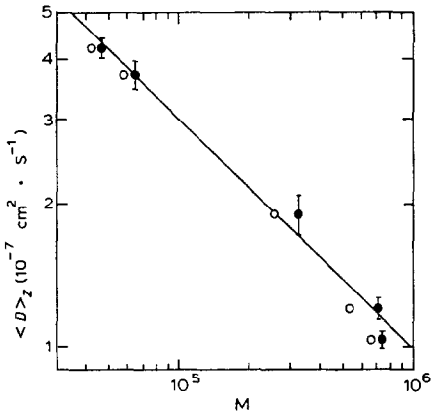

Fig. 5. Double-logarithmic plot of observed z-average diffusion coefficient *versus* molecular weight for dextrans. The open circles correspond to the weight-average molecular weight (M_W) and filled circles to the data corrected for polydispersity (M_D) *via* Eq. *17*.

are listed in Table II. The calculated values of ξ strongly suggest that dextran molecules behave as random coils in solution, and this result is quite consistent with the estimated α-value. The facts that α is a little smaller than 0.5 and that ξ is a little greater than 0.665 suggest that dextran molecules are somewhat more compact than ideal, random coils. This is ascribed to the effect of polymer branching in dextran molecules.

As has been shown here, the light-scattering properties of dextran solutions were well interpreted by taking the gel-permeation data into consideration. It will be of significance to refer here to the model for the molecular-weight distribution of dextrans. As gel-permeation chromatograms of dextrans show relatively narrow, unimodal, and symmetrical distributions, we may represent them by Gaussian distributions; that is,

$$P(v) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left[-\frac{(v - v_0)^2}{2\sigma^2}\right]. \tag{24}$$

Then, we obtain

$$\frac{M_W}{M_N} = \exp(b^2\sigma^2), \tag{25}$$

$$M_D = M_W \exp\left(\frac{1-\alpha}{2} b^2\sigma^2\right) = M_W \left(\frac{M_W}{M_N}\right)^{(1-\alpha)/2}, \tag{26}$$

$$\rho = \exp(\alpha^2 b^2 \sigma^2) - 1 = \left(\frac{M_W}{M_N}\right)^{\alpha^2} - 1, \tag{27}$$

TABLE III

RESULTS OF CALCULATIONS ACCORDING TO THE GAUSSIAN, GEL-CHROMATOGRAM MODEL, WITH $\alpha = 0.484$

Sample	M_D/M_W	ρ	C_ξ	ξ
T40	1.09	0.08	1.12	
T70	1.10	0.09	1.14	
T250	1.24	0.22	1.34	0.69
T500	1.34	0.31	1.49	0.86
ST500	1.11	0.10	1.16	0.71

$$C_\xi = \exp(\tfrac{3}{2}\alpha^2 b^2 \sigma^2) = \left(\frac{M_W}{M_N}\right)^{3\alpha^2/2} . \qquad (28)$$

Using only the values of M_W and M_W/M_N from gel-permeation data, we obtained $\langle D\rangle_z = 7.90 \times 10^{-5}\ M_D^{-0.484}\ cm^2.s^{-1}$ for the best-fit relation between investigated $\langle D\rangle_z$ and calculated M_D *via* Eqs. *16* and *26*. Values calculated according to Eqs. *26–28*, with $\alpha = 0.484$, are listed in Table III. The excellent agreement between Tables II and III convinced us that the Gaussian, gel-chromatogram model (in other words, a logarithmic, Gaussian distribution) is a very good model for the molecular-weight distribution of dextrans.

In summary, the random-coil behavior of dextran molecules in solution was verified by light-scattering experiments, and the efficiency of gel-permeation data on the light-scattering studies of polydisperse, macromolecular solutions was shown. The limitation of quasielastic, light-scattering experiments lay in the difficulty of data analyses in the case of such polydisperse samples as polysaccharide solutions. Now that we can relatively easily gain information about the size distribution of macromolecules by means of gel-permeation chromatography, quasielastic light-scattering should become a powerful technique for the study of polysaccharide solutions.

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