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AMYLOSE AND GLUCOSE SOLUTIONS. A COMPARATIVE STUDY

Abstract

Structural parameters of retrograded amylose chains in aqueous solution were determined by means of a comparative analysis with glucose solutions stored under the same conditions and the same specific volume.

Viscosimetric and polarimetric measurements were carried out in glucose and amylose (from potato) solutions during storage, within the same concentration range of 0.005–0.02 (g/cm^3), and with a fixed low concentration of a polymer complexing agent (5 10 $^{-5}$ g/cm^3), added as a polymer chain stiffener.

From the ratio of limit viscosity numbers obtained for polysaccharide and monosaccharide solutions, the average asymmetric parameter of rigid amylose chain was calculated, value of which points to a significant length expansion of retrograded α-D-glucopyranose coil.

Measurements of the concentration dependence of the optical rotatory dispersion in retrograded amylose and its monomer unit solutions were used to determine the degree of coiling into helix of polysaccharide in aqueous solution.

Materials and methods

Studies were performed on aqueous solutions of soluble potato amylose (POCh, Gliwice, Poland) and anh. D-glucose of analytical grade (POCh, Gliwice, Poland). The glucose solutions were prepared at room temperature, while the amylose was digested for 30 min in boiling re-distilled water (200 cm^3). At the end of gelling, the same small amount of Rose Bengal sodium salt (Sigma, Poland) was added to all samples of amylose and glucose. The concentration range studied for both compounds was 0.005–0.02 g/cm^3 and the fixed low concentration of the dye was 2.5 10^{-5} g/cm^3. The samples were stored in a darkness at 22.0 (± 0.5)°C. Viscosity measurements were carried out for samples stored for 3h, 24 h, 48 h and 100 h. The capillary method with the Ubbelhode viscometer was involved. Capillary diameter was 0.8 mm. Flow time for each sample was average of 5 to 8 runs.

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Optical rotatory dispersion (ORD) of the amylose and glucose solutions was measured at three wavelengths of 366 nm, 406 nm and 436 nm, respectively with a Polamat-A polarimeter (K. Zeiss, Jena, Germany) equipped with a 2 dm cell. Temperature was maintained constant.

Results and discussion

Viscosity measurements

Prolonged storage of amylose solutions resulted in significant structural and molecular changes in the system. The concentration dependence of the specific viscosity of amylose solution after 3 h, 24 h, two, and four days of ageing presented in Figure 1 relates to studies at low polymer concentration range, i. e. below 2%.

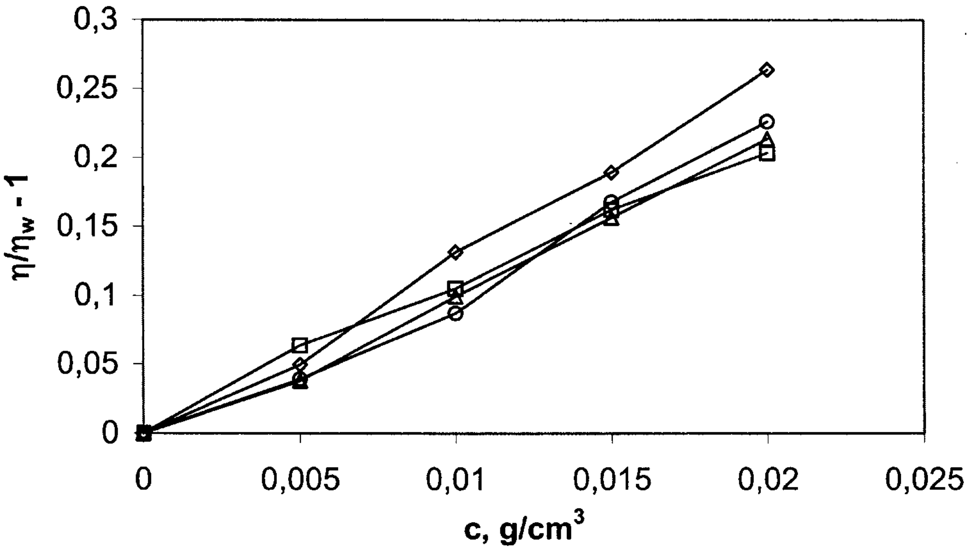

Fig. 1. The specific viscosity of a potato amylose solution in the presence of Rose Bengal (0.025 mM) as a function of polymer concentration after 3 h (□), 24 h (O), 48 h (Δ) and 100 h (◊) of storage at a constant temperature of 22°C.

In the first approximation the $[\eta/\eta_w - 1] = f(c)$ function for all samples is linear (Fig.1). Admixture of a small amount of Rose Bengal caused minor changes in the limit value of viscosity in comparison to pure water. The dye is known to complex to amylose [7] influencing in this manner the molecular motion of that polysaccharide chains in solution. The dynamic viscosity of water, $\eta_{w,}$, is lower than that of Rose Bengal solution, η_{RB}, but the difference is negligible in the first approximation.

Assuming a linearity between specific viscosity and amylose concentration in the solution, the limit viscosity number [η] was obtained. From the definition limit viscosity number $[\eta] = \lim \{[\eta/\eta_w - 1]/c\}$ when $c \rightarrow 0$. This number estimated for the amylose solution after 3, 24, and 48 hours of ageing is identical and reaches 10.6 ± 0.4 $(g/cm^3)^{-1}$, but it increases to 13.0 ± 0.4 on the 4-day amylose solution, where the effect of opacity in the 2% amylose concentration solution was observed.

The Flory's theory [3] of polymer solutions reveals that the limit viscosity number [η] is related to the size of the macromolecule coil, precisely to the mean square of the inertial diameter of a coil, $<s^2>$:

$$[\eta] = \Phi <s^2>^{3/2} / M_p, \tag{1}$$

where Φ is the universal constant, independent of the macromolecule character and solution. Its value of $4.2\ 10^{24}$ was calculated for the coil of a flexible polymer chain [6]; M_p – molecular weight of a polymer chain.

Based on Eq. (1), the ratio of $<s_r{}^2> / <s_f{}^2>$ was calculated for the mean square of the inertial diameter in amylose chain prior to retrogradation and in retrograded molecule. Resulting value of 1.36 indicates changes in the compactness of the random coil. Therefore, a significant increase in the inertial diameter of the amylose coil may lead to a break down in the phase continuity in the system. Independently of it, changes in the dynamics of the polymer motion in a liquid take place.

Let us compare the molecular dynamics of the amylose chain in water and its monomer unit, i.e. in glucose in solution of the same specific volume. We may consider the glucose solution as the limiting case of a non-branched α-D-glucan polymer solution. Thus, all differences between these two systems resulted from the polymerisation of small glucose molecules. The concentration dependence of the specific viscosity of the amylose and glucose solutions in the presence of Rose Bengal, after 100 h of storage is shown in Figure 2.

The limit viscosity number for the glucose - Rose Bengal solution is [η] = 3.0. Taking into account this value and that obtained for the α-D-glucopyranose chain, one may estimate an asymmetric parameter p for the amylose coil based on the relation between the limit viscosity number and the asymmetric parameter p in a suspension of ellipsoidal particles [1]:

$$[\eta] = v'\ 2.5 \left\{1 + \frac{0.4075}{2.5} (p - 1)^{1.508}\right\} \tag{2}$$

where v' – is the partial specific volume of the particle, p – is the asymmetric parameter of the ellipsoidal particle, the ratio of its semi-axes.

In our case, the specific volume of amylose, v'_{AM} and of glucose, v'_{GLU} in solution is the same, so we obtained value p for the fresh amylose chain p = 7.2, and for retrograded amylose p = 8.4.

An increase in the asymmetric parameter of the amylose coil in solution stored for a long time speaks in favour of a tendency of the polymer chain to expansion by decoiling. Amylose chains in aqueous solutions stray from the ideal spherical shape. Due to complexation of dye to polymer, conformation of the amylose chain is affected. Effect of Rose Bengal on the viscosity of 2% amylose and glucose solutions is shown in Figure 2.

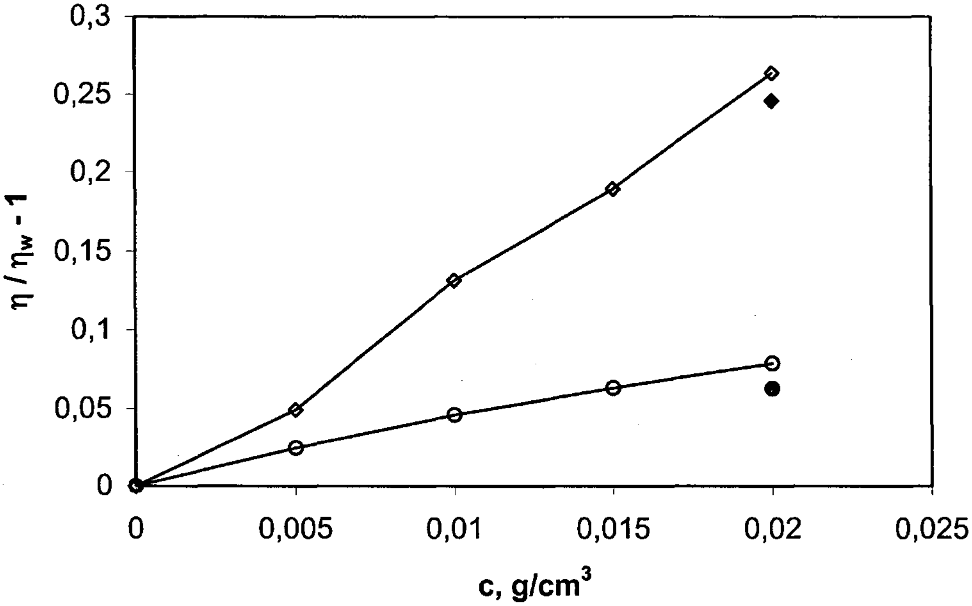

Fig. 2. Comparison of the concentration dependencies of relative viscosity of amylose (◊) and glucose (O) solution, stored for 100 h at the same conditions. (♦) and (•) - amylose and glucose solution without Rose Bengal, respectively.

Optical rotation measurements

The concentration dependence of the optical rotatory dispersion in glucose solutions, the monomer unit of the α-D-glucopyranose chain, and in amylose solutions of the same concentrations was measured during storage. The results of these measurements are presented in Table 1 and Figure 3.

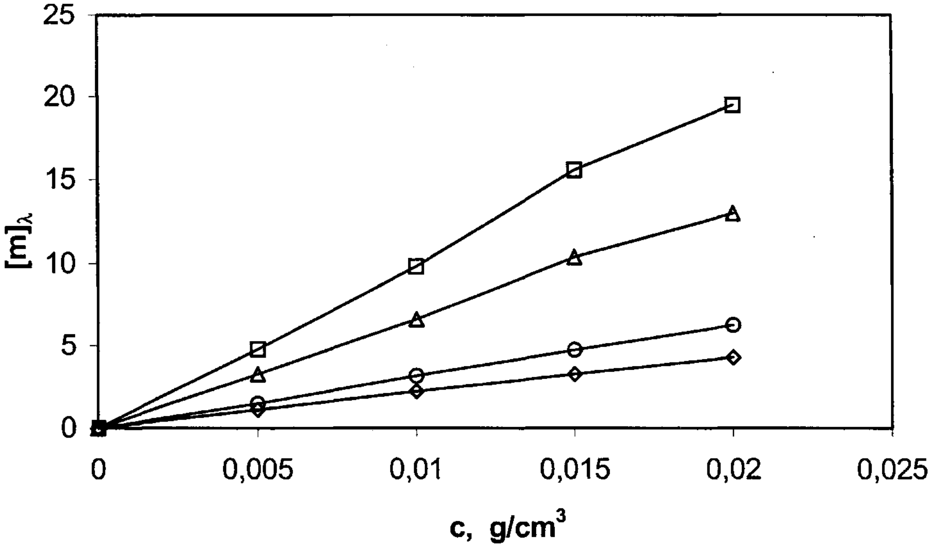

Fig. 3. Optical rotatory dispersion at two wavelengths of λ_1 = 366 nm and λ_2 = 436 nm, versus glucose (O, [366 nm], ◊ [436 nm]) and amylose (□, [366 nm], Δ [436 nm]) concentration.

Table 1

Optical rotatory (OR) dispersion vs concentration in glucose and amylose aqueous solution, measured at two wavelengths, λ_1 = 366 nm and λ_2 = 436 nm (after 48 h of storage).

No sample	Concentration, g/cm³, 10^{-2}	D-Glucose		α-D-glucopyranose	
		$[m_{366}]$,deg	$[m_{436}]$,deg	$[m_{366}]$,deg	$[m_{436}]$,deg
1	0.5	1.12	0.83	4.79	3.27
2	1.0	2.78	2.18	9.82	6.60
3	1.5	3.63	2.96	15.55	10.38
4	2.0	6.06	4.04	19.47	13.00
5*	2.0	7.54	5.01	20.33	13.50

* without Rose Bengal

Analysis of the data from these sources and calculated values of specific rotation, provided the degree of amylose coiling in solution during ageing. The calculations were based on the Moffitt equation [2, 8]:

$$[m]_\lambda = A\ f(\lambda^2) + B\ f(\lambda^2) \tag{3}$$

where: A, B – Moffitt's coefficients and $f(\lambda) = (\lambda_0{}^2 - 1)/\lambda^2$, λ_0 is a wavelength at maximum absorption.

Coefficient A depends on the contributions of the chiroptical centres of monomers in macromolecule to the optical rotation. Value of B depends on the contribution of the helices of the molecule to the optical rotation, and λ_0 is the wavelength corresponding to the maximum of the electronic transition of the molecule. Assuming for the amylose solution that $\lambda_0 = 153$ nm [5], both parameters of the Eq.(3) could be calculated. Due to the asymmetry of a helix, the helical structure is source of a some optical activity, which overlays the activity, induced by asymmetric carbon atoms in polymer chain. Comparing the optical rotation in the amylose and glucose solutions of the same number of asymmetric carbon atoms, one may assume, that the chain coiling in the polymer solution also contributes to optical activity. The average ratio of $[m]_\lambda$ for the amylose and glucose solutions, calculated from optical rotatory dispersion, equals to 3.1. This value is close to the ratio of the limit viscosity number for amylose and glucose stored in aqueous solution for less than 4 days. The degree of coiling, χ, can be obtained from Eq. (4);

$$\chi = ([m]_{AM} - [m]_{GLU})/[m]_{AM} \tag{4}$$

where $[m]_{AM}$ and $[m]_{GLU}$ – are optical rotations for amylose and glucose, respectively.

The value of $\chi = 0.68$ fit the data from the 1H-NMR method [5]. No changes in the degree of polymer coiling were observed in the amylose solution stored for 100 h.

Rose Bengal in amylose and glucose 2% solution decreased optical activity (Table 1).

The comparative analysis of behaviour of polymer and monomer in solution can be an important source of the information about structural changes and enables to determine some structural parameters of high-molecular systems.

REFERENCES

[1] Cvietkov V.N., Eskin W.J, Frenkel S.J.: Struktura makrocząstek w roztworach, WNT, Warszawa, (in Polish), 1968.

[2] Elmgren H.: Biopolymers, **23**, 1984, 2525.

[3] Flory P.J.: Principles of Polymer Chemistry, Cornell University Press, Ithaca, NY, 1953.

[4] Gessler K., Uson I., Takaha T., Krauss N., Smith S.M., Okada S., Sheldrick G.M., Saenger W.: Proc. Natl. Acad. Sci. USA, **96**, 1999, 4246.

[5] Maciejewska W., Napierała D., Hoffmann G. [in:] Properties of Water in Foods, Ed. by P.P.Lewicki, Warsaw Agric. Univ. Press, Warsaw, 1999, 7.

[6] Morawetz H.: Fizykochemia roztworów makrocząsteczek, PWN Warszawa (in Polish), 1970.

[7] Napierała D., Popenda M., Żywność. Technologia. Jakość, **2** (**7**) Supl., 1996, 28.
[8] Volkenstein M.W.: Molekularnaia Biofizika, Nauka, Moskva, (in Russian), 1975, 207.

BADANIA REOLOGICZNE I POLARYMETRYCZNE (ORD) ROZTWORÓW AMYLOZY I GLUKOZY

Streszczenie

Ze stosunku względnej lepkości roztworów poli- i monosacharydu obliczono średni parametr asymetrii sztywnego łańcucha amylozowego. Jego wartość wskazuje na znaczne wydłużenie łańcucha amylozy w trakcie retrogradacji.

Pomiary zależności stężeniowej ORD retrogradowanej amylozy oraz α-D-glukozy posłużyły do wyznaczenia w roztworze wodnym stopnia skręcenia łańcucha amylozy w heliks.