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(39) The first term on the right-hand side of eq 19 in Meier's theory[40] (derived on the basis of the Cahn–Hilliard formula with the assumed density distributions on $\phi_K(x)$ in eq 23) neglects the nonlocal aspects of the conformational entropy[26,28] which arises from the chemical bonding of segmental units, i.e., the requirement of a segmental unit at r having another segment a vector of distance b away. For a symmetric block an explicit formula for λ is obtained by minimizing ΔG with respect to λ[10]

$$\lambda = \frac{6T_A}{\chi_A Z_A} + \left[\left(\frac{6T_A}{\chi_A Z_A}\right)^2 + \frac{1}{6}\pi^2 t_D{}^2\right]^{1/2}$$

λ approaches λ_∞ with increasing molecular weight

$$\lambda \rightarrow \lambda_\infty = (\pi/6^{1/2})t_D$$

or

$$\lambda_l \rightarrow \lambda_{l,\infty} = 0.817t_D$$

The corresponding interfacial thickness a_I predicted from Helfand's theory is given, from eq 33, by

$$a_I = (2/6^{1/2})[(b^2/\chi) + \sigma_I{}^2]^{1/2}$$

and gives a thicker interphase than λ_l. a_I approaches $a_{I,0}$ for small values of b

$$a_I \rightarrow a_{I,0} = (2/6^{1/2})\sigma_I = 0.816\sigma_I$$

Thus in the limit of $b = 0$, the two results become identical.
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(41) The condition $g \gtrsim 0.035$ is necessary; otherwise a 10th-order peak should be resoluble.
(42) This does not mean that all three values t, λ_l, and a_I are in agreement. A comparison of the absolute values is shown in Table III.

Ionic Strength Dependence of the Average Dimension of Low Molecular Weight DNA

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ABSTRACT: Light scattering measurements are reported for sonicated calf-thymus DNA of known contour length (l) distribution in buffer solutions at five different ionic strengths ($5 \times 10^{-3} \leq I \leq 2 \times 10^{-1}$ M). The change with ionic strength of the root-mean-square radius of gyration as determined from these measurements for the relatively short macromolecules ($M_w = 3 \times 10^5$ g mol^{-1}, $l_w = 167$ nm) of high intrinsic rigidity is compared to theoretical predictions based on a wormlike chain with an electrostatic part of the persistence length (Odijk). The agreement is satisfactory if partial charge compensation (condensation) is taken into account.

One of the central problems in the physical chemistry of polyelectrolyte solutions is the influence of the charge interactions on the average dimension of the polyions. Recently a new theoretical approach to this problem was proposed independently by Odijk from this laboratory[1,2] and by Skolnick and Fixman in the U.S.A.[3] Considering a polyion as a wormlike chain with an intrinsic stiffness, characterized by a "bare" persistence length L_p, they calculated the increase in the stiffness due to the charges on the chain interacting through a Debye–Hückel potential with a screening length κ^{-1} depending on the ionic strength I of the solution. The expression for the additional "electrostatic" persistence length L_e is assumed to be valid under the condition that $L_p + L_e \gg \kappa^{-1}$ [3,4] and depends linearly on κ^{-2}. The total persistence length $L_t = L_p + L_e$ may be used to evaluate the average dimension of a polyion, e.g., its root-mean-square radius of gyration. As this quantity at infinite dilution may be obtained through light scattering measurements, these theoretical predictions can, in principle, be checked. A particularly suitable system should be DNA which even at considerably high ionic strength ($I = 0.2$ M) seems to behave in a way consistent with a description as a wormlike chain because of its high intrinsic stiffness.

We present here some experimental results obtained with DNA of low molecular weight (for which excluded-volume effects should be negligible) at five different ionic strengths. We show that the increase in the root-mean-square radius of gyration $\langle S^2\rangle^{1/2}$ over the range $I = 0.2$ M to $I = 0.005$ M is rather small (<5%) and in fair agreement with the theoretical predictions, at least if charge compensation (condensation) is taken into account. However, as the DNA system used was heterodisperse, this conclusion remains preliminary.

Materials and Methods

All chemicals were of analytical reagent grade. Water was deionized by a mixed-bed ion exchanger (Amberlite HB-1). The Na salt of calf-thymus DNA (Worthington Biochemical Co.) of low protein content (<0.9%) was sonicated for 9 min at a concentration of approximately 0.3 mg cm^{-3} in 0.2 NaCl/0.002 M EDTA/0.002 M Na_2HPO_4 buffer, saturated with chloroform, and at a frequency of 21 kHz. The sonicated DNA has been fully characterized as described elsewhere (as DNA II).[5] It was heterodisperse with number-, weight-, and z-average contour lengths

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Table I
Light Scattering Data for the Sonicated DNA Solutions at Different Ionic Strengths

I, M	N[a]	$(\mathrm{d}n/\mathrm{d}c)$,[b] cm^3 g^{-1}	$10^{-5}(M_w)_c$,[c] g mol^{-1}	10^2A_2,[d] mol cm^{-3} g^{-2}	$10^{-5}(M_w)_\theta$,[c] g mol	$\langle S^2\rangle_z^{1/2}$,[e] nm
2×10^{-1}	18	0.166 ± 0.003	3.02 ± 0.02	0.035 ± 0.003	3.08 ± 0.02	45.9 ± 0.5
5×10^{-1}	14	0.170 ± 0.002	3.03 ± 0.02	0.090 ± 0.001	3.08 ± 0.01	45.7 ± 0.3
2×10^{-2}	16	0.172 ± 0.004	3.06 ± 0.04	0.147 ± 0.005	3.12 ± 0.02	47.5 ± 0.5
10^{-2}	12	0.176 ± 0.002	2.97 ± 0.04	0.252 ± 0.006	3.00 ± 0.03	48.0 ± 0.5
5×10^{-3}	12	0.180 ±0.003	2.97 ± 0.06	0.40 ± 0.01	3.00 ± 0.02	47.9 ± 0.3

[a] N is the number of different solutions measured in the light scattering experiments at the I value given. [b] Determined from different solutions from those used in the light scattering experiments. [c] Standard deviations obtained from the standard deviations on the intercept of the linear least-squares fit. [d] Standard deviations obtained from the standard deviations on the slope of the linear least-squares fit. [e] Standard deviations obtained from the standard deviations on slope and intercept of the linear least-squares fit.

l of $l_n = 139$ nm, $l_w = 167$ nm, and $l_z = 190$ nm, respectively, as obtained from electron micrographs. The histograms used in calculating these averages were obtained by the determination of the individual length of a large number of molecules, with an estimated accuracy of 10 nm for a distribution covering a range $30 < l_i < 300$ nm.[5]

Conventional light scattering measurements were performed with a FICA-50 instrument with unpolarized light at $\lambda_0 = 436$ nm and cylindrical cells. All solutions were made dust free by repeated filtration through Millipore filters (0.22 μm). The intensity of the scattered light was measured at 25.0 ± 0.1 °C and at several angles between 30 and 150°. Calibration was performed with benzene of analytical grade. For each solution the intensity of the scattered radiation $I_{c,\theta}$ (in arbitrary units) was transformed into a Rayleigh ratio per unit volume $R_{c,\theta}$ by

$$R_{c,\theta} = \left[\frac{I_{c,\theta} - I_{0,\theta}}{I_{\mathrm{B},90}}\right]\left[\left(\frac{n_0}{n_\mathrm{B}}\right)^2 \sin\theta\right] R_{\mathrm{B},90} \qquad (1)$$

Here $I_{0,\theta}$ stands for the scattering intensity of the pure (complex) solvent at the same angle θ, $I_{\mathrm{B},90}$ for the scattered intensity of benzene at 90° (on the same intensity scale), and the factor $n_0^2n_\mathrm{B}^{-2}$ $\sin\theta$ for the scattering volume correction, with n_B and n_0 representing the refractive indices of benzene and of the solution (in practice, pure water), respectively. For the Rayleigh ratio of benzene at 90°, Coumou's value[6] was used, $R_{\mathrm{B},90} = 4.56 \times 10^{-5}$ cm^{-1}, although it is given at 23 °C. According to Kratohvil et al.[7] the temperature coefficient of $R_{\mathrm{B},90}$ is approximately 0.5% per degree and the total change from 23 to 25 °C is thus less than the uncertainty on the absolute value as given above.

The light scattering measurements were performed with five different sets of solutions obtained from the same sample of DNA and identical buffers of different ionic strengths (see Table I). The latter were the original buffer used in the sonication procedure and buffers obtained therefrom by dilution with water. The ionic strength of these solutions will in a first approximation be taken equal to the molarity of NaCl (e.g., a buffer indicated by $I = 5 \times 10^{-2}$ M is the original buffer diluted four times). At each ionic strength a series of solutions of varying concentration of DNA was prepared. For each series the solution of highest DNA concentration was first exhaustively dialyzed against the complex solvent (buffer solution) of corresponding ionic strength and dilutions were performed with the same.

For each ionic strength the highest concentration of DNA (in monomol L^{-1}) was at least a factor 3 smaller than the ionic strength of the buffer. After the scattering measurement the concentration of DNA in each solution was determined by extinction.

Refractive index increments at constant chemical potential of the buffer $(\mathrm{d}n/\mathrm{d}c)_\mu$ were determined with the help of a Brice-Phoenix differential refractometer at $\lambda_0 = 436$ nm and 25 °C. For each ionic strength at least five different DNA solutions, generally more concentrated than those used in the scattering experiments, were prepared and exhaustively dialyzed for several days against the pure buffer. The refractive index increment was found to increase slightly but significantly with decreasing I (see Table I). The value at $I = 0.2$ M was in excellent agreement with the value given by Godfrey and Eisenberg[11] for DNA in the same buffer.

No denaturation on dilution of the DNA is to be expected at the ionic strengths considered, as we have found that for the same sample of DNA but at a lower ionic strength $I = 3 \times 10^{-4}$ M no change in the extinction coefficient could be detected in the concentration range $10^{-4} < c < 2 \times 10^{-3}$ monomol L^{-1} (the monomolar mass of calf-thymus DNA being taken equal to 330.9 g monomol^{-1}).

Results

The scattering intensity of a large number of different DNA concentrations was measured at each ionic strength. For each solution the Rayleigh ratio $R_{c,\theta}$ was used to calculate the scattering function $K(\theta)c/R_{c,\theta}$, where c is the concentration (in g cm^{-3}) and $K(\theta)$ is given by

$$K(\theta) = \frac{2\pi n_0^2}{N_{\mathrm{Av}}\lambda_0^4}\left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)_\mu^2(1 + \cos^2\theta) \qquad (2)$$

with N_{Av} representing Avogadro's constant. These data were analyzed by the classic Zimm procedure, using the basic equation for the light scattering of macromolecular solutions which, for a homodisperse sample, is given by

$$\frac{K(\theta)c}{R_{c,\theta}} = M^{-1}\left[1 + \frac{1}{3}\left(\frac{4\pi n_0}{\lambda_0}\right)^2\langle S^2\rangle \sin^2\frac{\theta}{2} + \mathrm{O}\left(\sin^4\frac{\theta}{2}\right)\right] + 2A_2G(\theta)c + \mathrm{O}(c^2) \qquad (3)$$

Here M stands for the molar mass, $\langle S^2\rangle$ for the mean-square radius of gyration, A_2 for the second virial coefficient, and $G(\theta)$ for a factor depending on both inter- and intramolecular interference (with $G \to 1$ for $c \to 0$, $\theta \to 0$). The expression between square brackets in the first term on the right-hand side of (3) is the series expansion in powers of $\sin^2(\theta/2)$ of the single-particle scattering factor P^{-1}. For heterodisperse systems, M in (3) must be replaced by the weight-average molar mass M_w, $\langle S^2\rangle$ by the z-average mean-square radius of gyration $\langle S^2\rangle_z$, and A_2 by $\tilde{A}_2$ which is a rather complicated expression.[9]

For each solution (at constant c and I) the scattering function was found to depend linearly on $\sin^2(\theta/2)$ for angles $30 < \theta < 105°$. Values of $(Kc/R)_{\theta=0}$ could thus be obtained by a linear least-squares (unweighted) extrapolation of (Kc/R) against $\sin^2(\theta/2)$. These values were in turn extrapolated to zero concentration by another least-squares fit of $(Kc/R)_{\theta=0}$ vs. c, yielding a value of M_w indicated in Table I by $(M_w)_c$. From the slope of this least-squares straight line the value of $\tilde{A}_2$ was obtained. The molar mass was also found by another extrapolation: first Kc/R is extrapolated to zero concentration at constant angle followed by an extrapolation of $(Kc/R)_{c=0}$ to $\sin^2(\theta/2) = 0$, yielding $(M_w)_\theta$. From this value and the slope of the linear part of the curve $(Kc/R)_{c=0}$ vs. $\sin^2(\theta/2)$, $\langle S^2\rangle_z^{1/2}$ can be obtained (see Table I). Both extrapolations could be easily performed by a linear least-squares fit, the latter one being limited to seven angles between 30 and 105°. The least-squares straight lines $(Kc/R)_{c=0}$ vs. $\sin^2$

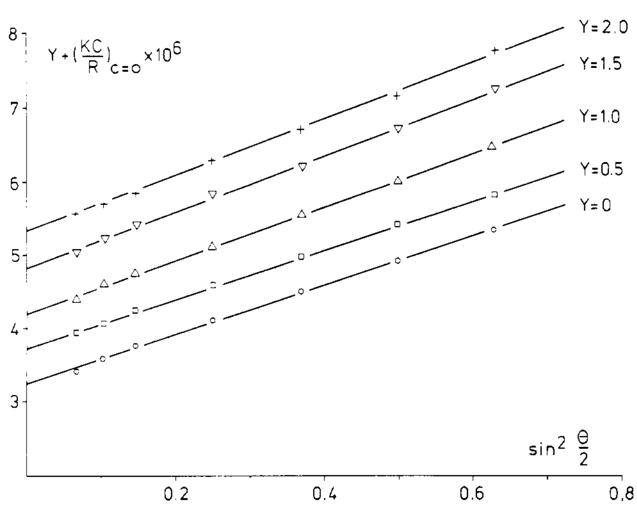

Figure 1. Limiting straight lines $(Kc/R)_{c=0}$ (in mol g^{-1} × 10^6) vs. sin^2 $(\theta/2)$ for different ionic strengths: (○) 2 × 10^{-1} M; (□) 5 × 10^{-2} M; (△) 2 × 10^{-2} M; (▽) 10^{-2} M; (+) 5 × 10^{-3} M. Each line is shifted upward with a constant Y.

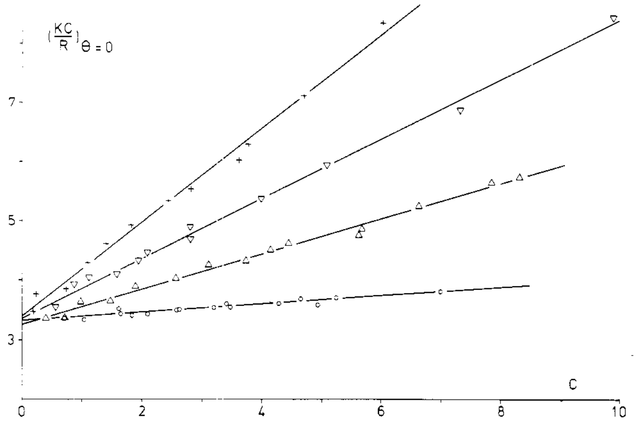

Figure 2. Limiting straight lines $(Kc/R)_{\theta=0}$ (in mol g^{-1} × 10^6) vs. concentration c (in g cm^{-3} × 10^4) for different ionic strengths (symbols as in Figure 1).

$(\theta/2)$ and $(Kc/R)_{\theta=0}$ vs. c for different sets of solutions are shown in Figures 1 and 2, respectively. Two representative Zimm plots for different values of I are given in Figures 3 and 4.

The values of M_w collected in Table I show that the agreement between $(M_w)_c$ and $(M_w)_\theta$ at each given I is reasonably good and that each of these M_w values is consistent with their mean calculated with all ten values available, $\bar{M}_w = (3.03 \pm 0.06) \times 10^5$ g mol^{-1} (the error given for this mean corresponds to the standard deviation with respect to that mean). This seems to indicate that the different least-squares fits are reliable and thus also the values of $\langle S^2\rangle_z^{1/2}$ obtained therefrom.

Discussion

From the values of the root-mean-square radius of gyration given in Table I it may be seen that the change with ionic strength in the average dimension of low molecular weight DNA at infinite dilution is surprisingly small. Over the range of ionic strength considered the maximum relative change in $\langle S^2\rangle_z^{1/2}$ is less than 5% whereas the *absolute* inaccuracy in each single value may be estimated to be 1% or more. This is in strong contrast to the relatively large increase in $\tilde{A}_2$ with decreasing I. All values of $\langle S^2\rangle_z^{1/2}$ are, however, still significantly smaller than the root-mean-square radius of gyration for completely stretched chains (see hereafter).

Assuming the low molecular weight DNA to behave as a wormlike chain at infinite dilution, we should, in principle, be able to relate the change of the root-mean-square radius of gyration to the change of the persistence length L_t by taking into account the polydispersity of the sample. The z-average mean-square radius of gyration of a polydisperse sample can be defined with the help of the distribution of contour lengths according to the expression

$$\langle S^2\rangle_z = \sum_i \langle S^2\rangle_i l_i^2 p_i \Big/ \sum_i l_i^2 p_i \qquad (4)$$

where $\langle S^2\rangle_i$ represents the mean-square radius of gyration of the molecules with contour length l_i characterized for the given sample by a distribution probability p_i. The value of $\langle S^2\rangle_i$ for relatively short chains without interactions can be evaluated according to the expression derived by Benoit and Doty[10]

$$\langle S^2\rangle_i = 2x_i l_i^2 \left[\frac{1}{6} - \frac{x_i}{2} + x_i^2 - x_i^3(1 - e^{-1/x_i}) \right] \qquad (5)$$

with $x_i \equiv L_t l_i^{-1}$. There are, however, two difficulties we have to face. The first is that the absolute value of $\langle S^2\rangle_z$ as obtained from light scattering may show a systematic error as no anisotropy correction has been applied, but this will probably be of minor importance. The other difficulty is that the distribution of contour lengths as obtained from the electron micrographs should be considered with proper care, as errors are introduced by the handling of the sample on the one hand and the actual determination of the l_i values on the micrographs on the other hand. Let us consider this more in detail.

The distribution as determined from the micrographs yields the values of l_n, l_w, and l_z as given above; in particular

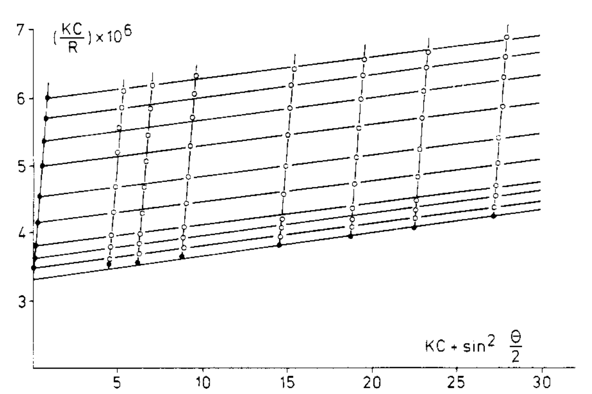

Figure 3. Zimm plot for DNA solutions at I = 5 × 10^{-2} M (k = 50000 cm^3 g^{-1}). For the sake of clarity not all solutions measured have been represented.

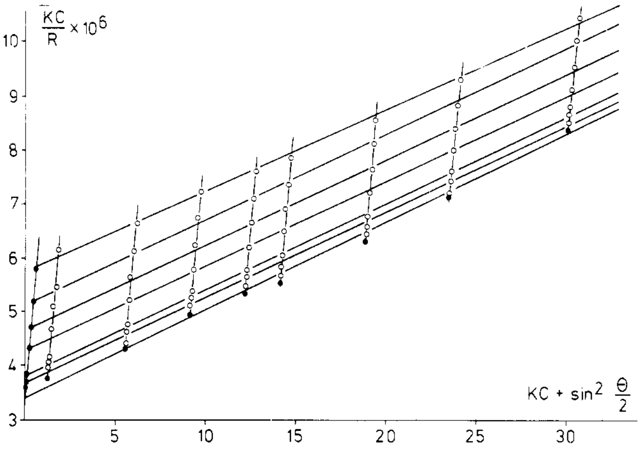

Figure 4. Zimm plot for DNA solutions at I = 5 × 10^{-3} M (k = 50000 cm^3 g^{-1}). For the sake of clarity not all solutions measured have been represented.

$l_w = 167$ nm. We can assume that the inaccuracy on this value is at least that on the individual contour length determination, i.e., 10 nm. With values $l_w = 167 \pm 10$ nm and $\bar{M}_w = (3.03 \pm 0.06) \times 10^5$ g mol^{-1} the molar mass per unit length M_l may be estimated to be $M_l = 1810 \pm 50$ g mol^{-1} nm^{-1}. This is in reasonable agreement with the value accepted for the B form of DNA; i.e., $M_l = 1950$ g mol^{-1} nm^{-1}. Next let us examine the value of $\langle S^2\rangle_z^{1/2}$ at the highest ionic strength ($I = 0.2$ M) for which the total persistence length of DNA has been extensively studied. According to the best estimates of L_t for DNA of different molar masses, as determined by light scattering,[11,12] sedimentation, and viscosity,[11,13-15] $L_t = 55 \pm 10$ nm at $I = 0.2$ M. With this value and the distribution of contour lengths of our sonicated DNA sample, we calculate $\langle S^2\rangle_z^{1/2} = 42 \pm 2$ nm, which is somewhat lower than our experimental value (see Table I). However, the former does not take into account the possible errors on the contour lengths. If we estimate $\langle S^2\rangle_z^{1/2}$ in a first approximation (and as a *lower limit*), using (5) and the z-average contour length $l_z = 190 \pm 10$ nm instead of the contour length distribution, we find $\langle S^2\rangle_z^{1/2} > 41 \pm 4$ nm, in agreement with the experimental value. It may thus be concluded that the inaccuracy on the absolute value of l_i has some influence on the theoretical evaluation of quantities averaged over the given distribution. Therefore, in order to compare the values found for $\langle S^2\rangle_z$ at different ionic strengths to the theoretical predictions, we shall not use absolute values for the z-average root-mean-square radius of gyration. We shall rather compare the change with ionic strength of the ratio r

$$r = [\langle S^2\rangle_z^{1/2}]_I / [\langle S^2\rangle_z^{1/2}]_{I=0.2\,\mathrm{M}} \tag{6}$$

for the values of Table I to the ratio calculated with the help of the theoretical expressions for L_t, assuming $L_t = 55$ nm at $I = 0.2$ M, and the distribution of contour lengths as found from electron micrographs. In this way we hope to reduce to some extent the influence of the inaccuracy on the absolute value of l_i in the comparison between experiments and theory.

For the theoretical value of the total persistence length, we shall use the following expression of Odijk,[1,2] derived for a wormlike chain having a homogeneous charge density with a potential of mean force between charges given by a Debye–Hückel potential:

$$L_t = L_p + L_e = L_p + \frac{Q}{4\kappa^2 A^2} f \tag{7}$$

Here $Q = q^2/\epsilon kT$ is the Bjerrum length (with q the elementary charge and ϵ the electric permittivity of the solvent, k the Boltzmann constant, and T the absolute temperature, all expressed in cgs units) and A is the contour distance between the charges on the chain. The Debye–Hückel screening length κ is defined by

$$\kappa^2 = 8\pi Q I \tag{8}$$

The factor f in (7) takes into account the possibility that through the strong interactions between polyion and the counterions part of the charges on the chain is effectively reduced. If no effective charge compensation occurs, $f = 1$; otherwise $0 \le f \le 1$. According to simple counterion condensation theory[16,17] $f = 1$ as long as the charge density parameter $\lambda \equiv Q/A$ is smaller than unity; if $\lambda > 1$, condensation increases the effective value of A to $A_{\mathrm{eff}} = Q$ ($\lambda_{\mathrm{eff}} = 1$) and $f = A^2/Q^2$.

At 25 °C the Bjerrum length in water is 0.71 nm. For DNA in the B form, assumed to exist predominantly in water, the value of A is 0.17 nm. Therefore $\lambda > 4$ and condensation should occur. We have estimated L_e with the help of (7), using two extreme values for f, i.e., L_e' with $f = A^2/Q^2$ and L_e'' with $f = 1$. The value of L_e' changes from 0.16 nm at $I = 0.2$ M to 6.54 nm at $I = 0.005$ M and for L_e'' from 2.9 to 116.6 nm under the same conditions. We have represented in Figure 5 as fully drawn curves the change of the theoretical value of r with ionic strength, using both L_e' and L_e'', if $L_t = 55$ nm is assumed at $I = 0.2$ M. (Note that the curve with L_e' would practically remain unchanged and the one with L_e'' only slightly lowered if we had used a somewhat larger value for L_t at $I = 0.2$ M, e.g., $L_t = 66$ nm.) The corresponding experimental values have also been indicated in the same figure. These turn out to lie close to the theoretical curve calculated with L_e'. Deviations with respect to the latter could arise among other things from the approximations involved in the derivation of (7) and an underestimation of f. Thus the agreement with the theoretical result, taking into account partial charge compensation, is reasonable. This may, of course, be due to some compensation of effects, as the adequacy of the simple model used in the theory for the electrostatic persistence length in as far as DNA is concerned may be questioned.

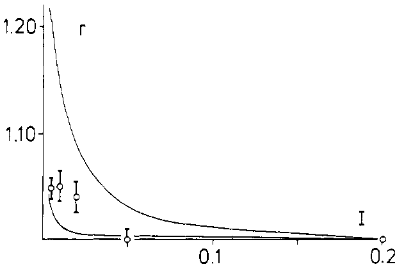

Figure 5. Comparison between the theoretical values of the ratio r (eq 6) and the experimental results (○): upper curve calculated using L_e''; lower curve calculated using L_e' (see text).

Certainly, even with all uncertainties involved in the present comparison, the theoretical predictions without any charge compensation (L_e'') fall outside consideration as is to be expected given the high charge density of DNA. Analogously, even at the lowest ionic strength considered here, the low molecular weight DNA, is, on the average, not yet fully stretched. For the given distribution of contour lengths rodlike particles would yield $\langle S^2\rangle_z^{1/2} = 57$ nm, not taking into account the absolute error on l_i. Using $l_z = 190 \pm 10$ nm to estimate the lower limit of that quantity would give $\langle S^2\rangle_z^{1/2} > 55 \pm 3$ nm. Both values are definitely larger than the root-mean-square radius of gyration found experimentally even at $I = 0.005$ M.

These results seem be be in conflict with the conclusions arrived at in a recent paper by Harrington[18] for T2 DNA. As shown by Odijk,[19], however, for the systems studied by Harrington, due to the high molar mass of the DNA ($M = 1.3 \times 10^8$ g mol^{-1}, $l = 7 \times 10^4$ nm), the excluded-volume effects are the dominant factor responsible for the observed expansion of the charged chains.

It may therefore by concluded that for rather short chains of high rigidity for which excluded-volume effects may be neglected, as is the case for low molecular weight DNA, the experimental results for the average dimensions as function of the ionic strength are in reasonably good agreement with the theory based on a wormlike chain with electrostatic interactions. It should be noted, however, that more experimental results, especially results obtained with homodisperse material, are needed before this tentative conclusion can be made more definite.

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Determination of Molecular Weight Distributions from Frequency-Analyzing (Dynamic) Light Scattering†

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ABSTRACT: A general method for the determination of molecular weight distributions from frequency-analyzing (=dynamic) light scattering is described. The measured autocorrelation functions are used to construct a curve which is not influenced by interaction between different particles and intramolecular interferences. This is done by a special extrapolation method. The method is applied to polystyrene samples with molecular weights $\bar{M}_w$ from 2.5×10^5 to 5×10^6 and different polydispersities, using toluene as solvent. The investigated system shows pronounced concentration and intramolecular interferences (scattering function and intramolecular chain motions) which are successfully eliminated. The molecular weights and polydispersities obtained by the new procedure are in good agreement with those from independent measurements.

For frequency-analyzing light scattering (FAN LS)[23] of polymers in solution a number of methods have been proposed[1–14] which permit characterization of the molecular weight distribution (MWD) of the scattering particles. Most of these methods are quite special and somewhat restricted in application. Often concentration effects are neglected, though this is not always permissible. We describe here a method which eliminates all dependencies on scattering parameter and concentration.

We introduce the autocorrelation function (ACF) in the usual form as a function of the scattering parameter $k = (4\pi n/\lambda_0) \sin (\vartheta/2)$ and the correlation time t, where λ_0, n, and ϑ have their usual meanings. The influence of concentration c is taken into account by regarding the ACF as a function of $h(c)$, which must strictly increase with c, be differentiable, and vanish for $c \rightarrow 0$; then the inverse function h^{-1} with $h^{-1}(h(c)) = c$ exists. The concentration effects, and with them the correlation of different particles, are eliminated by extrapolating the measured ACF to $h(c) \rightarrow 0$.

To provide a generally applicable procedure, it is necessary to construct from the measured autocorrelation curves a function such that the effects of intramolecular motions and of the scattering function are eliminated, while at the same time full information on the MWD is retained.

For the theoretical derivations we thus restrict ourselves to small scattering parameters k for which only translational diffusion influences the scattered light. In this case[15–18] the expression for the autocorrelation function $C_{\mathbf{ex}}{}^{\mathrm{hm}}$ of the homodyne technique is independent of the shape of the scattering particles and is given by

$$\lim_{h(c)\rightarrow 0} C_{\mathbf{ex}}{}^{\mathrm{hm}}[k, t, h(c)] = A\left\{\bar{M}_{\mathrm{w}}{}^{-1} \int_0^\infty Mf(M)P(k, M)e^{-k^2D_0(M)t}\, \mathrm{d}M\right\}^2 + B \quad (1)$$

where $f(M)$ is the weight-fraction MWD of the scattering molecules with molecular weight M, $P(k, M)$ is the scattering function, and $D_0(M)$ reflects the M dependence of the diffusion coefficient at vanishing concentration. A and B are linear parameters still to be specified. Application of eq 1 requires that values of k be sufficiently small; however, in general, it is not known just how small they have to be.

Theory

In order to solve the problem of using sufficiently small scattering parameters k, we replace the generalized coordinates $\{k, t\}$ of the optical system in the ACF by

$$\{g(k), t'\}: = \{g(k), k^2t\} \quad (2)$$

For $g(k)$ the same conditions as for $h(c)$ have to be fulfilled. With the generalized or optical time t' eq 1 then becomes

$$\lim_{h(c)\rightarrow 0} C_{\mathbf{ex}}{}^{\mathrm{hm}}[g(k), t', h(c)] = A\left\{\bar{M}_{\mathrm{w}}{}^{-1} \int_0^\infty Mf(M)P\{g^{-1}[g(k)], M\}e^{-D_0(M)t'}\, \mathrm{d}M\right\}^2 + B \quad (3)$$

For finite scattering parameters, eq 1 and 3 contain the same information about the scattering system and are thus equivalent. But when the identity

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† Dedicated to Professor G. V. Schulz on the occasion of his 75th birthday.

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