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Solution Properties of Pullulan

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ABSTRACT: Measurements of partial specific volume, intrinsic viscosity, sedimentation equilibrium, and photon correlation spectroscopy were carried out for aqueous solutions of eight pullulan fractions with narrow molecular weight distributions. The double-logarithmic plots for the intrinsic viscosity vs molecular weight, and for the diffusion coefficient at infinite dilution vs molecular weight deviate from the straight line at the lower molecular weight region. It was confirmed that pullulan molecules behave as random coils in water as a good solvent.

1. Introduction

Pullulan is a microbial polysaccharide and consists of α-1,6-linked maltotrioses. Since this is a linear polymer without branching, and easily water soluble, it has been used in many investigations. It has been proposed that pullulan can be used as a standard sample for water-soluble polysaccharide instead of dextran, which consists mainly of α-1,6-linked glucoses but contains some α-1,3 branchings. Kato et al.[1] and Kawahara et al.[2] have shown that pullulan behaves as a random coil in water, which acts as a good solvent. Brant et al.[3] studied the temperature and molecular weight dependence of the unperturbed dimensions of aqueous pullulan. However, the conformation of lower molecular weight fractions seems to remain ambiguous. In the present work, photon correlation spectroscopy (PCS), partial specific volume, intrinsic viscosity, and sedimentation equilibrium measurements were carried out on eight fractions of pullulan with different molecular weights and narrow molecular weight distributions.

2. Experimental Section

Materials. Eight pullulan fractions of different molecular weights with narrow molecular weight distributions were kindly supplied by Hayashibara Biochemical Laboratories, Ltd., Japan. All the measurements were carried out on 0.02% sodium azide solution to prevent bacterial degradation. Double-distilled water was used.

Measurements. Photon correlation spectroscopy (PCS) measurements were carried out by use of the Malvern dual-function type 7027 digital correlator equipped with a He–Ne laser (λ = 632.8 nm) and with an argon laser (λ = 488 nm) at 275 mW. Solutions were prepared at concentrations of 0.5 mg/cm^3 and were filtered through 0.45-μm filters in a dust-free box. Fluctuations in the intensity of the scattered light were monitored for solutions maintained at 25 °C, with the photomultiplier being positioned at an angle of 90° to the incident beam.

The partial specific volume (PSV) was determined by using a Anton Paar DMA 60 density meter equipped with a DMA 602 density measuring cell, which was maintained at 25 ± 0.01 °C, using a Hetofrig CB11 circulating water bath.

Sedimentation equilibrium measurements were carried out by using an analytical centrifuge (Hitachi 282). Rayleigh interference optics were used. The photographed fringe displacements were analyzed by using a profile projector (Nikon, V-16D) with a reversible counter and a digital printer. The relation between the fringe displacement and the concentration of the polymer was determined by using a synthetic boundary cell, and the calibration curve of dn/dc was made. A classical boundary formation technique[4] was used to determine the diffusion coefficients of lower molecular weight fractions.

Intrinsic viscosities were determined by using the Ubbelohde viscometers. The flow times for the solvent (0.02 wt % sodium azide solution) were 130 and 475 s. No kinetic energy corrections were necessary.

3. Results and Discussion

Partial Specific Volume. Figure 1 shows the density of pullulan solutions as a function of polymer concentration. Since the relation between the density d and the concentration c of pullulan solutions is linear, the slope of the straight line gives the value $(d - d_0)/c$, where d_0 represents the density of the solvent. Therefore, the partial specific volume

$$\bar{v} = \lim_{c \to 0} \frac{1}{d_0}\left(1 - \frac{d - d_0}{c}\right)$$

is independent of the concentration lower than 0.0025 g/mL.

The values of partial specific volume $\bar{v}$ determined in this way are shown in Table I. $\bar{v}$ is almost independent of the molecular weight within experimental error.

Sedimentation Equilibrium. The weight-average molecular weight was evaluated by the following equations proposed by Fujita.[5]

$$\begin{aligned} M_{\mathrm{app}} &= (c_{\mathrm{b}} - c_{\mathrm{a}})/\lambda c_0 \\ &= (1 - \bar{v} d_0)(r_{\mathrm{b}}^2 - r_{\mathrm{a}}^2)\omega^2/2RT \end{aligned}$$

$$M_{\mathrm{app}}^{-1} = M_{\mathrm{w}}^{-1} + 2A_2(1 + \delta_1)\bar{c}$$

$$\bar{c} = (c_{\mathrm{a}} + c_{\mathrm{b}})/2$$

Rotor speed was chosen so that the product of M_{app} and λ might be close to unity for each run. Here, r_{a} and r_{b} are the radial distances from the center of rotation to the meniscus and to the bottom of the solution; c_0 is the initial concentration; $\bar{v}$ is the partial specific volume of the polymer; d_0 is the density of the solvent; ω is the angular velocity; R is the gas constant; T is the absolute temperature; A_2 is the second virial coefficient; δ_1 is the correction term for polydispersity and is taken to be zero in the present work. Observed values of M_{app}^{-1} were plotted

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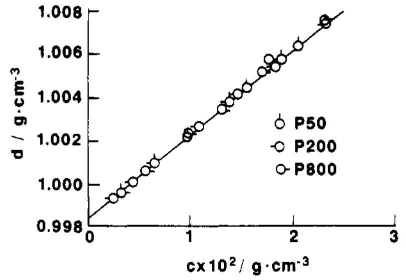

Figure 1. Concentration dependence of the density of aqueous pullulan solutions at 25 °C: (o̍) P50; (-o) P200; (o-) P800.

Table I
Molecular Characteristics of Pullulan Samples at 25 °C

sample	$\bar{v}$, cm³/g	$M_w \times 10^{-3}$	$M_z \times 10^{-3}$	M_z/M_w	$A_2 \times 10^4$, cm³/g
P5	0.594	5.3	5.0	0.95	10.1
P10	0.635	12.3	12.1	0.98	5.0
P20	0.606	23.6	23.8	1.03	5.0
P50	0.609	53.9	58.8	1.09	6.1
P100	0.617	112	123	1.10	3.1
P200	0.619	228	256	1.12	3.5
P400	0.600	478	531	1.11	3.4
P800	0.606	1015	1094	1.08	1.9

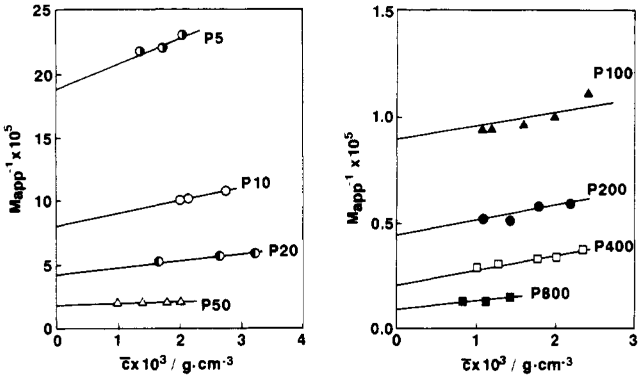

Figure 2. Plots of M_{app}^{-1} against c for an aqueous pullulan solution at 25 °C.

against $\bar{c}$, extrapolated to infinite dilution, and shown in Figure 2 for eight pullulan samples. The weight-average molecular weights were determined from the intercept of these straight lines, while the second virial coefficients were estimated from the slopes of these straight lines and are shown in the third and sixth columns of Table I.

The z-average molecular weight was determined by Norisuye's method;[6] from the observed values of c_0, c_a, c_b, r_a, and r_b and the derivatives $(\partial c/\partial r^2)_{r=r_a}$ and

$$Q = \frac{(c_b - c_a)^2}{c_0(r_b^2 - r_a^2)[(\partial c/\partial r^2)_{r_b} - (\partial c/\partial r^2)_{r_a}]}$$

$(\partial c/\partial r^2)_{r=r_b}$ was evaluated and was equaled to

$$Q = (M_w/M_z)[1 + A_2M_w(1 + \delta_2)\bar{c} + ...)$$

where

$$\delta_2 = \lambda^2(M_wM_z/12)(3M_{z+1}/M_w - 2M_z/M_w - 1) + O(\lambda^4)$$

Extrapolating to infinite dilution gives the value of M_z. Values of M_z are shown in the fourth column of Table I.

The ratios M_z/M_w for most of the samples were smaller than 1.1, indicating that the molecular weight distribution is quite narrow. Since M_z was determined by a graphical method, the high precision was not to be expected; however, the error should be less than 10%.

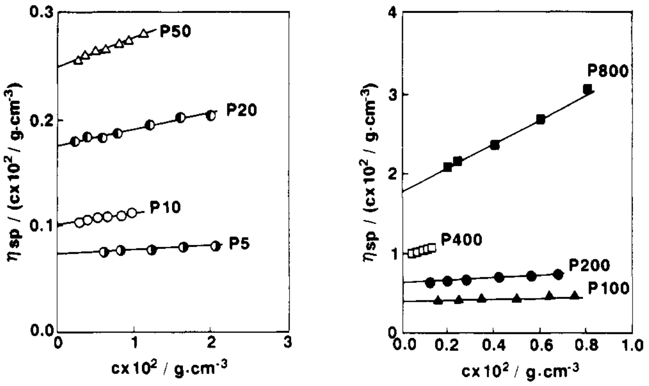

Figure 3. Plots of η_{sp}/c against c for aqueous pullulan solutions at 25 °C.

Table II
Viscometric Characteristics of Pullulan Samples at 25 °C

sample	$[\eta]$/ cm³·g⁻¹	k'	α_η^2	sample	$[\eta]$/ cm³·g⁻¹	k'	α_η^2
P5	7.3	0.74	0.73	P100	40.4	0.50	1.13
P10	10.0	1.15	0.65	P200	64.1	0.37	1.14
P20	17.4	0.51	1.00	P400	101.5	0.40	1.20
P50	24.8	0.44	1.08	P800	177.8	0.47	1.06

Second virial coefficients A_2 estimated from the slopes of the straight lines in Figure 2 are shown in the sixth column of Table I. A_2 is a decreasing function of the molecular weight. The double-logarithmic plot of A_2 against M_w gives the relation $A_2 \sim M_w^{-0.33}$ although the data scattered. The exponent −0.33 agrees fairly well with that obtained by Kawahara et al.[2] and with −0.30 obtained by Brant et al.[3] from light scattering measurements. The concentration dependence of the diffusion coefficients for lower molecular weight fractions P5, P10, and P20 determined by the classical boundary formation technique is shown in Figure 6b together with the results for higher molecular weight fractions determined by PCS.

Viscosity. Figure 3 shows the plot of reduced viscosity η_{sp}/c against concentration, where $\eta_{sp} = (\eta - \eta_0)/\eta_0$; η and η_0 represent the viscosity of the solution and solvent, respectively. Huggins' coefficient k' is defined by[7]

$$\eta = \eta_0(1 + [\eta]c + k'[\eta]^2c^2 + ...)$$

Unfortunately, many different numerical estimates for k' have been published even for rigid-sphere molecules. Most of the theories for flexible molecules concern the calculation of k' at the Θ point.[9]

Sakai extended the Peterson–Fixman theory to the good solvent case and proposed an empirical equation[10]

$$k' = \frac{1}{2} + \frac{\bar{v}}{2[\eta]} - \frac{3(\alpha_\eta^2 - 1)}{4\alpha_\eta^4}$$

where α_η is the viscosity expansion factor defined as $\alpha_\eta^3 = [\eta]/[\eta]_\Theta$ and $[\eta]_\Theta$ represents the intrinsic viscosity of the polymer at the Θ point. Substituting the observed values of k', $\bar{v}$, and $[\eta]$, we can estimate α_η^2.

Table II shows the observed values of $[\eta]$ and k' and the estimated values of α_η^2 for eight pullulan samples. The values of Huggins' coefficient and of the viscosity expansion factor changed at the molecular weight around 2 × 10⁴.

Figure 4 shows the double-logarithmic plot of the intrinsic viscosity against the weight-average molecular weight. The exponent in this Mark–Houwink–Sakurada equation is predicted as 0.5 in a Θ solvent and 0.8 in a good solvent by the Zimm model for flexible polymers.[8] However, the observed values of the exponent for many flexible

Table III
Diffusion Coefficients of Pullulan Samples

sample	D_0/ 10^{-7} cm^2·s^{-1}	R_H/ 10^{-6} cm	V_h/ cm^3·g^{-1}	$(k_D)_{obs}$	$(k_D)_y$	k_f	$k_f/[\eta]$	k_{f0}	$(k_D)_{PF}$(min)	$(k_D)_{PF}$(max)
P5	12.0	0.204	4.04	−7.0	−0.36	17.1	2.34	4.23	−18.8	1.09
P10	9.56	0.256	3.45	−15.0	1.98	26.7	2.67	11.41	−13.0	4.01
P20	5.94	0.43	7.51	−6.2	1.33	29.2	1.68	3.89	−30.8	6.25
P50	3.45	0.710	16.7	−17.3	9.00	82.5	3.33	4.940	−54.4	28.0
P100	2.93	0.837	13.4	−15.7	13.8	84.5	2.09	6.32	−27.1	38.9
P200	2.21	1.11	15.1	−7.2	48.1	166	2.59	11.0	50.9	125
P400	1.40	1.750	28.2	0	101.1	324	3.19	11.5	122	262
P800	0.97	2.53	40.3	46	113.1	339	1.91	8.41	96.9	297

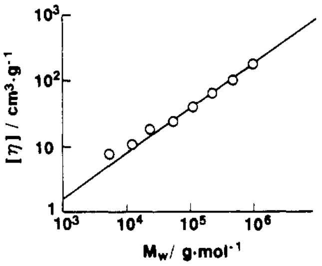

Figure 4. Double-logarithmic plot of $[\eta]$ against M_w for pullulan samples at 25 °C.

polymers in good solvents were smaller than 0.8, perhaps because the molecular weight was not sufficiently high for the asymptotic behavior to be observed.[8] The observed exponent 0.65 for pullulan suggests that the pullulan molecule in water behaves as a random coil. This agrees fairly well with the data and conclusion obtained by Kawahara et al.[2] and Brant et al.[3] However, the data for lower molecular weight fractions deviate from the straight line to the upper side. The reason why the double-logarithmic plot of the intrinsic viscosity against the weight-average molecular weight deviates from the straight line is attributed to the following:[9]

(1) The molecular chain is too short to obey the Gaussian statistics.

(2) The finite cross section of the molecular chain, which is negligible in long-chain molecules, becomes important.

(3) Partial free draining of coiled molecules begins to become effective.

Figure 5 shows the Burchard–Stockmayer–Fixman plot

$$\frac{[\eta]}{M_w^{\;1/2}} = K_0 + 0.51\Phi_0 B M_w^{\;1/2}$$

where

$$K_0 = \Phi_0\left(\frac{6\langle S^2\rangle_0}{M_w}\right)^{3/2}, \quad B = \beta/M_w^{\;2}$$

Φ_0 is a universal constant 4.19×10^{24}, $\langle S^2\rangle_0$ represents the mean-square unperturbed radius of gyration, β is a binary cluster integral.[9] From the least-squares fitting, we obtain $K_0 = 0.091$, $0.51\Phi_0 B = 8.44 \times 10^{-5}$.

This leads to

$$\langle S^2\rangle_0 = 1.30 \times 10^{-17} M_w$$

$$\beta = 3.95 \times 10^{-29} M_w^{\;2}$$

Photon Correlation Spectrometry. Figure 6 shows the concentration dependence of the diffusion coefficient for five pullulan samples determined by PCS together with the results for three pullulan samples determined by the classical boundary formation technique. This dependence seems to be a linear relation up to a concentration of about 0.02 g/mL, and then the extrapolation to infinite dilution was made to determine D_0 from the intercept. Since the measurements were done at the regime $kR_g \ll 1$ (k, scattering vector; R_g, radius of gyration), only the overall

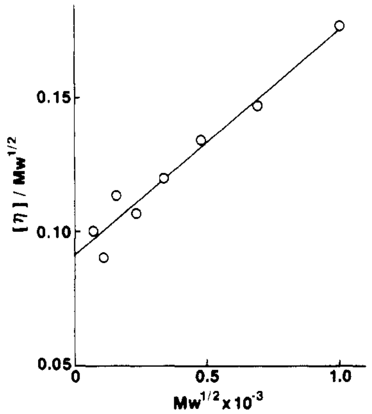

Figure 5. Burchard–Stockmayer–Fixman plot for pullulan samples at 25 °C.

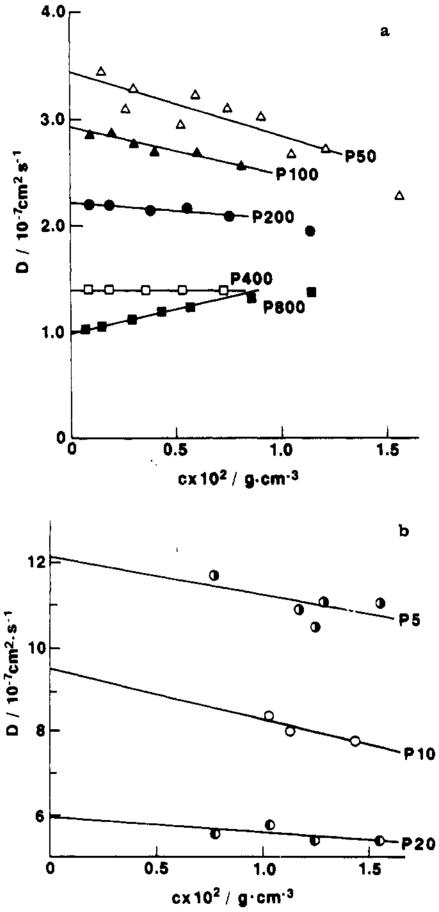

Figure 6. Concentration dependence of the diffusion coefficient for pullulan samples at 25 °C: (a) from PCS, (b) from the classical boundary formation.

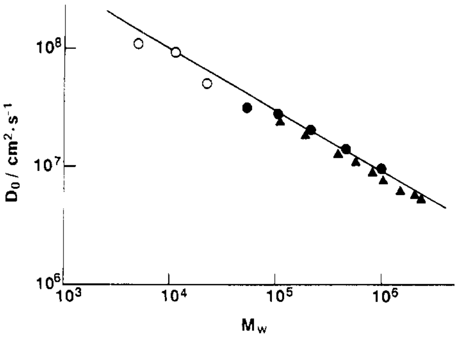

Figure 7. Double-logarithmic plot of D_0 against M_w for pullulan samples at 25 °C: (●) from PCS, (○) from the classical boundary formation, (▲) from ref 1.

translational motion of the polymer was observed.[8] The so-called diffusion coefficient, second virial coefficient k_D, was evaluated from the slope. The values of k_D were negative for seven lower molecular weight fractions and positive for the highest molecular weight fraction P800.

Yamakawa proposed a theory for k_D as[10,12]

$$k_D = 0.8A_2M - V_h - \bar{v}$$

where V_h is the specific hydrodynamic volume and is given by $V_h = 4\pi R_H{}^3N_A/3M$. R_H is the hydrodynamic radius and can be estimated by the Einstein–Stokes equation $R_H = kT/6\pi\eta_0D_0$. In Yamakawa's equation, the contribution from $\bar{v}$ was negligibly small for all eight samples. The calculated values of k_D are shown in the sixth column of Table III. Although the calculated value of k_D decreased with decreasing molecular weight, it is always positive and cannot explain the negative values of observed k_D.

Pyun and Fixman also proposed an equation for k_D[13]

$$k_D = 2A_2M - k_f - \bar{v}$$

where $k_f = k_{f0}V_h$ and $k_{f0} = 2.23$ for the soft-sphere model at the Θ point and 7.16 for the hard-sphere model in the asymptotic limit of the excluded-volume effect. However, this theory also gives values of k_D too large for higher molecular weight fractions P200, P400, and P800. This is different from the conclusion of Kato et al.,[1] and a reasonable explanation for negative k_D values must be found.

Figure 7 shows the double-logarithmic plot of the translational diffusion coefficient at infinite dilution, D_0, against the weight-average molecular weight, M_w. Although values of D_0 were determined by PCS for higher molecular weight fractions and by the hydrodynamic method for lower molecular weight fractions, these were located in a straight line. Least-squares fitting gives a relation

$$D_0 \sim M_w{}^{-0.51}$$

which agrees with the result of Kato et al.[1] although they determined the weight-average molecular weight from light scattering. The Zimm model for flexible polymers predicts the exponent 0.5 in a Θ solvent and 0.6 in a good solvent. However, observed values of the exponent for many flexible polymers in good solvent were about 0.55, which is slightly smaller than the theoretical value. This discrepancy is attributed to the same reason as in the case of the exponent in the Mark–Houwink–Sakurada equation in the intrinsic viscosity–molecular weight relationship.[8] Therefore, the observed exponent 0.51 for pullulan must not be attributed to the poorness of the water solvent but may be to the not sufficiently high enough molecular weight of the polymer to reach the asymptotic behavior.

Kawahara et al.[2] suggested that pullulan molecules behave as flexible random coils in the molecular weight range from 20 000 to 800 000 based on the sedimentation equilibrium measurements.

It is required to do measurements using higher and lower molecular weight fractions to confirm the conclusion.

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