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Hydrodynamic properties of the fractions of mannan formed by *Rhodotorula rubra* yeast

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Aqueous solutions of fractions of an extracellular linear mannan formed by *Rhodotorula rubra* yeast have been investigated by hydrodynamic methods (high-speed sedimentation, translation isothermic diffusion and viscometry). The molecular weight was determined according to Svedberg (M_{sD}) and the polydispersity parameters of the initial sample were also determined ($M_w/M_n = 1{\cdot}20$ and $M_z/M_w = 1{\cdot}21$). Relationships between the molecular weight (M) and s_o, D_o and $[\eta]$ in the range $21 < M_{sD} \times 10^{-3} < 180$ were: $[\eta] = 2{\cdot}33 \times 10^{-2} M^{0{\cdot}75}$, $D_o = 1{\cdot}65 \times 10^{-4} M^{0{\cdot}58}$, $s_o = 2{\cdot}24 \times 10^{-15} M^{0{\cdot}43}$. The equilibrium rigidity and hydrodynamic diameter of chains representing mannan molecules were evaluated.

INTRODUCTION

Many microorganisms can synthesize extracellular polysaccharides. Polysaccharides exhibiting biological activity are of particular interest. They include extracellular mannans formed by *Rhodotorula* and *Sporobolomyces* yeast. Mannan *Rhodotorula rubra* exhibits antitumoral and fibrinolitic activity of the macrophage, and modifies the activity of some enzymes (Elinov *et al.*, 1988; Gurina *et al.*, 1988). The molecular weight and the polydispersity of polysaccharide samples may play a role in their biological activity. However, the molecular characteristics of mannans have not been investigated in detail (Yalpani, 1988).

In the present work the methods of molecular hydrodynamics (Tanford, 1961; Tsvetkov *et al.*, 1970; Cantor & Schimmel, 1980) were used for the investigation of a non-fractionated sample and fractions of *Rhodotorula rubra* mannan (I) and a sample of mannan identical to sample I but produced by *Sporobolomyces* (II).

EXPERIMENTAL

Materials and methods

In order to obtain extracellular mannan, the yeast was grown on a synthetic medium with glucose and vitamins. After the cells had been separated from the enzymatic mass by precipitation with ethanol, crude polysaccharide was obtained. Mannans were isolated by using a specific purification by Fehling's solution (Elinov & Vitovskaya, 1970). The chemical structure of these mannans was established by the methods of carbohydrate chemistry and ^{13}C-NMR spectroscopy. They are linear polymers, the repeating unit of which contains β-1,3 and β-1,4 bonded residues of mannose in equimolecular ratio, i.e. they are the molecules of β-1,3 and β-1,4 polymannopyranosyl-D-mannose (Elinov & Vitovskaya, 1970, 1979).

Sample I was fractionated by fractional precipitation in the water-acetone system from an initial polymer concentration $C = 10$ kg/m^3 (Fig. 1). The fractions were lyophilically dried and stored in a dessicator under $CaCl_2$.

High-speed sedimentation of these fractions was investigated using an MOM 3170 analytical ultracentrifuge (Hungary) generally at a rotor speed of 40×10^3 rpm in a double sector synthetic boundary cell (the height of the centerpiece was 12 mm). For several fractions the concentration dependence of the sedimentation coeffient s was studied. It satisfied the equation $s^{-1} = s_o^{-1}\,(1 + (k_s + \bar{v})c + \ldots)$ (Yamakawa, 1971) (Fig. 2), where $\bar{v}$ is the specific partial volume of the polymer in the solvent. The relationship between s

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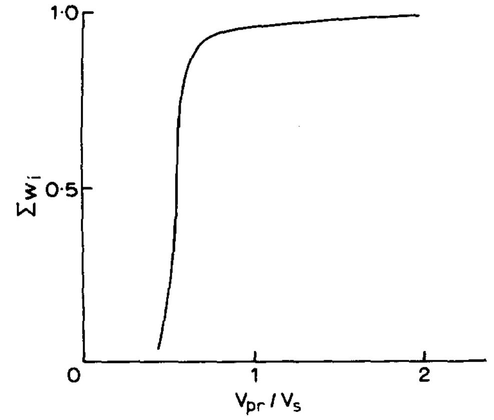

Fig. 1. Cumulative fractionation curve for sample I: w_i, weight fraction of fraction i; V_{pr}, volume of precipitant; V_s, initial volume of the solution.

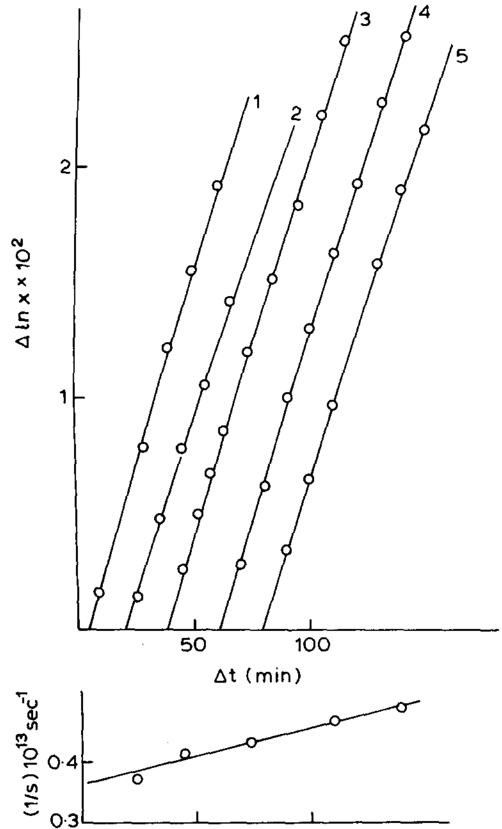

Fig. 2. Dependences of $\Delta \ln x$ on sedimentation time Δt, where x is the position of the sedimentation peak maximum for solutions of sample I at concentrations $C \times 10^2$ g/cm³ = 0·278 (1); 0·203 (2); 0·147 (3) (at rotor speed = 48 × 10³ rpm; h = 12 mm) and C = 0·092 (4); 0·052 (5) (rotor speed = 44 × 10³ rpm; h = 30 mm). Underneath is the concentration dependence of s corresponding to the main graph.

and the concentration sedimentation coefficient k_s obtained was: $k_s = 11{\cdot}4\, s_0^{2{\cdot}0 \pm 0{\cdot}6}$, and this was used to allow for the concentration effect in measurements carried out at a single concentration. The average value of the dimensionless parameter $k_s/[\eta]$, introduced by Newman and Eirich (1950) and used by Wales and van Holde (1954) and Creeth and Knight (1965) for the fractions investigated here, was 1·0 ± 0·5.

The translational diffusion coefficient D was determined from the time dependencies of dispersion of the diffusion boundary formed in a glass cell of optical path length, h = 30 mm at an average solution concentration C = 0·3 kg/m³. The values of D obtained at these low concentrations were assumed to be the values extrapolated to zero concentration D_0. The optical system of recording the solution–solvent boundary in sedimentation–diffusion analyses was a Lebedev's polarizing interferometer (Tsvetkov *et al.*, 1970) (Fig. 3). The refractive index increment $\Delta n/\Delta c$ (Table 1) was determined from the area spanned by the interference curve, and its average value $(\Delta n/\Delta c)$ was (0·12 ± 0·012) × 10 m³/kg at λ = 550 nm.

Viscometric measurements were carried out in an Ostwald viscometer (Fig. 4). The values of intrinsic viscosities $[\eta]$ and Huggins parameters k' are shown in Table 1 (the average value of k' was 0·50 ± 0·07). The temperature coefficient of $[\eta]$ was measured in the temperature range $0{\cdot}5 < T$ (°C) $< 72{\cdot}5$ for the unfractionated sample I$(\Delta \ln [\eta]/\Delta T = -3{\cdot}2 \times 10^{-3}\ \mathrm{K}^{-1})$. The intrinsic viscosity for one of the samples was measured in NaCl solution in the salt concentration range 0·15 M $\leqslant C_{\mathrm{NaCl}} \leqslant$ 1 M. The differences in $[\eta]$ in the range of $0 \leqslant C_{\mathrm{NaCl}} \leqslant$ 1 M did not exceed 3%, i.e. they are within experimental error.

Hydrodynamic parameters were measured in water at 25 °C or were reduced to this temperature according to standard equations (Tanford, 1961; Tsvetkov *et al.*, 1970; Cantor & Schimmel, 1980). The density increment $(1 - \bar{v}\rho_0)$ was determined picnometrically and found to be 0·35 ± 0·015. The experimental data are given in Table 1.

RESULTS AND DISCUSSION

The hydrodynamic invariant $A_0 = \eta_0 D_0 (M[\eta])^{1/3} T^{-1} = [R(D)^2(s)[\eta]]^{1/3}$ (Tsvetkov *et al.*, 1970) was calculated from experimental data. Its average value over all the fractions was $(3{\cdot}2 \pm 0{\cdot}16) \times 10^{-17}\ \mathrm{J\,K^{-1}\,mol^{1/3}}$. The average value of the sedimentation parameter $\beta_s = N_A[s]k_s^{1/3}M^{-2/3} = N_A(R^{-2}[D]^2[s]k_s)^{1/3}$ (Pavlov & Frenkel, 1988) was $(1{\cdot}06 \pm 0{\cdot}12) \times 10^7\ \mathrm{mol}^{1/3}$ where N_A is Avogadro's number, R the gas constant, η_0 is the solvent viscosity, $[D] = D_0\eta_0T^{-1}$ and $[s] = s_0\eta_0(1 - \bar{v}\rho_0)^{-1}$. These average values are characteristic of linear polymers, in particular of cellulose and its derivatives (Tsvetkov *et al.*, 1984; Pavlov, 1989).

The molecular weight was calculated from Svedberg's equation $M_{sD} = [\mathrm{RT}/(1 - \bar{v}\rho_0)](s_0/D_0)$ (Svedberg & Pedersen, 1940). On the basis of the results of fractionation and M_{sD}, an integral distribution curve was plotted

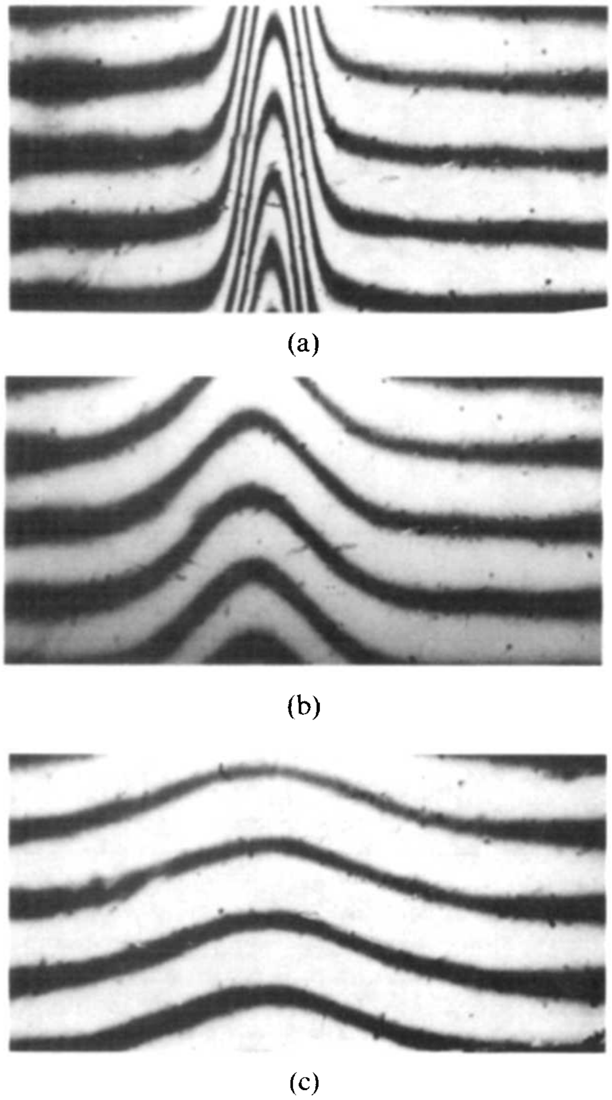

Fig. 3. Interference image of the diffusion boundary of the fraction 4 in water at time t_i after the formation of the boundary: (a) t_1 = 10 min, (b) t_2 = 310 min, (c) t_3 = 910 min (c = 0·025 × 10^{-2} g/cm^3).

(Fig. 5) by the Schulz method (Tung, 1967). This distribution corresponds to the following average molecular weights $M_n = 43{\cdot}6 \times 10^3$, $M_w = 52{\cdot}3 \times 10^3$, $M_z = 63{\cdot}3 \times 10^3$ and the values characterizing the distribution width $M_w/M_n = 1{\cdot}20$ and $M_z/M_w = 1{\cdot}21$. These values indicate that the molecular weight distribution of the mannan studied was relatively narrow.

Let us consider the molecular weight dependencies of the hydrodynamic parameters (Fig. 6). It is assumed that the equimolecular ratio of β-1,3 and β-1,4 of glycosidic bonds is retained for the fractions. It follows from Fig. 6 that the characteristics of non-fractionated samples and those of the fractions are described by the same dependencies. Hence, the computations were carried out by the least-squares method for all data given in Table 1. Table 2 lists the parameters of the Kuhn-Mark-Houwink equations ($Pr_i = K_i M^b$, where $Pr_i = [\eta]$, s_o or D_o) and linear correlation coefficients r_i from which the mean-square errors were calculated: $(\Delta b/b)^2 = (1 - r^2)/[(n-2)r^2]$ and $\Delta \log K = \Delta b[\Sigma(\log M_i)^2/n]^{1/2}$, where n is the number of points through which the straight line is drawn (Pavlov *et al.*, 1985). The relationship $b_D = 1 - b_s = (1 + b_\eta)/3$ characteristic of polymer homologues is satisfactorily obeyed.

The value of b_η (0·74) is greater than that expected (0·5); this may be due either to the excluded volume effects or to free draining effects. If it is supposed by analogy with the behaviour of the macromolecules of β-1,4 glucans (cellulose and its derivatives; Tsvetkov, 1989) and soluble chitin derivatives (Pavlov & Selunin, 1986) that volume effects may be neglected in the first approximation, then the experimental results can be described by the following dependencies:

$$[s]N_A P_o = (M^2\Phi_o/[\eta])^{1/3} = (M_L/A)^{1/2}M^{1/2}$$
$$+ (P_o M_L/3\pi)(\ln A/d - \phi(0))$$

where $P_o = 5{\cdot}11$ and $\Phi_o = 2{\cdot}87 \times 10^{23}$ are the Flory hydrodynamic parameters (Yamakawa, 1971), L is the

Table 1. Hydrodynamic parameters of mannan fractions in water at 25°C and their molecular weight

Fraction number	$[\eta]$ 10^3 (m^3/kg) ±2	k' ±0·1	D_o 10^{11} (m^2/s) ±0·07	$\Delta n/\Delta c$ 10^3 (m^3/kg) ±0·01	s_o 10^{13} (s) ±0·05	k_s 10^3 (m^3/kg) ±7	M_{sD} 10^{-3}	A_o 10^{17} (JK^{-1} mol$^{1/3}$)	β_s 10^{-7} (mol$^{1/3}$)
1	135	0·50	2·35	0·114	3·4	136	103	3·65	1·22
2	115	0·40	2·2	0·104	2·9	—	93	3·14	—
3	104	0·56	—	—	3·0	95	90	—	1·05
4	98	0·50	2·5	0·144	2·55	—	72	3·10	—
5	82	0·48	2·7	0·120	2·55	120	67	3·08	1·12
6	73	0·39	3·4	0·121	2·05	—	43	3·21	—
7	70	0·59	3·8	0·123	1·95	—	36	3·35	—
8	62	0·65	3·5	0·120	2·2	—	45	3·17	—
9	50·5	0·51	4·25	—	1·9	22	32	3·21	0·82
10	37	0·44	4·8	0·110	1·8	44	27	3·08	1·09
11	—	—	5·5	—	1·65	—	21	—	—
I	84	0·44	2·7	0·100	2·6	107	68	3·12	1·14
II	175	0·48	1·55	0·117	4·0	131	180	3·20	0·98

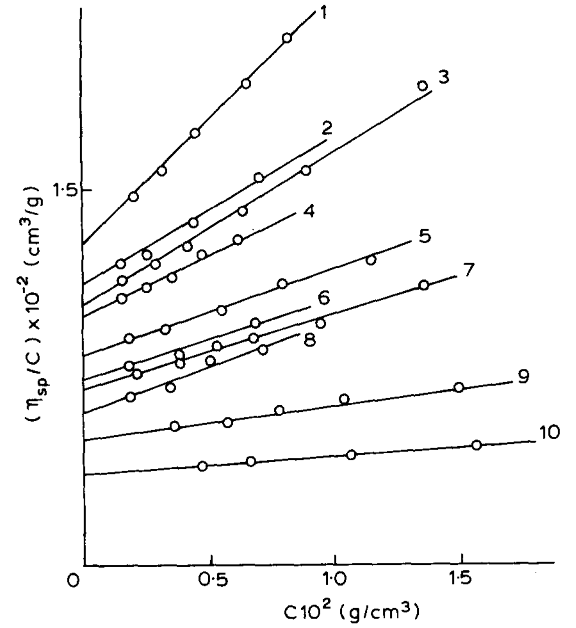

Fig. 4. Plots of η_{sp}/c against c for mannan in water at 25 °C. The numbers indicate the fraction numbers in Table 1.

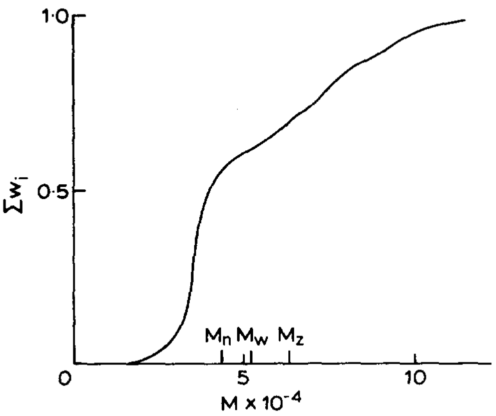

Fig. 5. Integral molecular-weight distribution of the unfractionated sample I obtained from the results on the separate fractions.

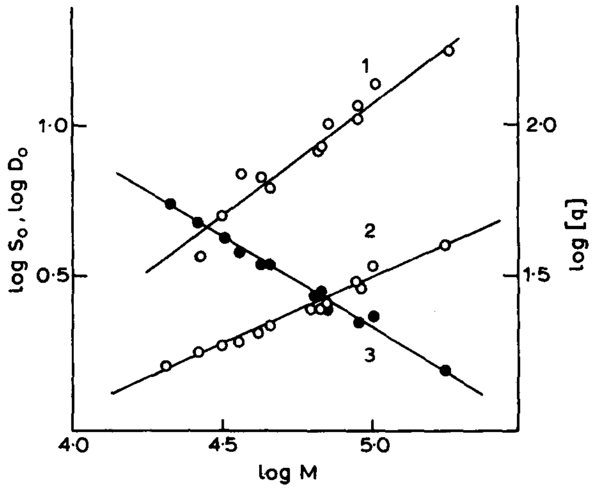

Fig. 6. Kuhn-Mark-Houwink dependences: 1 – $[\eta]$ – M; 2 – s_0 – M; 3 – D_0 – M.

Table 2. Parameters of Kuhn-Mark-Houwink equations for β-1,3-β-1,4 polymannopyranosil-D-mannose in water ($20 < m \times 10^{-3} < 180$)

ε	$\log K_i \pm \log K_i$	K_i	$b_i \pm \Delta b_i$	r_i
$[\eta]$	$-1{\cdot}63 \pm 0{\cdot}31$	$2{\cdot}33 \times 10^{-2}$	$0{\cdot}75 \pm 0{\cdot}065$	0·964
D_0	$3{\cdot}218 \pm 0{\cdot}098$	$1{\cdot}65 \times 10^{-4}$	$-0{\cdot}58 \pm 0{\cdot}021$	0·994
s_0	$-1{\cdot}649 \pm 0{\cdot}090$	$2{\cdot}24 \times 10^{-15}$	$0{\cdot}43 \pm 0{\cdot}019$	0·990

contour length of the molecule, M_L is the mass per unit length of the macromolecules.

The dependence $s_0 = f(M^{1/2})$ is an analytical expression that follows from the Hearst-Stockmayer ($\phi(0) = 1{\cdot}431$) and Yamakawa-Fujii ($\phi(0) = 1{\cdot}056$) theories for the case of $L/A > 2{\cdot}3$ (Yamakawa, 1971; Tsvetkov, 1989), the dependence $(M^2/[\eta])^{1/3} = f'(M^{1/2})$ is developed by Bushin-Tsvetkov (Tsvetkov, 1989). These dependences (Fig. 7) make it possible to estimate both the length of the Kuhn-segment A characterizing the equilibrium chain rigidity and the hydrodynamic diameter d. These evaluations require the knowledge of $M_l = M_0/\lambda$ where M_0 is the molecular weight of the repeating unit and λ is the projection of the unit in the chain direction. The value of λ used here was an average of the values obtained from X-ray data by Winner *et al.* (1975) ($\lambda = 0{\cdot}923$ nm) and by Marchessault *et al.* (1980) ($\lambda = 0{\cdot}95$ nm). These values were obtained for polysaccharides, the repeating unit of which consists of two pyranose rings jointed successively by β-1,4 and β-1,3 bonds: it is deduced therefore for the present case that M_L is (346 ± 5) nm^{-1}.

Table 3 gives the evaluations of A and d obtained with an assumption of the absence of volume effects ($\varepsilon = 0$). The values of A exceed those usually obtained for flexible chain polymers (Tsvetkov *et al.*, 1970) $A \approx 2{\cdot}0$ nm. However, they are lower than for cellulose ($A > 10$ nm; Pavlov & Shildiaeva, 1988) and even to a great extent than for its derivatives ($A > 20$ nm; Tsvetkov, 1989). Moderate values of A do not rule out the possible influence of volume effects on the chain dimensions of the mannan investigated.

At present it is not possible to separate the contributions to the difference between b_η (or b_D) from 0·5 provided by the effects of hydrodynamic draining and excluded volume effects taking into account the dependence of these contributions on the reduced length L/A of the macromolecule. Hence, we can estimate the lower limit of A values assuming that all the difference between b_η and 0·5 is due to volume effects, as is done for flexible-chain polymers. In this case, to evaluate A over the entire molecular weight range, the theory of Gray *et al.* (1967) may be used. Then the experimental results are described by the following dependencies:

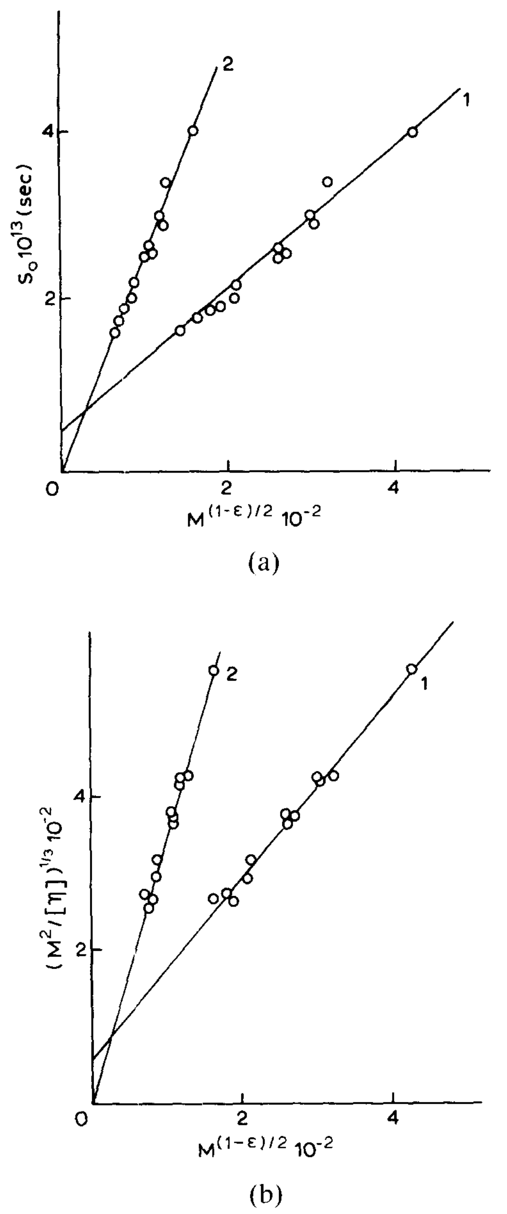

Fig. 7. Dependences of (a) s_0 and (b) $(M^2/[\eta])^{1/3}$ on $M^{(1-\varepsilon)/2}$ at (1) $\varepsilon = 0$ and (2) $\varepsilon = 0{\cdot}16$.

$$[s]N_AP_0 = [3/(1-\varepsilon)(3-\varepsilon)]\,[M_L^{(1+\varepsilon)/2}/A^{(1-\varepsilon)/2}]\,M^{(1-\varepsilon)/2} + (M_LP_0/3\pi)\,[\ln A/d - 1/3(A/d)^{-1} - \phi(\varepsilon)] \qquad (1)$$

where ε is the parameter characterizing volume effects (Ptitsyn and Eizner, 1959) and $\phi(\varepsilon)$ is the function tabulated by Gray *et al.* (1967).

According to Ptitsyn and Eizner (1959), we have $\varepsilon = (2b_\eta - 1)/3 = (2b_D - 1)$ and then $\varepsilon = 0{\cdot}16$ and $\phi(\varepsilon) = 1{\cdot}970$.

The computations related to the $[\eta]$ data were made by using the principle of equivalence (or similarity) of chain size in translational and rotational friction and assuming that the hydrodynamic invariant A_0 is constant in the homologous series of the polymer. It may be written (Pavlov *et al.*, 1990) that

Table 3. Values of equilibrium rigidity *A* and hydrodynamic diameter *d* of β-1,3-β-1,4 polymannopyranosil-D-mannose molecules in water

Excluded volume parameter, ε	Translational friction (s_0, D_0)		Viscosity ($[\eta]$)	
	$A \pm \Delta A$ (nm)	$d \pm \Delta d$ (nm)	$A \pm \Delta A$ (nm)	$d \pm \Delta d$ (nm)
0	73 ± 6·3	4·8 ± 1·5	55 ± 4·5	1·9 ± 0·7
0·08	59 ± 7·3	6·7 ± 2·9	43 ± 5·6	3·0 ± 1·7
0·16	44 ± 4·7	10·4 ± 4·0	31 ± 3·1	5·5 ± 2·4

$$[s]P_0N_A = (M^2\Phi_0/[\eta])^{1/3} \qquad (2)$$

Hence the viscosity data will be considered in a system of coordinates $(M^2\Phi_0/[\eta])^{1/3} = f(M^{(1-\varepsilon)/2})$, where the type of the function is given on the right-hand side of eqn (1). The plots corresponding to eqns (1) and (2) are shown in Fig. 7. The values of A and d at $\varepsilon = 0{\cdot}16$ are given in Table 3. Table 3 also lists the values obtained at an average value of $\varepsilon = 0{\cdot}08$ ($\phi(\varepsilon) = 1{\cdot}681$).

According to the results of translational friction (s_0 or D_0), $4{\cdot}4 < A_f\,(\text{nm}) < 7{\cdot}3$ was obtained and according to the results of rotational friction we have $3{\cdot}1 < A_\eta\,(\text{nm}) < 5{\cdot}5$. The differences in A_η and A_f values are normal and reflect the absence of agreement between the theories describing translational and rotational friction of macromolecules (Tsvetkov, 1989). The values of hydrodynamic diameter obtained from viscometric data at $\varepsilon = 0$ are too low compared to the geometrical chain diameter.

These values of A are lower than those of β-1,4-glucan and indicate that equilibrium chain rigidity of linear uncharged polysaccharides is profoundly affected by the type of pyranose ring insertion into the main chain (Rees & Scott, 1971; Rees, 1982).

The equilibrium rigidity of molecules of β-1,4 and β-1,3 polysaccharides may be determined both by different intrachain hydrogen bonding in different polysaccharides and by different angles of rotation axis in polysaccharide chains on passing from β-1,4 to β-1,3 bonds.

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