Effect of Temperature on the Flexibility of Cellulose Tricarbanilate in Dioxane and Ethyl Acetate by Dielectric Measurements

A. K. Gupta,[1a] E. Marchal,*[1a] and W. Burchard[1b]

Centre de Recherches sur les Macromolécules (C.N.R.S.), 67083 Strasbourg Cedex, France, and the Institute of Macromolecular Chemistry, University of Freiburg, Freiburg im Breisgau, West Germany. *Received May 13, 1975*

ABSTRACT: Dielectric measurements on cellulose tricarbanilate (CC) in dioxane and ethyl acetate were made in the frequency range 100 Hz–2 MHz, in order to study the stiffness of the molecules in these solvents at different temperatures. In both these solvents CC was found to undergo a reversible thermally induced conformational change from a less flexible to a more flexible conformation as temperature increased. In both solvents, the temperature range in which this change occurs was higher for higher molecular weight samples, whereas for a given molecular weight it was higher in the case of ethyl acetate than in dioxane. The pronounced chain length dependence of the transition temperatures is compared with similar effects observed with polypeptides and polynucleotides, and is shown to be in agreement with theories based on the Ising model of finite chains. Persistence lengths of CC in dioxane were calculated from these data and its values were 108 Å at 25°C and 50 Å at 90°C, in the range of molecular weights 4×10^4–4×10^6. These values are about 25% smaller than those obtained from light scattering and optical stress coefficient measurements of other authors. The reason for this deviation is discussed.

Polymers whose repeat unit has a dipole moment component parallel to the chain contour will show low-frequency molecular weight dependent dielectric relaxation in solution. Cellulose derivatives belong to this class of polymers. An investigation on the dielectric behavior of cellulose acetate and ethyl cellulose has been reported.[2] For the present investigation high molecular weight cellulose tricarbanilate (CC) was used. Its optical and hydrodynamic properties show marked deviations from those which would be observed for chains whose conformation is characterized by Gaussian statistics.[3-5] The aim of this work is to study the influence of temperature on the chain stiffness of CC in dioxane and ethyl acetate.

The end-over-end rotational time and dipole moment of CC were evaluated from the frequency dependence of the dielectric permittivity in dilute solution. The critical frequency f_c was taken to be that at which $\epsilon' = (\epsilon_0 + \epsilon_\infty)/2$, ϵ_0 and ϵ_∞ being respectively the low- and high-frequency limits of the dielectric constant, ϵ', of the solution. It is related to the rotational diffusion constant D by $D = \pi f_c = \frac{1}{2}\tau$ where τ is the relaxation time. For rodlike molecules of length L and radius b rotating about their minor axis (i.e., if the dipole moment is parallel to the major axis), in a solvent of viscosity η

$$f_c = \frac{3kT}{\pi^2 \eta L^3} [\ln (L/b) - \gamma] \tag{1}$$

Different values of γ have been given by several authors. A plot of $\log f_c$ vs. $\log M$ in the range $10 < L/2b < 100$ gives a straight line of slope $\beta = -2.54$ if Broersma's expression[6] for γ is used. For Gaussian coils whose dipole moment vector is proportional to the end-to-end distance, this slope depends on the exponent in the Mark–Houwink expression between limiting viscosity index and M. The relation between the critical frequency relative to the first normal mode of vibration and M[7] is

$$f_c = CT/M[\eta]\eta \tag{2}$$

C is a factor depending on the degree of hydrodynamic shielding and other constants. Thus, for Gaussian coils the $\log f_c - \log M$ slope β satisfies

$$1.5 \leqslant \beta < 2.0$$

For polydisperse samples f_c defined above cannot be related in a simple manner to any definite molecular weight average. One of the authors[8] has shown that the value of β obtained by the exact procedure taking polydispersity into account does not differ very much from the case where f_c is associated with M_w, in the case of semiflexible helices. In the present work, polydispersity, which is low, is not taken account of.

The relation between $\epsilon_0 - \epsilon_\infty$ and the mean-square dipole moment, $\overline{\mu^2}$, of the molecule depends on the theories relating the applied field and the other orienting field acting on the particle. We use the Barriol and Weissbecker[9] formulation, based on the Onsager internal field concept. A discussion on its applicability to macromolecules has been given in an earlier publication.[35] Assuming $\Delta\epsilon = \epsilon_0 - \epsilon_\infty$ is small compared with the dielectric constant of the solvent ϵ this relation is:

$$\frac{\overline{\mu^2}}{M} = \frac{9kT}{4\pi N_a} \frac{2\epsilon^2 + n^4}{\epsilon^2 (n^2 + 2)^2 d} \frac{\Delta\epsilon}{w} \tag{3}$$

where w is the weight fraction of the solute, d the density of the solution, n the refractive index of the solvent, N_a Avogadro's number, and k the Boltzmann constant.

The polar groups in CC are situated in the main chain as well as the side chain (see Figure 1); the possible occurrence of intramolecular bonds has been discussed elsewhere.[3]

Experimental Section

Apparatus. Two impedance bridges, already described elsewhere,[10] were used to cover the frequency range 100 Hz to 20 KHz and 20 KHz to 2 MHz. The two cells used were made of stainless steel cylindrical electrodes having air capacitances 89 and 196 pF. The cell was enclosed in a cylindrical copper chamber connected to the thermostat to maintain its temperature constant within ±0.1°C.

Light-scattering measurements were made on Fica photogoniometers (Model 50 and Model 42000) at the wavelengths γ_0 3650, 4360, and 5460 Å. Details are given elsewhere.[4] Refractive index increments of 0.163 at 4360 Å and 0.151 at 5460 Å in dioxane at 20°C were used.[4]

Materials. The CC samples were prepared by treating bleached linters with phenyl isocyanate in hot pyridine.[11] High molecular weight samples were obtained by fractionation of the original CC in acetone with water as precipitant. To get lower molecular weights the bleached linters were degraded by hydrolysis with HCl solution at 80°C. After conversion to carbamate derivatives the product was fractionated as before. Special care was given to the purification of the CC products from biphenyl urea and phenyl

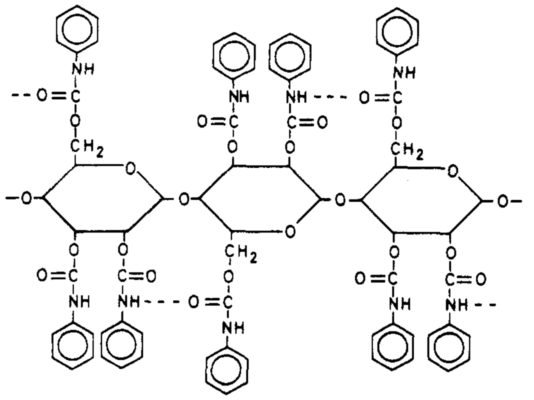

Figure 1. Structure of cellulose tricarbanilate. Dotted lines represent the hydrogen bonds.

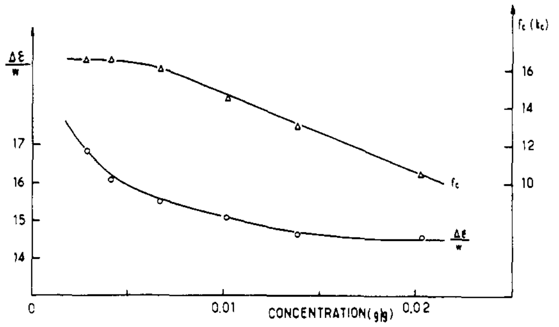

Figure 2. Variation of $\Delta\epsilon/w$ and critical frequency of CC (M = 230000) in ethyl acetate with concentration at 25°C.

methyl urethane. Extraction with hot methanol and a twofold reprecipitation from dioxane in water were applied. The degree of substitution was determined by analysis of nitrogen, which was found to lie between 8.15 and 8.30%, theoretical 8.09%. The heterogeneity of some of the fractions was determined by analytic fractionation and found to be $M_w/M_n = 1.10 \pm 0.05$.

The molecular weights were determined by light scattering and lie in the range $43600 < M < 385000$ for the fractions studied.

Freshly distilled dioxane and ethyl acetate were used and care was taken to minimize exposure to atmosphere in order to avoid absorption of humidity. The polymer was allowed to dissolve at room temperature at least 12 hr before measurements. Concentrations were in the range of $1 \times 10^{-3} < w < 2 \times 10^{-2}$ g/g.

Results

(a) Concentration Effect. In ethyl acetate the dipole moment was found to vary with concentration for all molecular weights. This apparent dipole moment showed a continuous increase with decreasing concentration. Critical frequency, on the other hand, first increased as concentration was lowered, then attained a constant value for $w < 5 \times 10^{-3}$ g/g. In dioxane constant values of both dipole moment and critical frequency were obtained at concentrations lower than 7×10^{-3} g/g. Typical curves of this concentration effect are shown in Figures 2 and 3. Thus in dioxane, there seems to be no interaction between polymer molecules in the lower concentration region. In ethyl acetate the reason for the continuous increase in apparent dipole moment with decreasing concentration may be very complicated and we limited our investigations to those concerning f_c in this solvent.

(b) Temperature Effect. Both in dioxane and ethyl acetate the quantity $\eta f_c/T$ was found to vary with temperature as shown in Figures 4 and 5. This change was reversible. The increase of $\eta f_c/T$ with increasing temperature indicates that the molecules have larger overall dimensions at lower temperatures. Furthermore, the S-shaped character of the curve could imply some cooperativity in this conformational change although it is not a sufficient condition. It may be due to the breaking of intramolecular hydrogen bonds indicated by dots in Figure 1. The range of temperature in which this change occurs, i.e., ~40°C, is rather broad compared, for example, with that of the helix-coil transition of polypeptides.[12] Furthermore, the temperature range of the conformational change was found to depend on molecular weight. The higher the molecular weight, the higher was the temperature of such a transition. In addition for the same molecular weight sample it was higher in ethyl acetate than in dioxane. This is clearly seen in Figures 6 and 7 where these transition curves for various molecular weights are shown using an arbitrary scale on the ordinate axis. The values of $\eta f_c/T$ at various temperatures

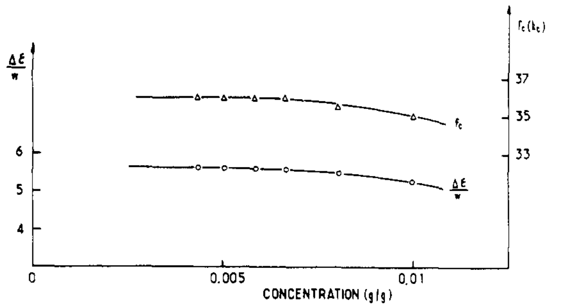

Figure 3. Variation of $\Delta\epsilon/w$ and critical frequency of CC (M = 63000) in dioxane with concentration at 25°C.

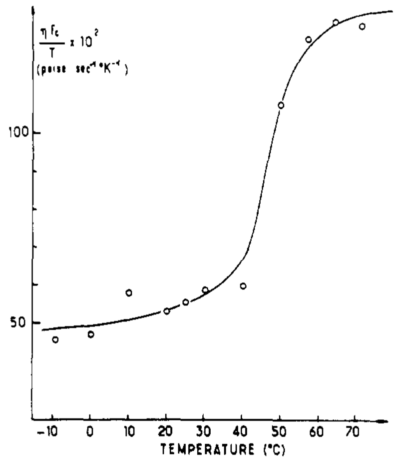

Figure 4. Variation of $\eta f_c/T$ with temperature for CC (M = 130000) in ethyl acetate.

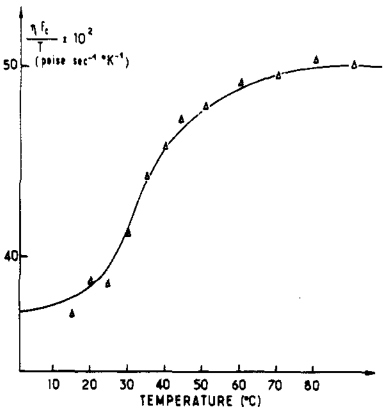

Figure 5. Variation of $\eta f_c/T$ with temperature for CC (M = 130000) in dioxane.

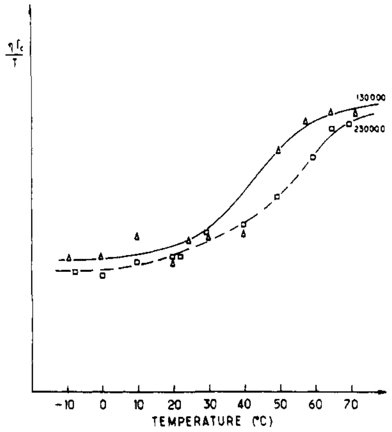

Figure 6. Variation of $\eta f_c/T$ with temperature for two samples of CC in ethyl acetate. Arbitrary scale has been used on the ordinate axis.

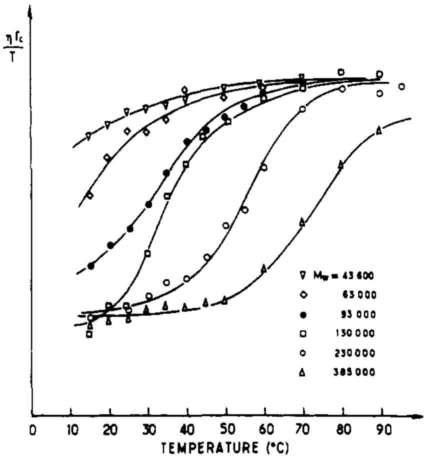

Figure 7. Variation of $\eta f_c/T$ with temperature for different molecular weight samples of CC in dioxane. Arbitrary scale has been used on the ordinate axis.

are given in Tables I and II. The experimental range of temperature was limited by the boiling and freezing points of the solvents.

Variation of the apparent dipole moment at one concentration during the thermal transition is shown in Figure 8. If we look at these variations as incomplete S-shaped curves two remarks can be made: (i) the conformational change measured by critical frequency appears at higher temperature than that obtained from dipole moment values; (ii) the range of temperatures in which it occurs is broader for dipole moment than for critical frequency. A similar observation has been reported[13] for the helix-coil transition of polypeptides and has been attributed to a larger effect of conformational changes on dipole moments at the early stage of flexibility change of the rigid particle.

Discussion

The striking feature of the $\eta f_c/T$ curves, Figure 7, is the strong chain-length dependence of the temperature range where a conformational change from a comparatively stiff chain to a more flexible one occurs. As already mentioned, the appearance of two plateau regions and the S-shaped transition curves are strong indications for well-defined conformational states of the chains similar to those of the helical and coil states of polypeptides and polynucleic acids. Hence our experiments confirm structural peculiarities of CC as were suggested by one of the authors ten years ago as a result of light-scattering measurements in various solvents.[14] A closer comparison of our results with those obtained from polypeptides[15,16] and oligo adenylic acid (oligo A)[17] reveals that the chain-length dependence of the transition temperature observed with CC is also typical of strong linear nearest neighbor interaction. In fact, the theory of strongly cooperative processes of order–disorder along chains of finite lengths predicts a more or less pronounced chain-length dependence of the transition temperature.[18–20] Its magnitude is partly caused by the difference in the free-energy content of the chain elements at the end of the chain to that of an internal chain element in the H-bonded state. This different free energy content is caused, on the one hand, by the fact that the chain units at the end can be fixed in space by only half of the H bonds (see Figure 1); on the other hand, it has its origin in the bulky additional carbamate residues in respectively the C_1 and C_4 positions of the glucose rings at the ends.

The different chemical structure of CC with respect to polypeptides should also affect the characteristic cooperativity parameters, which again affect the chain-length dependence. For polypeptides there is a drastic change in persistence length as the thermal transition proceeds (of the order of 100-fold), whereas the change is small for CC

Table I
Values of $\eta f_c/T$ for CC in Ethyl Acetate

Temp, °C	$\eta f_c/T$, P sec^{-1} °K^{-1} M = 130000	M = 230000
0	0.469	0.132
20	0.528	0.183
40	0.599	0.275
64	1.284	
65		0.545

Table II
Values of $\eta f_c/T$ for CC in Dioxane

	$\eta f_c/T$, P sec^{-1} °K^{-1}					
Temp, °C	M = 43600	M = 63000	M = 93000	M = 130000	M = 230000	M = 385000
15	2.690	1.385	0.769	0.370	0.149	0.058
25	2.825	1.466	0.868	0.386	0.154	0.062
40	2.875	1.667	1.092	0.460	0.169	0.067
60	2.940	1.666	1.193	0.493	0.226	0.086
80			1.224	0.506	0.268	0.141

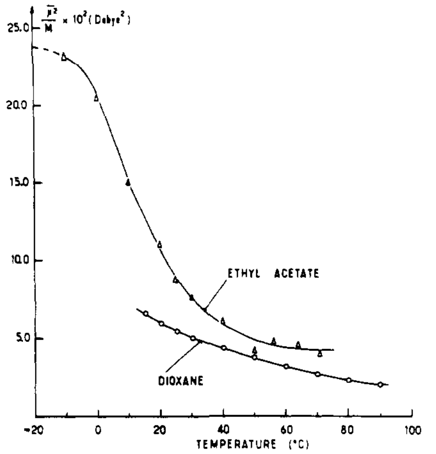

Figure 8. Variation of dipole moment with temperature of CC (M = 130000) in dioxane and in ethyl acetate; w = 0.005 g/g in both solvents.

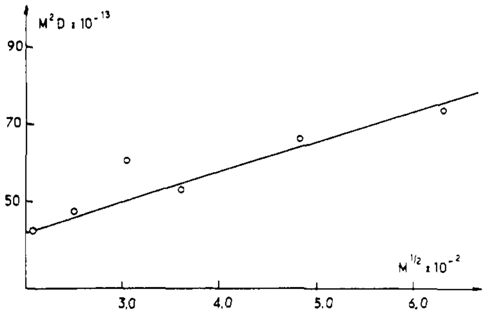

Figure 9. Variation of M^2D with $M^{1/2}$ for CC in dioxane at 25°C.

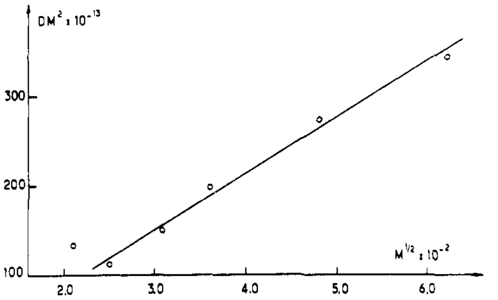

Figure 10. Variation of M^2D with $M^{1/2}$ for CC in ethyl acetate at 25°C.

(about a factor of 2, as will be shown). In other words, the nucleation parameter σ of the helix-coil transition theory would be considerably larger for the present polymer than for polypeptides. Reference to the book of Poland and Scheraga[21] may be helpful for comparison with polypeptides and polynucleotides.

The chain-length dependence of the transition region is unusually large compared with those known for polybenzyl glutamate and oligo A. While for polypeptides and polynucleic acids its effect remains restricted to relatively low DP's, it is still pronounced at high DP's for CC. Figure 7 demonstrates that at 25°C the CC molecular weight must exceed 200000, i.e., DP > 380, before the chains are stabilized in their stiffened state. We may say that in a plateau region the molecules have the same structures, and that the determination of persistence lengths as molecular constants should be sufficient to characterize them entirely. Below DP 380 at 25°C, however, the chains exist in states somewhere between the two limits of the plateau regions, and if characterized by persistence lengths these are expected to be dependent on the chain length and smaller than for chains above DP 380.

These facts have to be kept in mind if a persistence length of CC is determined by application of the various methods. The Hearst theory[22] for the *rotatory diffusion constant* of wormlike chains was applied to the results from the present dielectric relaxation measurements. The variation of M^2D with $M^{1/2}$ was found to be linear (Figures 9 and 10) with slopes leading to values of the persistence lengths a = 108 Å for CC in dioxane and a = 52 Å in ethyl acetate at 25°C. In this calculation we used monomer length l_0 = 5.15 Å obtained from X-ray crystallographic data from cellulose[23] thus giving mass per unit length = 519/5.15 = 101 Å^{-1}. The straight line in Figures 9 or 10 represents only an average persistence length; low M samples are already outside the low-temperature plateau region at 25°C and are characterized by smaller a values. In fact, within the limits of experimental error a slightly bent curve could very well be drawn with smaller limiting slopes at high molecular weights to give a larger persistence length.

Table III
Persistence Length of CC in Dioxane at Different Temperatures

Temp, °C	Persistence length, Å Our data (from M^2D vs. $M^{1/2}$ plot)	Noordermeer's data[24]
15	131	
20	136[a]	
25	108	129
35	90	
50	73	109
75	57	109
90	50	99

[a] Value from light-scattering data.[4]

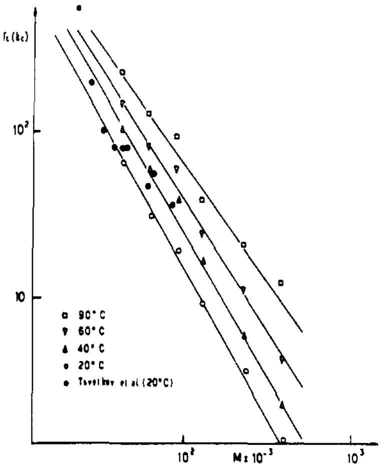

Figure 11. Variation of log f_c with log M for CC in dioxane at different temperatures. The "filled circles" represent the data of Tsvetkov et al.[25] for CC in dioxane at 20°C.

Calculation of persistence length at high temperature leads to a value of 50 Å for CC in dioxane at 90°C. This value, however, has a low precision because of low dielectric increment at such high temperatures. Mass per unit length, M_0/l_0, was taken the same for all temperatures. Table III shows the values of persistence length calculated from the present data at different temperatures using the M^2D vs. $M^{1/2}$ plot. A decrease of persistence length with rising temperature in dioxane was also found by Noordermeer;[24] his values are also given in Table III.

The tendency of increasing flexibility with increasing temperature of CC as shown by normal thermal transition curves is also seen from the variation of log f_c vs. log M (Figure 11) of CC in dioxane. This variation was found to be linear for the molecular weight range investigated. The slopes of these linear plots at two extreme temperatures lead to the following relations

$$f_c = 2.1 \times 10^{13} M^{-1.83} \quad \text{at } 20°\text{C}$$

$$f_c = 1.0 \times 10^{12} M^{-1.44} \quad \text{at } 90°\text{C}$$

Values obtained from rotational relaxation time reported by Tsvetkov et al.[25] are also shown in Figure 11. The slight differences with our values may be due to the different degrees of substitution of their samples. In ethyl acetate the variation of log f_c with log M was also found to be linear in this range of molecular weight as shown in Figure 12. The slope of this plot was 1.4 at 25°C as compared with 1.8 in dioxane, indicating that the molecules in ethyl acetate are more flexible than in dioxane.

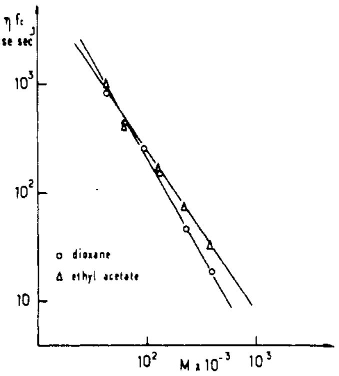

Figure 12. Variation of log ηf_c with log M for CC in ethyl acetate and dioxane at 25°C.

The value 1.44 of the exponent in the above relation at 90°C in dioxane is the same as that obtained in ethyl acetate at 25°C. This is consistent with the fact that persistence lengths were found to be the same in dioxane at 90°C and ethyl acetate at 25°C. The conformations of CC in high- and low-temperature plateau regions are not the same in the two solvents. Thus a solvent-induced conformational change is expected at ordinary room temperature for high molecular weight CC in dioxane–ethyl acetate mixture. Such a change is observed for CC in a mixture of dioxane and phenyl benzoate.[24]

From the present data one can calculate the persistence *dipole moment* of the CC chain in the following manner. For a macromolecule bearing dipole moments along the chain skeleton, the Kratky and Porod treatment[26] can be applied for dipole moment vectors:

$$\langle \mu^2 \rangle = 2\mu_a^2 \left[\frac{n\mu_0}{\mu_a} - 1 + \exp(-n\mu_0/\mu_a) \right] \quad (4)$$

μ is the molecular dipole moment, μ_0 the monomer dipole component along the chain, n the degree of polymerization, and μ_a the persistence dipole moment, which is related to the persistence length by $\mu_a = a\mu_0/l_0$. On Figure 13 the experimental values of μ/n at 25°C vs. n are compared with those calculated for a = 108 Å, the value obtained from f_c analysis, and μ_0 = 0.9 D. The fit is good except at low molecular weights where the molecules are not in the plateau region at this temperature; the lower experimental values in the low molecular weight range can be attributed to the shift in the μ variation vs. temperature with respect to that of f_c as already mentioned. The value of μ_0 thus obtained is slightly lower than the value 1.15 D determined from electric birefringence and translational diffusion constant measurements.[25]

An estimation of the variation of a with temperature was then made from eq 4 using the experimentally determined values of molecular dipole moments and assuming the variation of μ_0 with temperature to be negligible. For the sample of M = 130000, the variation is shown in Figure 14. The values agree with those obtained from the Hearst theory based on rotatory diffusion constant determinations for several molecular weights discussed above (Table III).

In a previous light-scattering study[4] the persistence length a was determined from the molecular weight dependence of the mean-square radius of gyration $\langle S^2 \rangle_0$ in a Θ solvent at 20°C, using Benoit and Doty's formula[27] for radius of gyration.

$$D_n \equiv \frac{6\,\langle S^2 \rangle_0}{n l_0^2} = (a/l_0)[2 - 6x^{-1} + 12x^{-2} - 12x^{-3}(1 - e^{-x})] \quad (5)$$

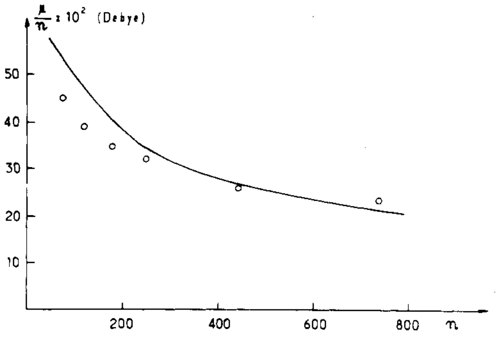

Figure 13. Variation of μ/n with n obtained from the Kratky–Porod model, eq 7, for persistence dipole moment using μ_0 = 0.9 D and a = 108 Å (continuous curve). Experimental values are shown by circles.

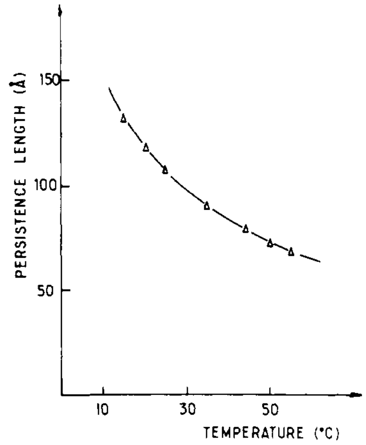

Figure 14. Variation of persistence length of CC in dioxane with temperature for sample M = 130000 (from eq 4) with the dipole moment data, using $a_{25°C}$ = 108 Å and μ_0 = 0.9 D.

$x = nl_0/a$. For infinitely long chains D_n becomes equal to Flory's characteristic ratio C_n

$$D_\infty = C_\infty = 2a/l_0$$

Thus eq 5 may be written

$$D_n = D_\infty - f[a,l_0,n] \quad (6)$$

In this equation the bond length l_0 and persistence length a were considered as constant parameters to be fitted. The best fit was obtained with l_0 = 4.15 Å and a = 169 Å. Similar results were obtained from the molecular weight dependence of the intrinsic viscosity and friction coefficient[28,29] when applying the current theories of stiff-chain molecules. Since we have shown the persistence length a to be a decreasing function of n below n = 380, the former interpretation of the light-scattering results has to be revised.

As a matter of fact, the apparent persistence length at any chain length and temperature may be expressed as

$$a(n,T) = a_s g(n,T) + a_f[1 - g(n,T)]$$

where a_s refers to the persistence length of the chain in the stiff state (low-temperature plateau region) and a_f refers to the chain in the flexible state (high-temperature plateau region) and $g(n,t)$ is an empirical function having its limits of 1 and 0 at the low- and high-temperature bounds. Without going into detail it can be shown that the limiting value of a at large n,a_∞, is smaller than a determined under the assumption of a constant persistence length. This is because a was determined with the aid of the expression $f[a,l_0,n]$ in eq 6 whose value increases with increasing per-

Table IV
Values of Radius of Gyration of CC (M = 130000) in Dioxane at Various Temperatures

Temp, °C	Radius of gyration,[a] Å
20	288
30	283
40	280
50	267

[a] From light-scattering measurements.

sistence length and decreasing n. Since now the persistence length is decreased for n below $n = 380$ eq 6 should be written more correctly

$$D_n = D_\infty[1 - \phi(n,T)] - f[a(n,T)l_0,n]$$

$$D_\infty = 2a_\infty/l_0$$

where $\phi(n,T)$ takes account of the variability of the persistence length. From this relationship the values for $f[a(n,T),l_0,n]$ and consequently a are found to be smaller than under the incorrect assumption $\phi(n,T) = 0$. A smaller value of a_∞ in the limit of large n necessarily results in a larger value for l_0. Unfortunately, the correct l_0 value is at present not known from X-ray diffraction measurements but if again we consider the value $l_0 = 5.15$ Å for the unsubstituted cellulose as being correct for CC too, one obtains from $l_0^2D_\infty = 2al_0 = 1400$ Å^2 instead of $a = 169$ Å, a persistence length of $a = 136$ Å at 20°C. Stress optical coefficient measured in dioxane by Noordermeer[24] gave $a = 129$ Å at 25°C in a wide molecular weight range up to $M = 2 \times 10^6$. Tsvetkov et al[25] from translational diffusion coefficient measurements in dioxane, using theories of Kuhn and Silberberg[30] and Hearst and Stockmayer,[31] found $a = 80$ Å. The reason for this lower value could be again the lower molecular weight ($14000 \leq M \leq 107000$) used but also because Tsvetkov used incompletely substituted CC. We noticed that incomplete substitution reduces drastically the intrinsic viscosity. Other reported values of persistence length are 76.8 Å in benzophenone at 55°C[5] and 108 Å in acetone obtained from X-ray small-angle scattering measurements.[32]

Light Scattering. Values of radius of gyration of CC (M = 130000) in dioxane obtained by light-scattering measurements are shown in Table IV. The trend of decreasing radius of gyration with increasing temperature has also been found for higher molecular weight samples; limiting viscosity numbers were also found to decrease strongly with rising temperature.[24,33]

For a quantitative comparison with the dielectric measurements we make a very crude assumption, that the particles behave like rigid cylinders. Their mean-square end-to-end distance $\overline{L^2} = 12\langle S^2\rangle$ is compared with the length of the rod calculated according to eq 1, using $b = 9.2$ Å as deduced from small-angle X-ray scattering measurements on acetone solution[34]

$$(\overline{L^2})^{1/2} \text{ (from } \langle S^2\rangle) = 970 \text{ Å}$$

$$L(\text{from D}) = 1300 \text{ Å}$$

It can also be assumed that the real volume V of the rod is equal to its hydrodynamic volume. Using the relations

$$V = V_{\text{sp}}M/N_{\text{a}} \tag{7}$$

and

$$\frac{1}{D} = \frac{8\eta V_{\text{sp}}}{RT}Mr^{-1}(p) \tag{8}$$

where $p = L/b$ and $r(p) = 6p^{-2}(\ln 2p - \gamma)$ and taking $V_{\text{sp}} = 0.72$ cm^3/g,[25,29] we find $L = 720$ Å. Keeping in mind that the model is very crude, values of L determined from light scattering and rotational diffusion constant do not greatly differ in their order of magnitude. This shows that we are dealing with an end-over-end rotational relaxation. Furthermore, the corresponding trend of variation of L with temperature is also in agreement. In ethyl acetate the relative variation of $\eta f_c/T$ with T during the transition is larger than in dioxane (Tables I and II). The overall length seems to undergo a greater change in this solvent.

High-Frequency Absorption. At the high-frequency side of the absorption spectrum the dielectric constant was not found to be equal to that of the solvent. The difference was always higher than that calculated for induced polarization, $\Delta\epsilon/w = 2.8 \pm 0.4$ in dioxane, meaning that there exists another region of dielectric absorption at higher frequencies. This may be due to the rotation of components of the dipole moment perpendicular to the chain such as the polar side groups, in agreement with the fact that $\Delta\epsilon/w$ was found to be independent of molecular weight. This is contrary to Tsvetkov et al.'s[25] statement that the degree of correlation of orientation of perpendicular components of the dipole moments depends on the molecular weight, a higher correlation existing for lower molecular weights. However, recalling that the lower molecular weight samples are already in the transition region at 25°C and that dipole moment and the optical anisotropy of Kuhn segment ($\alpha_1 - \alpha_2$) decrease as the transition proceeds, a comparison of the molecules in their "plateau" region where they are in the same state would increase μ^2 and thus the Kerr constant which would raise the experimental values obtained by these authors, at low values of X ($X = nl_0/a$ in our notation) (see Figure 9 of ref 25), increase being highest at lowest X. The points would then have a tendency to lie on the curve calculated for high b values and their conclusion would be modified.

In ethyl acetate the corresponding value of $\Delta\epsilon/w$ is 1.1 for the high-frequency absorption. Taking account of the higher value of dielectric constant ϵ of ethyl acetate (ϵ = 6.02) than that of dioxane (ϵ = 2.209) this leads to a value of $\overline{\mu^2}/M$ lower than found in dioxane. This may be due to the difference in solvation of the lateral groups by the two solvents.

Conclusion

In ethyl acetate and in dioxane cellulose tricarbanilate undergoes a temperature-dependent conformational change. Furthermore, at a given temperature the conformational state of the molecule is dependent on molecular weight. This must be taken into account in the interpretation of the measurements made on different molecular weights at constant temperature.

Note Added in Proof. After the communication of the present report, a recent study (J. T. Guthrie, M. B. Huglin, R. W. Richards, V. I. Shah, and A. H. Simpson *Eur. Polym. J.*, **11**, 527 (1975)) about temperature effect on CC in solution came to the authors' notice.

Acknowledgment. The authors thank Professor H. Benoit for his useful suggestions and Dr. C. Strazielle for the radius of gyration determinations.

References and Notes

(1) (a) Centre de Recherches sur les Macromolécules; (b) Institute of Macromolecular Chemistry.

(2) (a) P. C. Scherer, D. W. Levi, and M. C. Hawkins, *J. Polym. Sci.*, **24**, 19 (1957); (b) P. C. Scherer, M. C. Hawkins, and D. W. Levi, *ibid.*, **37**, 369 (1959).
(3) W. Burchard, *Makromol. Chem.*, 88, 11 (1965).
(4) W. Burchard, *Br. Polym. J.*, 3, 214 (1971).
(5) H. Janeschitz-Kriegel and W. Burchard, *J. Polym. Sci., Part A-2*, **6**, 1953 (1968).
(6) S. Broersma, *J. Chem. Phys.*, **32**, 1626 (1960).
(7) B. H. Zimm, *J. Chem. Phys.*, **24**, 269 (1956).
(8) E. Marchal and J. Marchal, *J. Chim. Phys. Phys.-Chim. Biol.*, **64**, 1607 (1967).
(9) J. Barriol and A. Weissbecker, *C. R. Hebd. Seances Acad. Sci.*, **259**, 2831 (1964).
(10) E. Marchal, Thesis, University of Strasbourg, 1964.
(11) W. Burchard and E. Husemann, *Makromol. Chem.*, **121**, 1 (1969).
(12) C. Dufour and E. Marchal, *Biopolymers*, **11**, 1021 (1972).
(13) E. Marchal, C. Dufour, and C. Strazielle, *Eur. Polym. J.*, **6**, 1147 (1970).
(14) W. Burchard, *Z. Phys. Chem.*, **42**, 293 (1964).
(15) B. H. Zimm, P. Doty, and K. Iso, *Proc. Natl. Acad. Sci. U.S.*, **45**, 1601 (1959).
(16) A. K. Gupta, C. Dufour, E. Marchal, and H. Benoit, *Biopolymers*, **14**, 641 (1975).
(17) J. Applequist and V. Damle, *J. Am. Chem. Soc.*, **87**, 1450 (1965).
(18) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(19) S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).
(20) K. Nagai, *J. Chem. Phys.*, **34**, 887 (1961).
(21) D. Poland and H. A. Scheraga, "Theory of Helix-Coil transitions in Biopolymers", Academic Press, New York, N.Y., 1970.
(22) J. E. Hearst, *J. Chem. Phys.*, **38**, 1062 (1963).
(23) K. H. Meyer and L. Misch, *Ber. Dtsch. Chem. Ges. B*, **70**, 266 (1937).
(24) J. W. M. Noordermeer, Thesis, Technische Hogeschool, Delft, 1974; J. W. M. Noordermeer, R. Daryanani, and H. Janeschitz-Kriegl, *Polymer*, **16**, 359 (1975).
(25) V. N. Tsvetkov, E. I. Rjumtsev, L. N. Andreva, N. V. Pogodina, P. N. Levrenko, and L. I. Kutsenko, *Eur. Polym. J.*, **10**, 563 (1974).
(26) O. Kratky and G. Porod, *Recl. Trav. Chim. Pays-Bas*, **68**, 1106 (1949).
(27) H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).
(28) W. Sutter and W. Burchard, to be published.
(29) W. Sutter, Ph.D. Thesis, University of Freiburg, 1970.
(30) H. Kuhn, W. Kuhn, and A. Silberberg, *J. Polym. Sci.*, **14**, 193 (1953).
(31) I. Hearst and W. Stockmayer, *J. Chem. Phys.*, **37**, 1425 (1962).
(32) W. Burchard and K. Kajiwara, *Proc. R. Soc. London, Ser. A*, **316**, 185 (1970).
(33) W. Burchard, unpublished results.
(34) O. Kratky, I. Pilz, and W. Burchard, unpublished results.
(35) A. K. Gupta, C. Dufour, and E. Marchal, *Biopolymers*, **13**, 1293 (1974).

Solvent Contributions to Small-Angle X-Ray Scattering from Macromolecular Solutions

Arthur S. Hyman

Department of Chemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228. *Received May 19, 1975*

ABSTRACT: Theoretical methods are developed which quantitatively account for solvent contributions to the inner portion of small-angle X-ray patterns obtained from macromolecular solutions. The usual assumption of a homogeneous solvent is shown to be inadequate. The presence of solvent is shown to significantly affect observed values of the radius of gyration for certain cases. The effect is strongly dependent on the macromolecular size, relative mean electron density of the macromolecule and solvent, and the degree of inner solvation. Polyelectrolyte–counterion systems are considered in detail. An interpretation of recent data on the binding of cations to phenylalanine specific *t*-RNA is included.

I. Introduction

Several attempts to include the effects of solvent into the formalism of small-angle X-ray scattering from macromolecular solutions have been made previously. Stuhrmann and Kirste[1,2] suggest a method for eliminating solvent effects which arise because of intramolecular fluctuations in electron density. Their method involves studying the macromolecule successively in several solvents which differ in mean electron density. This technique was employed to study myoglobin in glycerin–water, glucose–water, and saccharose–water mixed solvents. One difficulty with this approach lies in finding several solvents which differ suitably in mean electron density while at the same time leave the macromolecular structure unaltered. Hyman and Vaughan[3] have described the effect of solvent for sphere-like molecules. Luzzati and coworkers[4] have developed theoretical methods which apply to polyelectrolyte systems. Their theory involves the parameterization of fundamental scattering relations. Each of these methods have merit, but they suffer from a common defect: each assumes a homogeneous solvent. This assumption appears reasonable since internal solvent structure is short ranged and will not be observed at small angles. We have no quarrel with this argument. However, there are other problems which arise from the homogeneous solvent model which are not related to internal solvent structure. In a loose sense the problems arise from a consideration of where the macromolecular domain ends and the domain of the solvent begins. We illustrate the problem below.

In the small-angle region the exact electron density, $\rho(\mathbf{r})$, of the scattering system is not observed. Instead a locally averaged electron density is perceived. This local average is not well defined, and may vary over the volume occupied by one or several atoms. If, after this averaging, inhomogeneities of macromolecular scope persist, small-angle scattering is observed. To illustrate the difficulties with the assumption of a homogeneous solvent, consider a hypothetical one-component, two-phase, monatomic system in which solid-phase fragments of macromolecular dimensions are dispersed in a liquid phase. We assume that the solid phase has frozen out with a mean electron density identical with that of the liquid. Would this system exhibit small-angle scattering? If we simply assume a homogeneous solvent we are inclined to think not, because of the equal electron densities of the dispersed and liquid phases. However, this is not the case. One would observe small-angle scattering, however small, due to a discontinuity of the locally averaged electron density at the phase boundaries, represented by a depletion of electron density at the interface. The situation is illustrated in Figure 1 for a one-dimensional case. The atoms of a solid phase fragment are depicted as points. Although at small angles atoms scatter as points, they do not interact physically with each other as points and consequently their centers are restricted to minimum distances of approach. The atomic centers in the liquid phase are as-