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Dilute solution properties of pullulan by dynamic light scattering

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Dedicated to Prof. Dragutin Fleš on the occasion of his 70th birthday

Summary

Pullulan, produced by the fungus Aureobasidium, has been studied in dilute solution by viscometry, static low angle light scattering and dynamic light scattering at temperatures of 25, 40, 60 and 80 °C. The samples used were fractions obtained by partial hydrolysis of native pullulan and successive fractionation with ethanol. The mass-average molar mass, M_w, of the studied pullulans were in the range of $1.67*10^5$ to $1.0*10^6$ g/mol. The translational diffusion coefficient at infinite dilution, Do, was obtained from the reduced first cumulant of the intensity autocorrelation function of the light scattered by extrapolation to zero scattering angle and zero polymer concentration. The activation energy of diffusion, E_D, of pullulan macromolecules was calculated from $Do = D^o\exp(-E_D/RT)$. It was found that in the given temperature range $E_D = 16.5 \pm 0.5$ $kJmol^{-1}$ for all samples.

Introduction

Pullulan is a linear polysaccharide consisting of maltotriose as the repeating unit connected through α-1,6-glucosidic linkages /BRANT et al., 1981/. It is usually otained by cultivation of the fungus Aureobasidium pullulans on glucose. The molar mass of the pullulan synthesized by these bacteria is very high. Pullulans of desired molar masses are produced by hydrolysis in dilute acid.

Since pullulan is water soluble and biodegradable, interest in this polymer has greatly increased in the last ten years. It is expected that the use of pullulan in the cosmetical, pharmaceutical and food industry will increase in the near future. The interest in pullulan also originates from the fact that its solution properties are not completely understood /KATO et al., 1982, 1984, BULIGA et al., 1987/. Although the solution properties at the point of transition from dilute to the semi-dilute regime are also of some practical and theoretical interest, in this paper we present only results concerning the dilute regime, i. e., at concentrations lower than the coil overlap concentration C^*, which is usually defined as $C^* = 1.08/[\eta]$, where $[\eta]$ is the limiting viscosity number of the polymer. Viscosity and static low angle light scattering measurements were carried out at 25 °C while dynamic light scattering measurements were performed at temperatures of 25, 40, 60 and 80 °C.

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Experimental part

The pullulan used in this work was obtained by cultivation of the fungus Aureobasidium pullulans on glucose. Pullulans of certain molar masses were produced by the partial hydrolysis of the so obtained pullulan with 0.08M HCl and by successive fractionation with ethanol from aqueous solutions. The mass-average molar masses of the used pullulans, $\overline{M}_w$, were determined by static low angle light scattering measurements with a KMX-6 photometer.
The intrinsic viscosities, $[\eta]$, of the samples used were determined in water at 25 °C using an Ostwald-type viscometer. The relative viscosities were adjusted to be in the range 1.10 - 1.60.

Pullulan solutions for dynamic light scattering measurements were prepared in bidistilled water in which 0.02 % (w/w) of sodium azide had been added. Solutions were held at 80 °C for about 4 hours with shaking. Solutions were clarified by filtration into the clean scattering cell through 0.22 μm Millipore filters.

Dynamic light scattering measurements at various temperatures were carried out in a specially designed apparatus. The detailed information is given elsewhere /NORDMEIER et al., 1989/. A Spectra-Physics He-Ne laser (Model 107S/207; λ_o = 633 nm) was used as the light source. The temperature in the sample cell was controlled to within ± 0.01 °C. Light scattering fluctuations were detected in the angle range of 30 to 120°. The output signal was correlated by a correlator ALV-3000 (ALV, Germany) and then analysed by a Peacock computer. Evaluation of the data was done by expanding the experimentally measured normalized autocorrelation function g_1 (t) in a series /KOPPEL, 1972, BROWN et al., 1975/:

$$g_1(t) = \exp(-q^2 D_{app} {*} t)(1 + \mu_2 t^2/2! - \mu_3 t^3/3! + ...) \quad (1)$$

where D_{app} is the apparent translational diffusion coefficient, μ_i the moments related to the molar mass distribution of the polymer and q the scattering vector expressed as:

$$q = 4\pi/\lambda \ \sin\theta/2 \quad (2)$$

where θ is the scattering angle and λ is the wavelength of the light in the scattering medium. Small changes of λ with temperature were taken in account through $\lambda = \lambda_o/n$, n being approximated with the refractive index of the solvent at the given temperature.

In the region of dilute solution, the translational diffusion coefficient, Do, of an isolated polymer chain in solution at infinite dilution can be estimated from the reduced first cumulant, Γ/q^2, of the scattering intensity autocorrelation function measured directly by dynamic light scattering using the following equations:

$$\Gamma/q^2 = D_{app} = D_z(c)\,(1 + B\,R_G^2 q^2 - ...) \quad (3)$$

$$\text{with} \quad D_z(c) = Do(1 + k_D c + \tilde{k}_D c^2 + ...) \quad (4)$$

where Γ is the first cumulant, R_G the radius of gyration, $D_z(c)$ the diffusion coefficient at the limit $q \rightarrow 0$, B is a characteristic constant that depends on the molecular architecture and polydispersity, and k_D and $\tilde{k}_D$ the second and third hydrodynamic virial coefficients.

Results and Discussion

Intrinsic viscosity data and the data of the static low angle light scattering measurements are presented in Table 1. The Kuhn-Mark-Houwink viscosity equation was obtained by double-logarithmic plots of $[\eta]$ against $\overline{M}_W$ as:

$$[\eta] = 7.38*10^{-3}\ \overline{M}_W{}^{0.72},\ cm^3g^{-1} \tag{5}$$

Table 1. $\overline{M}_W$ and $[\eta]$ of studied pullulan samples

Sample code	$\overline{M}_W*10^{-5}$ (g mol^{-1})	$[\eta]$ at 25 °C (cm^3 g^{-1})
P-1	1.67	42.5
P-2	3.03	65.3
P-3	5.13	95.4
P-4	10.00	154.2

This equation is in fair agreement with the viscosity equations from the literature /KATO et al., 1982, 1984/.

The reduced first cumulants, $\Gamma/q^2 = D_z(c,q)$, for P-1 at 25 °C for various polymer concentrations are plotted against q^2 in Figure 1. It is seen that there is no dependence of $D_z(c,q)$ on the scattering vector, q. The same holds for all other samples

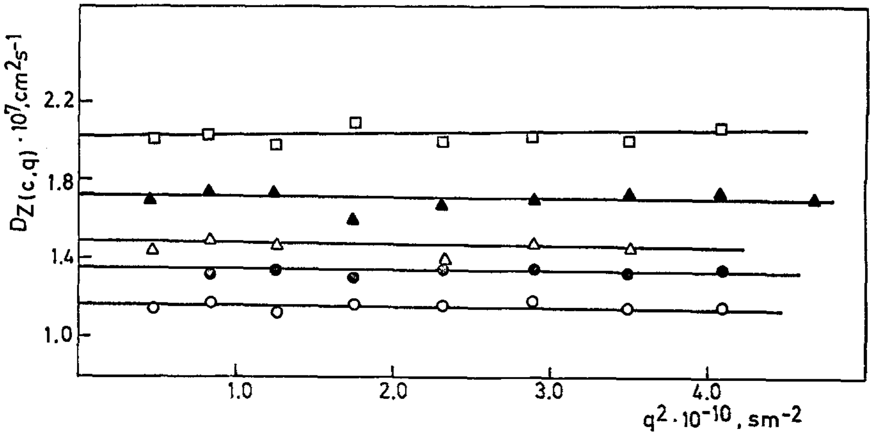

Figure 1. Plots of the reduced first cumulant, $\Gamma/q^2 = D_z(c,q)$, against q^2 for sample P-1 at 25 °C. Sample concentrations (from the top): = $0.571*10^{-2}$, $1.683*10^{-2}$ and $2.860*10^{-2}$ g cm^{-3}.

of pullulan at 25 °C. This finding is somewhat in disagreement with that of Kato et al. /KATO et al., 1984/ who found a small q-dependence of the reduced first cumulant. Likely, our pullulan samples possess a somewhat other polydispersity than those of Kato. Figure 2 shows plots of $D_z(c,q)$, against q^2 for sample P-1 at 80 °C for various polymer concentrations. Obviously, the q^2-dependence of $D_z(c,q)$ at 80 °C is quite different from that seen at 25 °C. The constant B is negative now, indicating that the molecular architecture of pullulan changes as T is increased. Very similar behaviour was found for all other studied samples of pullulan. Figure 3 shows a plot of $D_z(c)$ at $q^2 = 0$ against the polymer concentration, c, for P-1 at 25 °C. Analogous relation-

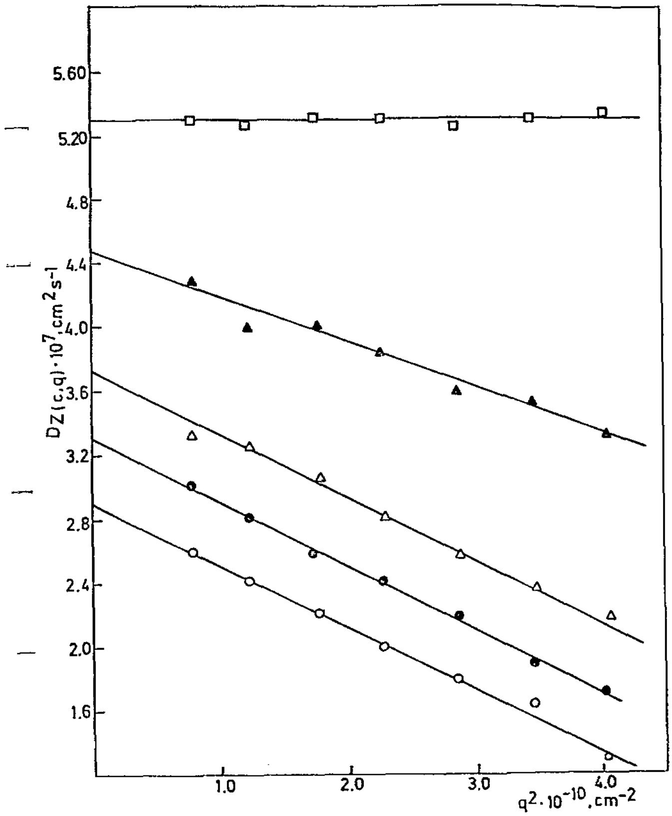

Figure 2. Plots of the reduced first cumulant, $\Gamma/q^2 = D_z(c,q)$, against q^2 for sample P-1 at 80 °C. Sample concentrations (from the top): $0.555*10^{-2}$, $1.060*10^{-2}$, $1.636*10^{-2}$, $2.232*10^{-2}$ and $2.780*10^{-2}$ g cm^{-3}.

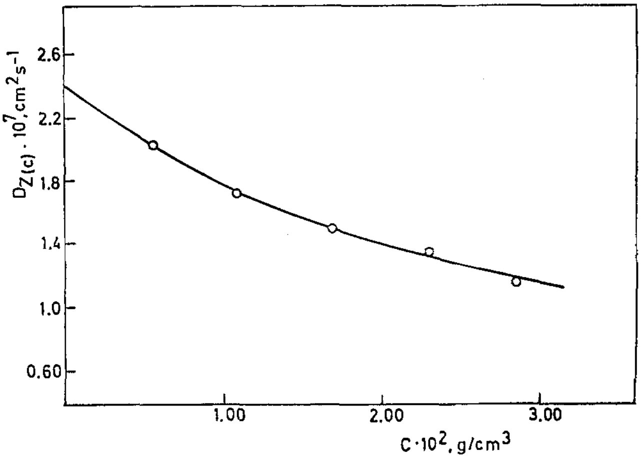

Figure 3. Plot of $D_z(c)$ against polymer concentration for P-1 at 25 °C.

ships were found for temperatures of 40, 60 and 80 °C. It is obvious that the concentration dependence of $D_z(c)$ for pullulan P-1 is not linear over the concentration range investigated. Second order linear regression was performed in order to estimate the translational diffusion coefficient at infinite dilution, Do, and the k_D value in eq. 4. The k_D values were found to be negative at all temperatures studied as seen from Table 2.

Figure 4 shows a plot of $D_z(c)$ versus polymer concentration, c, for P-2 at 25 °C. It is seen that $D_z(c)$ vs. c has a zero slope, thus, k_D in eq. 4 is zero. The same holds

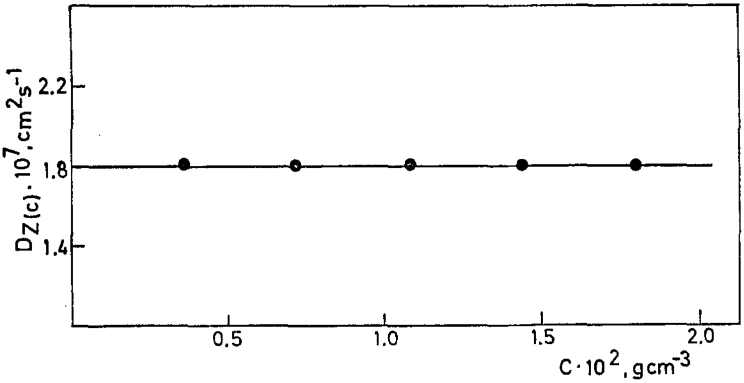

Figure 4. Plot of $D_z(c)$ vs. polymer concentration for P-2 at 25 °C.

for T = 40 °C, while for 60 and 80 °C, k_D has small positive values (see Table 2).

The concentration dependence of $D_z(c)$ for pullulans P-3 and P-4 at all studied temperatures is linear over the concentration range investigated. A typical relationship is presented in Fig. 5.

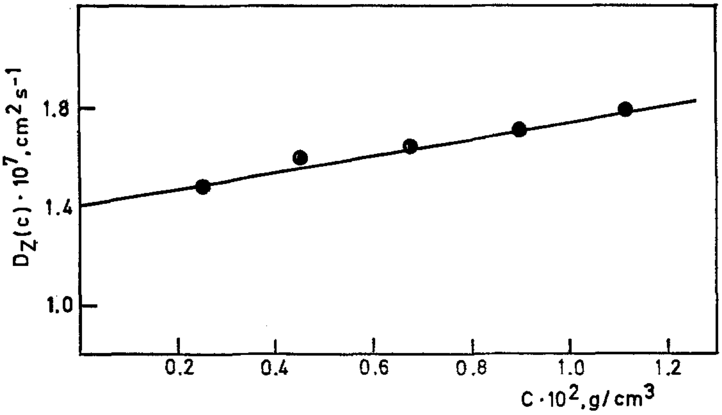

Figure 5. Plot of $D_z(c)$ vs. polymer concentration for P-3 at 25 °C.

Table 2. Results of dynamic light scattering for pullulan samples at different temperatures

sample	temperature °C	$Do*10^7$ cm^2s^{-1}	R_H nm	k_D cm^3g^{-1}	E_D $kJ\ mol^{-1}$
P-1	25.0	2.36	10.7	-32.0	16.2
	40.0	3.20	10.8	-37.1	
	60.0	4.30	12.0	-35.3	
	80.0	6.70	10.8	-46.3	
P-2	25.0	1.80	13.2	0	16.9
	40.0	2.50	13.8	0	
	60.0	4.10	12.6	5.1	
	80.0	5.00	14.5	12.8	
P-3	25.0	1.40	17.9	23.6	16.1
	40.0	2.00	17.3	28.0	
	60.0	3.00	17.2	26.7	
	80.0	3.80	19.0	27.5	
P-4	25.0	1.00	25.1	55.0	16.8
	40.0	1.40	24.7	65.7	
	60.0	1.90	27.2	84.2	
	80.0	2.95	24.5	54.9	

Do and k_D values for all studied pullulans at each given temperature are shown in Table 2, together with the hydrodynamic radii, R_H calculated according to the Stokes-Einstein relation:

$$Do = k_BT/6\pi\eta_oR_H \tag{6}$$

where k_B is absolute Boltzmann's constant, T the temperature and η_o the viscosity of the solvent.

The molar mass dependence of Do at 25 °C is shown in Fig. 6 and described by the following equation:

$$Do = 7.41*10^{-5}\ \overline{M}_w^{-0.48},\ cm^2s^{-1} \tag{7}$$

The exponent in the molar mass dependence of Do is smaller than the theoretical value (0.60) but it is close to the value given by Kato et al. /KATO et al., 1984/.

The temperature dependence of Do is expressed by an Arrhenius type relation:

$$Do = D^o \exp(-E_D/RT) \tag{8}$$

where E_D is the activation energy of diffusion of macromolecules. Figure 7 presents plots of ln[Do] vs. the inverse absolute temperature. The E_D values for all studied pullulans were calculated from the slope of the corresponding curve and are given in Table 2. It is seen that E_D is independent of the molar mass of pullulan.

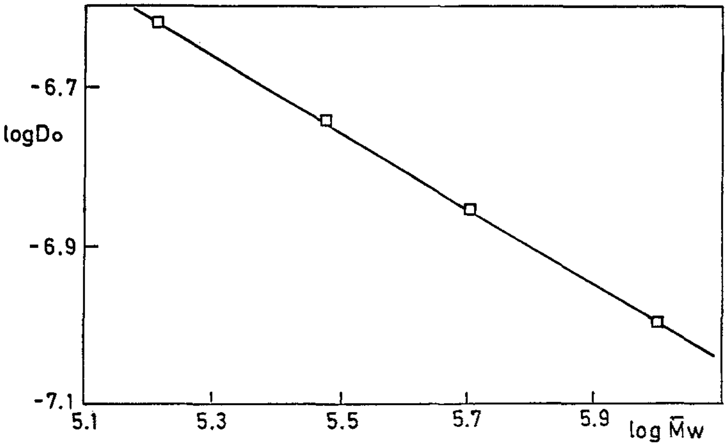

Figure 6. Double-logarithmic plot of Do against $\overline{M}_w$ for pullulan in water at 25 °C.

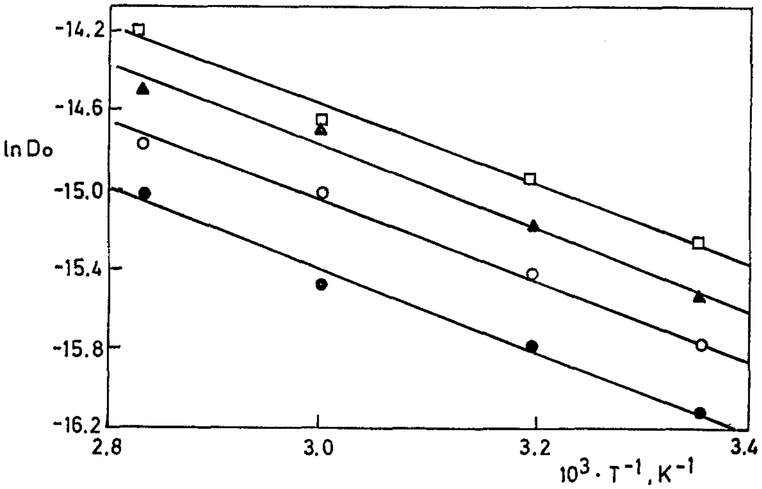

Figure 7. ln[Do] vs. T^{-1} plot for pullulan in water.

The second hydrodynamic virial coefficient, k_D, increases with increasing molar mass of pullulan, while the temperature dependence of k_D seems to be rather complex.

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