Dielectric Study of Amylose Tricarbanilate in Dioxane and Ethyl Acetate

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ABSTRACT: Dielectric measurements on amylose tricarbanilate (AC) in dioxane and ethyl acetate were made in the frequency range 20 Hz–2 MHz. In both of these solvents AC was found to undergo a reversible thermally induced conformational change from a less flexible to a more flexible conformation as temperature increased. In dioxane, the temperature range in which this change occurs was higher for higher molecular weight samples, as in the case of cellulose tricarbanilate (CC). For AC the persistence dipole moment μ_a = 65 D and the monomer dipole component μ_0 = 1.8 D in the low temperature conformational state are higher than those for CC. In dioxane the critical frequency variation with molecular weight obeys the following laws: $f_c = 3.7 \times 10^{13}\ M^{-1.77}$ (low temperature) and $f_c = 9.7 \times 10^{11}\ M^{-1.36}$ (high temperature) for the studied range 125 < degree of polymerization < 1520.

Recently reported dielectric investigation[1] as well as light scattering[2] and stress-optical coefficient[3] measurements on cellulose tricarbanilate (CC) have revealed many facts about the molecular conformation and chain stiffness of CC in two classes of solvents: ketonic and ester solvents. A temperature-induced transition from one state of flexibility to another was observed for CC in dioxane and ethyl acetate and furthermore it was found that at any given temperature flexibility was different in the two solvents.

In the present work the dielectric study of amylose tricarbanilate (AC) in dioxane and ethyl acetate is presented. AC differs from CC in the way the anhydroglucose rings are linked together: in cellulose the glucose rings are joined in their equatorial planes (β-glucosidic bond) whereas in amylose the subsequent rings are attached axially to the ring of the preceding unit. It has been shown from light-scattering measurements[2] that AC differs from CC in chain stiffness and in the length of the monomer unit in the chain direction.

Experimental Section

Amylose samples of low polydispersity ($M_w/M_n < 1.01$), obtained by enzymatic synthesis of glucose 1-phosphate, were used as starting material.[5,6] The amylose tricarbanilates were prepared by treatment with phenyl isocyanate in pyridine at 100 °C. The different samples studied are denoted as AC(125), AC(190), AC(340), AC(560), AC(600), AC(760), AC(1130), and AC(1520), where the numbers in the parentheses represent their degree of polymerization. The solvents, dioxane and ethyl acetate, were freshly distilled.

Dielectric measurements in the frequency range 20 Hz–2 MHz were carried out on the General Radio 1616 capacitance measuring assembly and two impedance bridges were described in detail elsewhere.[4] A Cylindrical cell of 1-mm electrode separation and 196 pF air capacitance was used. Its temperature was maintained within ±0.1 °C by enclosing it in a thermostated copper chamber.

Theory

The dielectric data are interpreted in terms of (i) specific dielectric increment $\Delta\epsilon/w$ ($\Delta\epsilon$ being equal to $\epsilon_0 - \epsilon_\infty$, where ϵ_0 and ϵ_∞ are the limiting values of the real part of dielectric constant at low and high frequencies, respectively, and w is the weight fraction of solute), and (ii) the critical frequency f_c which is the frequency at which the dielectric constant acquires the value $(\epsilon_0 + \epsilon_\infty)/2$. The quantity $\Delta\epsilon/w$ is related to the mean square dipole moment $\bar{\mu}^2$ of solute particules, while f_c is related to the rotational diffusion constant D of the solute particles ($D = \pi f_c$).

The molecular weight dependent dielectric absorption which occurs in this frequency range is due to the orientation polarization of these rigid molecules bearing dipole moment components in the direction of the chain. Ignoring long-range interactions, the resultant mean-square dipole moment should be proportional to the mean-square end-to-end distance. This leads us to define the "persistence dipole moment", which like the persistence length defined for the Kratky–Porod wormlike chain[70] characterizes the chain stiffness. This can be determined by using the Benoît–Doty equation[8] for the mean-square end-to-end distance of a wormlike chain. The mean-square dipole moment can thus be expressed as:

$$\bar{\mu}^2 = 2\mu_a^2\left[\frac{n\mu_0}{\mu_a} - 1 + \exp\left(-\frac{n\mu_0}{\mu_a}\right)\right] \qquad (1)$$

where μ_0 is the monomer dipole moment, n is the degree of polymerization, and μ_a is the persistence dipole moment which is related to persistence length a by $\mu_a = a\mu_0/l_0$ (l_0 being the monomer length).

Results and Discussion

The AC solutions in dioxane and ethyl acetate show concentration effects which are qualitatively similar to those observed for CC in these respective solvents.[1] The present investigation on AC in dioxane is, therefore, made in a range of concentration (below $\sim 10^{-3}$ g/g) where such effects are inappreciable. For AC in ethyl acetate the concentration effects are not absent even at low concentration, thus the experiments are carried out at the lowest possible concentrations.

1. **Effect of Temperature.** The variation of $\eta_0 f_c/T$ and $T\Delta\epsilon/w$ with temperature for AC in dioxane gives S-shaped curves such as that shown in Figure 1 for AC(560). η_0 is the solvent viscosity at temperature T; since $\eta_0 f_c/T$ is invariant for a particular sample if no intramolecular change occurs, the observed behavior suggests the occurrence of a conformational thermally induced transition, as was also observed for CC in dioxane. Unlike the case of CC, the transition curves for the two quantities $\Delta\epsilon/w$ and f_c for any given sample show no shifts relative to each other on the temperature scale. This is observed on all samples in dioxane and might be due to the different chain stiffness of AC and CC. The temperatures corresponding

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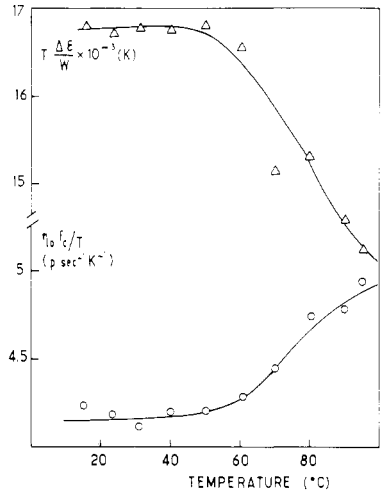

Figure 1. Variation of $T(\Delta\epsilon/w)$ and $\eta_0 f_c/T$ with temperature for AC(DP-560) in dioxane.

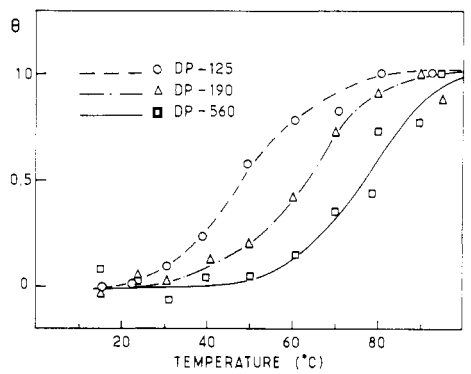

Figure 2. Normalized thermal transition curves for three samples of AC in dioxane.

Table I
Values of $\eta_0 f_c/T$ for AC in Dioxane

temp, °C	$\eta_0 f_c/T$, P s^{-1} K^{-1}			
	DP-125	DP-190	DP-560	DP-760
15	3.508	2.164	0.423	0.142
23	3.515	2.186	0.418	0.125
40	3.580	2.212	0.420	0.128
60	3.732	2.304	0.428	0.119
80	3.797	2.458	0.474	0.122
95	3.796	2.451	0.496	0.120

to the midpoint of the $\eta_0 f_c/T$ curves (transition temperature $T_{1/2}$) for identical molecular weight samples are slightly higher for AC than for CC in dioxane.

The variation of transition temperatures for AC in dioxane is shown in Figure 2, where the normalized transition curves for three samples are plotted. Data on other samples, shown in Table I, could not be plotted on this figure as the normalization is not possible due to lack of data in both plateau regions. The normalized data are represented in terms of the parameter θ defined as:

$$\theta = \frac{(\eta_0 f_c/T) - (\eta_0 f_c/T)_{min}}{(\eta_0 f_c/T)_{max} - (\eta_0 f_c/T)_{min}}$$

where $(\eta_0 f_c/T)_{max}$ and $(\eta_0 f_c/T)_{min}$ denote the limiting values of $(\eta_0 f_c/T)$ at high and low temperatures, respectively. Thus we find for AC in dioxane that the higher the molecular weight, the higher is the transition temperature.

The high-temperature plateaux in these thermal transition curves correspond to a lower flexibility state of the molecules, as has been shown[1] in the case of CC. The variations of critical frequency with molecular weight,

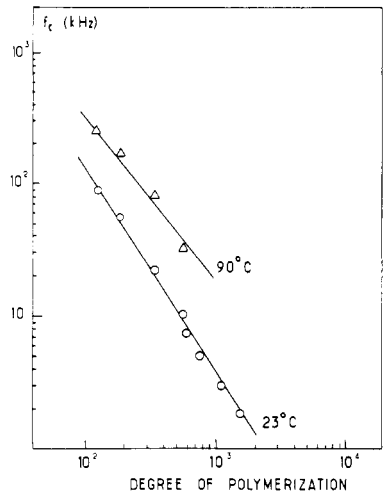

Figure 3. Variation of log f_c with log (DP) for AC in dioxane at two extreme temperatures.

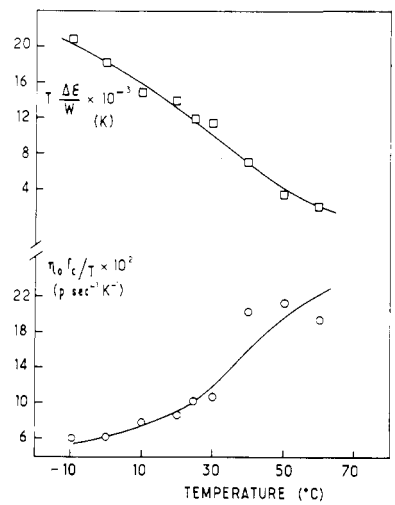

Figure 4. Variation of $T(\Delta\epsilon/w)$ and $\eta_0 f_c/T$ with temperature for AC (DP-760) in ethyl acetate.

shown in Figure 3, fit the following equations for low- and high-temperature plateau values of f_c for AC in dioxane: $f_c = 3.7 \times 10^{13}$ M$^{-1.77}$ (low temperature); $f_c = 9.7 \times 10^{11}$ M$^{-1.36}$ (high temperature). Like in the case of CC, the lower value of the exponent at high temperature suggests higher flexibility at high temperatures for AC in dioxane.

The effect of temperature on AC in ethyl acetate is studied for only a few samples. Although these results might have some ambiguity because the intermolecular interactions are not completely absent in this solvent, the variations of $\eta_0 f_c/T$ and $T(\Delta\epsilon/w)$ with T for one sample, AC(760), in ethyl acetate are shown in Figure 4. The variation is similar to that found for AC in dioxane and for CC in dioxane or ethyl acetate, but the change for AC in ethyl acetate is broader and well-defined plateau regions are not reached in the accessible range of temperature. The broader transition region is not necessarily an indication for a lower cooperativity. In a common helix–coil transition the width of the transition from dipole moment variation is proportional to the van't Hoff transition enthalpy.[9] The cooperativity parameter $\sigma^{1/2}$, however, is defined as the ratio of $\Delta H_{van't\ Hoff}/\Delta H_{residue}$, and $\Delta H_{residue}$, the enthalpy changer per monomer unit in the chain, will be highly influenced by the solvation power of the different solvents. For instance if the solvent interaction with the monomer unit is strong $\Delta H_{residue}$ may become rather small and consequently $\Delta H_{van't\ Hoff}$ will be fairly low while the cooperativity parameter is unchanged.

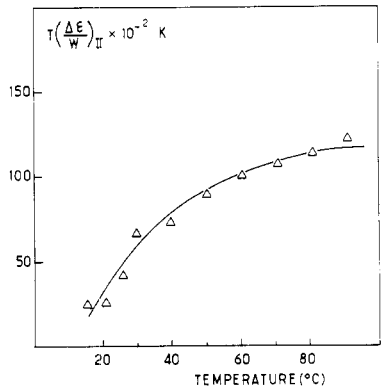

Figure 5. Variation of $T(\Delta\epsilon/w)_{II}$ with temperature for AC-(DP-600) in dioxane.

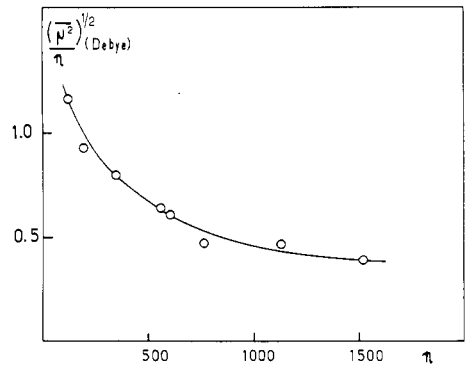

Figure 6. Variation of $(\mu^2)^{1/2}$ with n, the degree of polymerization for AC in dioxane at lower limiting temperature. The theoretical curve (eq 1) using μ_0 = 1.8 D and μ_a = 65 D is shown by the continuous line.

2. High-Frequency Absorption. The high-frequency limiting value of the dielectric constant for this low-frequency absorption is found to be higher than that of the pure solvent. Values of $(\Delta\epsilon/w)_{II}$ are 1.0–4.0 g/g, depending on temperature. This indicates the existence of another absorption in the high-frequency range. It was, however, difficult to obtain precise results for such low dielectric increments. Thus its variation with concentration could not be attempted because of the low precision on very dilute solutions. The effect of temperature on this high-frequency absorption (which we will call "second absorption" and use a subscript II on the quantities) has been studied with care and reproducible results were obtained. Figure 5 shows the temperature behavior of the dielectric increment for the second absorption $(\Delta\epsilon/w)_{II}$ for sample AC(600) at 1 MHz and concentration 2.5×10^{-3} g/g. The absorption curves are horizontal around that frequency indicating thereby the complete absence of any influence of the first (low frequency) absorption. We notice a similar effect of temperature on the high-frequency absorption of CC.

The second absorption might be due to higher internal modes of motion due to increasing flexibility with temperature, but the corresponding permittivity changes are quite high. It seems rather to be due to the component of the dipole moment perpendicular to the main chain. The low value of $(\Delta\epsilon/w)_{II}$ at low temperature may suggest that due to high chain stiffness these perpendicular components are highly correlated and for a sufficiently long molecule they would cancel each other, leading to a zero average perpendicular component of the dipole moment of the whole molecule. The behavior of AC second absorption is similar to that of CC which is surprising because of the different structure of the two molecules. In CC the substituents on alternate glucose rings point in opposite directions (as in syndiotactic chains) while in AC these are always in the same direction (as in isotactic chains). Thus in the rigid chain of CC, these components should cancel but not in AC if the chain would have a similar extended conformation. Such cancellation for AC would be possible for a helically wound chain. The amylose backbone tends, indeed, to a certain helix formation because of the regular $\alpha(1\text{–}4)$ glucosidic bonds between adjacent sugar rings. This helix will probably not be well organized, but even relatively short helical sections will reduce the perpendicular dipole moment considerably. As the temperature increases the decreasing correlation of the chain segments, as deduced from the first absorption, will allow segmental motion to set in. At the same time, the decreasing correlation of the perpendicular dipole moments will increase the dielectric increment relative to that motion. Thus increasing flexibility of AC chain with temperature supports the observed behavior, which may be a consequence of side-chain mobility.

We also note on Figure 5, and this has been confirmed on other samples, that the change in $(\Delta\epsilon/w)_{II}$ occurs at lower temperatures than that in $(\Delta\epsilon/w)_I$ and in $(\eta_0 f_c)_I$. This seems to show that the second absorption is very sensitive to the first stages of the increase of side-chain mobility while the overall molecular conformation is still frozen.

3. Flexibility at Low Temperature. The rotatory diffusion constant D for a wormlike bead model has been formulated by Hearst[10] on the basis of the general theory of Kirkwood.[11] According to this the slope of the plot $\eta_0 M^2 D/kT$ vs. $M^{1/2}$ must be $0.72(M_L/2a)^{3/2}$, where a is the persistence length and M_L the mass per unit length of the chain. From the low-temperature plateau values of f_c for AC in dioxane the plot of M^2D vs. $M^{1/2}$ is not linear in this range of M and thus it is not possible to determine a persistence length. In the previous study on CC, a linear fit was obtained when values of D measured at 25 °C were taken. These values were not necessarily in plateau regions for all the samples. Had the plateau values been taken in the case of CC, a curvature would also have been observed. This curvature is difficult to explain and may be due to the failure of the theory. A defect of the Hearst–Stockmayer[12] theory has earlier been remarked on by Yamakawa et al.[13] It is worth pointing out that AC is not a really rigid molecule, since the persistence length appears to have no more than about 36 monomer units. Furthermore, the finite cross-section of the chain cannot, at that persistence length, be fully neglected. It appears conceivable and likely that these finite cross dimensions cause the failure of the Hearst theory which does not take chain cross dimensions properly into account.

We further attempted to apply Hearst's equation for a weakly bending rod[10] although the condition $M/4M_L a \ll 1$ needed for its use is not satisfied in this case. The values of M_L = 12.4 Å^{-1} and a = 150 Å are those obtained from light-scattering measurements.[2] In using the Hearst equation, we assume that the distance b between frictional elements is equal to the diameter a of the Stokes sphere whose translational friction f is considered. The persistence length thus determined on the sample AC(125) in dioxane is very sensitive to b. For b = 22 Å the persistence length is 63 Å and for b = 11 Å the persistence length is 300 Å. Thus the determination of persistence length from these data would be rather unreliable.

However, the determination of dipole moments and the use of eq 1 allows investigations on molecular flexibility. The best fit of experimental data for AC in dioxane and the theory is obtained for μ_a = 65 D and μ_0 = 1.8 D as shown in Figure 6. For AC the number of monomers per

persistence dipole moment is 36 whereas for CC it is 21, suggesting a more rigid conformation of AC which is in qualitative agreement with the molecular parameters deduced from light-scattering measurements.[2] The monomer dipole moment in the AC chain is higher than the corresponding value $\mu_0 = 0.9$ D found for CC, which is a further proof of the different conformations of the two polysaccharides.

Conclusion

On the basis of dipole moment determinations, the rigidity as well as the monomer dipole moment in the chain direction are higher for AC than for CC, which implies that their conformations are different. As in the case of CC, a thermally induced conformational transition was found for AC in both dioxane and ethyl acetate. This transition shifts with molecular weight for both molecules. The occurrence of a high-frequency absorption whose magnitude is temperature dependent is a noteworthy consequence of this transitional change.

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Water Binding on Collagen by Inverse Phase Gas Chromatography: Thermodynamic Considerations

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ABSTRACT: In a previous publication, the details of using inverse phase gas chromatography (IPGC) for determining the moisture sorption isotherms of proteins were outlined. In the present article, the moisture sorption isotherms of collagen at five temperatures in the range of 40–60 °C are described using this method. The thermodynamics of the sorption process are considered by application of the BET equation, the Clausius–Clapeyron equation, and the Zimm–Lundberg cluster theory. Almost the entire region of the isotherms studied was found to be in or below the monomolecular layer as defined by the BET equation. The calculated values of the Zimm–Lundberg cluster function were negative throughout, indicating the absence of water clustering. The isosteric heat of adsorption was found to be highest at the lowest moisture contents studied and then dropped slightly before leveling off at a constant value of 6.8 kcal/mol characteristic of hydrogen bonding.

The theories of gas chromatography (GC) which relate peak shape to sorption isotherms also include retention volume as part of the definition of equilibrium conditions as derived from plate theory. The following equations described by Kiselev and Yashin[1] illustrate the application of these theories to actual conditions:

$$u = m_p S_p / ms \tag{1}$$

$$p = m_p q h R T / s w \tag{2}$$

where u = uptake (in our specific case water on collagen), g/g; m_p = mass of probe (water), g; S_p = total area under curve, cm^2; m = mass of stationary phase (collagen), g; s = peak area, cm^2; p = partial pressure of probe in carrier gas, atm; q = chart speed, cm/min; h = peak height, cm; T = temperature, K; w = corrected carrier gas flow rate, cm^3/min; and R = universal gas constant, (cm^3 atm)/(mol K).

BET Isotherm. The isotherm model in most general use is that of Brunauer et al.[2] This general equation is known as the BET isotherm and can be derived kinetically, from statistical mechanics and from thermodynamic considerations, as described by Adamson:[3]

$$\frac{V}{V_m} = \frac{Ca}{(1-a)[1+(C-1)a]} \tag{3}$$

where V = amount sorbed, V_m = monolayer value, a = water activity, and $C = Ke^{(Q_s/RT)}$ [K = (accomodation coefficient)/(frequency factor) $\simeq 1$, Q_s = heat of adsorption, R = universal gas constant, and T = temperature (K)]. Generally, eq 3 is rearranged to the form:

$$\frac{a}{(1-a)V} = \frac{1}{V_m C} + \frac{a(C-1)}{V_m C} \tag{4}$$

A plot of $a/(1-a)V$ vs. a should give a straight line. From the slope and intercept of this line, the monolayer coverage value, V_m, can be calculated.

Zimm–Lundberg Cluster Theory. Zimm and Lundberg[4] proposed a "cluster theory" in order to explain the sorption of vapors by high polymers. The theory defines a function that measures the tendency of the absorbed molecules to cluster.

Zimm[5] derived the following relationship between the activity coefficient ($\gamma_1 = a_1/\phi_1$) and the cluster integral G_{11}

$$G_{11}/V_1 = -\phi_2[\partial(a_1/\phi_1)/\partial a_1]_{p,T^{-1}} \tag{5}$$

where ϕ_2 is the volume fraction of the polymer and is equal to $(1-\phi_1)$, while V_1 is the partial molar volume.

The clustering function G_{11}/V_1 is the mean number of water molecules in the neighborhood of a given water

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