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Physio-chemical properties of polymyxan 88A–water system

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Abstract

The system exopolysaccharide polymyxan 88A–water was studied at several temperatures. The temperature dependence of viscosity at cooling and heating was obtained in order to estimate the phase separation temperature (T_s) and the gelation temperature (T_g). The experimental values of T_s and T_g were used to plot the phase diagram of the system under study at polymer concentrations below 1.5 wt%. Viscous flow in the system was examined by the cylinder–cylinder rotation method. It has been found that: (i) at shear rates within 1–100 s^{-1} the dependence of viscosity on shear rate can be fairly expressed by the power low; (ii) the activation enthalpy of viscous flow practically does not depend on shear rate; and (iii) the activation entropy of viscous flow is negative, most likely due to an orienting action of mechanical field.

Keywords: Microbial exopolysaccharides; Phase diagram; Phase separation

1. Introduction

Microbial exopolysaccharides were under comprehensive studies during the last decades, due to their important role in the structure and metabolism of microorganisms. Another point of interest is a diversity in their physico-chemical structure, which leads to a variety of their properties and to the possibility to classify these polymers as an individual group of commercial polymers (Crescenzi, 1994; Sutherland, 1986). In some cases, microbial exopolysaccharides can be used as an alternative to natural or synthetic polymers, in other cases they seem novel polymers to be used as suspending, emulsifying, gel-forming agents changing the rheological characteristics of aqueous systems (Crescenzi, 1995; Sandford, 1983).

The nomenclature of microorganisms employed for industrial synthesis of exopolysaccharides constantly expands, and, as a consequence, the number of biopolymers with various structural and physico-chemical properties is being increased, these polymers used in various branches of industry.

The ability of polysaccharide synthesis is characteristic of many species of p. *Bacillus*. These microorganisms produce homo- and heteropolysaccharides of neutral and acidic nature. The exoglycanes of *Bacillus polymixa* are characterised by high viscosity, thermal stability, and stability to biodegradation (Cox, Steer & Steer, 1981; Fukui, Tanaka & Misaki, 1985; Glukhova, Shenderov, Panasenko, Deryabin & Ignatov, 1986; Madden, Dea & Steer, 1985; Mitsuda, Miyata & Hirota, 1981; Murphy, 1952). We have succeeded in getting a polysaccharide named polymyxan 88A with a high viscosity (Porozhnyakova, Shipin, Matora, Zhemerichkin, Ignatova & Panasenko, 1992). By now, polymyxan 88A has been shown to be a promising material for some branches of industry (e.g. food and oil industry). Therefore, studies of its physico-chemical properties are of practical interest. The goal of the present work was to plot the phase diagram of the system polymyxan 88A–water and to study its rheological properties.

2. Materials and methods

2.1. B. polymixa strain and polysaccharides produced

The bacterial strain-producent *B. polymyxa* 88A was obtained from the museum strain CCM 1459 by means of an exposure to short-term powerful microwave (2375 MHz) radiation. The cultivation of this producent and polysaccharide isolation were carried out in accordance with the method developed earlier (Porozhnyakova et al., 1992).

Recently, ion-exchange chromatography showed *B. polymyxa* 88A strain to produce an acidic polysaccharide and (in small amounts) a neutral one. Both fractions give single symmetrical peaks on their gel-chromatogram, which evidences their homogeneous nature. The molecular mass of the neutral and acidic polysaccharide varies within 100–300 kDa and 1–10 MDa, respectively. The neutral

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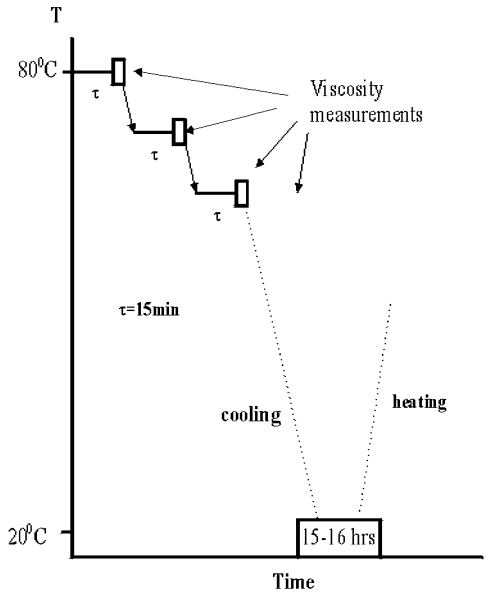

Fig. 1. Scheme of experiment realisation for determination of phase separation temperature using viscosity measurements.

polysaccharide has turned out to be glucomannan with approximately equal amounts of the corresponding monosaccharides and trace amounts of uronic acids. The acidic polysaccharide is composed of glucose, mannose, galactose, and glucoronic acid in an approximate molar ratio 5:5:3:1. The specific optical rotation of the neutral and acidic polysaccharide is $[\alpha]_D^{20} = +61°$ (C 0.032, water) and $[\alpha]_D^{20} = +72°$ (C 0.054, water), respectively (Matora et al., 1992).

Solutions of the acidic exopolysaccharide *B. polymyxa* 88A feature the higher viscosity while the contribution of the neutral one to the overall rheological characteristics is negligible. Our further studies were made on the acidic polysaccharide named by us as polymyxan 88A.

While trying to perform a ^{13}C-NMR study on it, the authors faced a difficulty in preparing rather concentrated solutions; moreover, after its lyophilisation, the polysaccharide ceased to be soluble in water. Hence the solution of this polysaccharide was subjected to an ultrasound treatment for partial depolymerisation. This decreased the molecular mass down to 100–200 kDa, the viscosity by 15–20 times; after lyophilisation no solubility was lost. At the same time, the ^{13}C-NMR spectra of the native and degraded polysaccharides are identical. Their interpretation was difficult, possibly because of structure irregularity (Matora et al., 1992).

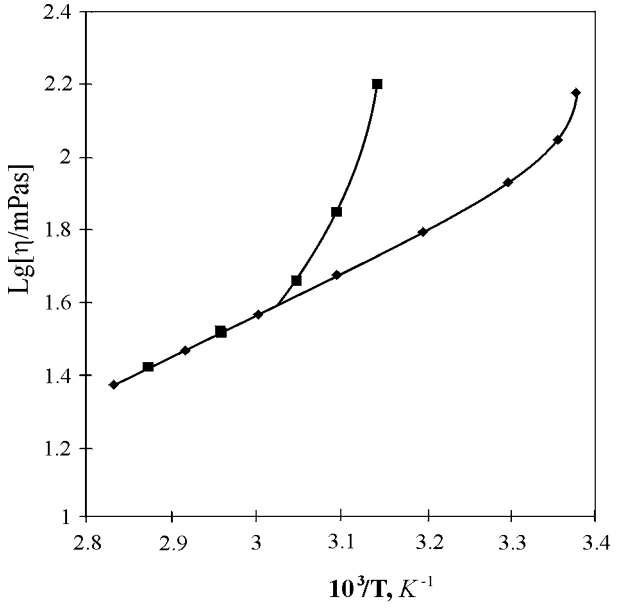

Fig. 2. Temperature-dependence of viscosity on (◆) cooling and (■) heating for the polymyxan–water sistem at $C = 0.5$ wt%, illustrating determination of the phase separation temperature (T_s).

2.2. Physico-chemical methods

The phase diagram was plotted according to the development of viscosity with temperature, the solutions being cooled or heated—this method had been proposed earlier (Ptitchkina, Panina, Karmanova & Novikova, 1996). The viscosity measurements were made at a constant shear stress chosen within 4–10 Pa using a Heppler viscometer. A hot (80°C) polymyxan solution was poured into the viscometer preheated up to 80°C, kept for 15 min, and then being cooled by 3–5 K at a rate 1 K/min; a 15 min incubation followed, by a subsequent measurement of the viscosity. This cycle was repeated several times (Fig. 1). On the final cooling, the system was kept at room temperature for 15–16 h, then a similar experiment, but now with heating, was performed.

The gel strength was examined on a Valenta device with a hemispherical plunger ($S = 1.5$ cm^2). The strength was thought as the minimal pressure for the plunger to penetrate into the gel, having broken the surface layer (Pernas, Smidsrod, Larsen & Haug, 1967).

Two sets of experiments were carried out to reveal how the concentration of polymyxan and temperature of gel formation affect the gel strength. In the first case, samples with concentrations 0.5, 1.0, and 1.5 wt% were prepared and kept at 20°C during 25 h before strength measurements. In the second case, three samples of the same concentration (1.0 wt%) were kept for 24 h at 20, 30, and 40°C, respectively, then thermostated at 20°C for 1 h before strength examination.

Viscous flow in the system was studied with the aid of a Rheotest-2 (made in the former DDR). This viscometer provides a constant shear rate (D) which may be specified within 0.17–1200 s^{-1}, having chosen an appropriate subrange. A hot (80°C) solution placed in the viscometer was cooled at a rate of 2 K/min down to the temperature of the corresponding experiment; after being thermostated for 15 min, the viscosity was measured on the first subrange ($D = 0.17$ s^{-1}); then the device was switched to the second

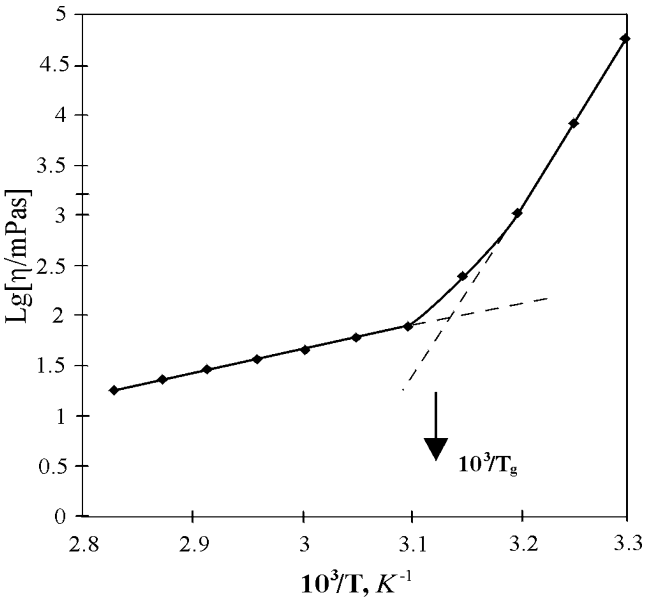

Fig. 3. Temperature-dependence of viscosity on cooling for the polymyxan–water system at $C = 1$ wt% illustrating determination of the gelation temperature (T_g).

subrange ($D = 0.37\ s^{-1}$), the third one ($D = 0.5\ s^{-1}$), and so on, going over the whole range of possible values of shear rate.

3. Results and discussion

Fig. 2 illustrates the method used for determination of the phase separation temperature. The curves obtained at cooling and heating are seen to have converged above a certain temperature denoted as T_s. Any system may be regarded as being in thermodynamic equilibrium if its state does not depend on the specific pathway which this state has been reached by. We therefore assume T_s to be a good estimation for the temperature of phase separation.

The gelation temperature was determined as shown in Fig. 3. The onset of gel formation in the course of cooling can be detected by a sharp increase of the temperature dependence of viscosity. There were two linear regions on this dependence (at lower and higher temperatures), they were extrapolated, and the point of intersection of the corresponding straight lines gave the gelation temperature T_g.

The experimental values of T_g and T_s were used for plotting the phase diagram of the polymyxan–water system below 1.5 wt%. It should be mentioned that our work with the system under study at concentrations above 1.5 wt% was extremely difficult due to its high viscosity. This is the reason for our failure to expand the phase diagram towards higher concentrations.

As is seen from Fig. 4, the concentration dependences of T_g and T_s divide the space of possible phase states into three areas. The range above the T_s vs C curve corresponds to the single-phase state. The system here is homogeneous and stable. Below this curve, the system gets unstable and separates into two phases. The mechanism of this phase separation (liquid–liquid or liquid–crystal) is still vague and cannot be clarified by our experiments. However, we have noticed that the A → B transition is accompanied by the system getting turbid, detected visually. This light scattering is apparently caused by formation of new-phase droplets or particles.

When $T < T_g$ (C), the system forms a thermally reversible gel. Its considerable turbidity obviously points to its two-phase nature. Gels with concentrations above 1 wt% seem rather strong. For example, cylindrical samples with a diameter 5 cm and a height 3 cm preserved their shape well. As the concentration decreases, the strength of gels drops sharply (see Fig. 5a). On the other hand, the gel strength substantially depends on the temperature of formation (Fig. 5b). As the temperature is raised, a sharp monotonic decrease of the gel strength was observed. Having extrapolated the curves shown in Fig. 5 to the zero value of gel strength, we obtained the concentrations where the system started to gelate at 20°C (Fig. 5a) and the temperatures above which no gelation of a 1 wt% solution occurred (Fig. 5b). It should be noted that these values agree fairly with the corresponding values obtained from the phase diagram (Fig. 4).

Fig. 6 demonstrates the dependence of viscosity (η) on shear rate (D) for a 1 wt% solution in the log–log coordinates. This dependence is linear within a range of shear rate 1–100 s^{-1}, so the power law

$$\eta = K \cdot D^{n-1}$$

is obeyed.

As the solution concentration is increased from 0.1 to

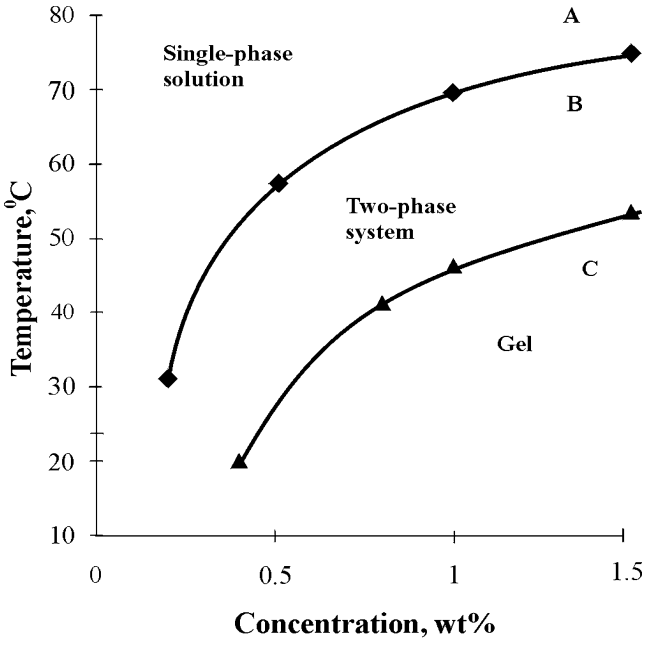

Fig. 4. Phase diagram of the polymyxan–water system.

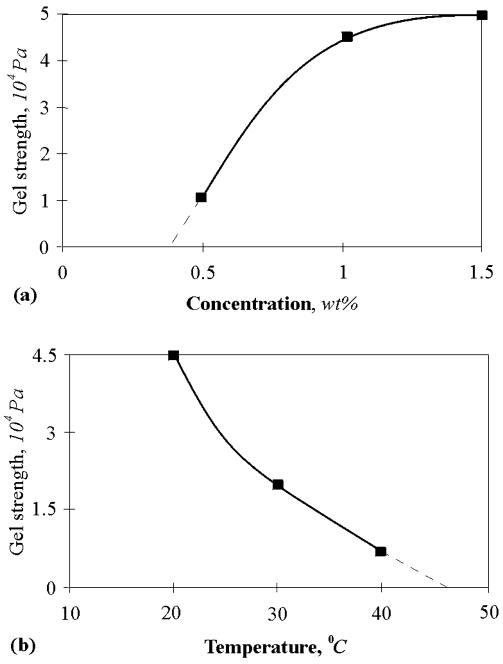

Fig. 5. (a) Dependence of gel strength on polymer concentration at 20°C. Gels were stored at 20°C for 25 h. (b) Dependence of gel strength on the temperature of gel formation for 24 h. Concentration of polymyxan of $C = 1$ wt%. Before measurements, samples were kept at 20°C for 1 h.

1.5 wt%, the flow behaviour index decreases from 0.25 down to 0.15, practically being independent of temperature.

The temperature dependence of the viscosity of a 1 wt% solution for several shear rates is shown in Fig. 7 which is useful to be compared with Fig. 3. This comparison reveals a substantial difference in the rheological behaviour of the same system, which can be explained by: (i) a different rate of cooling; and (ii) a different mode of the mechanical action on the system.

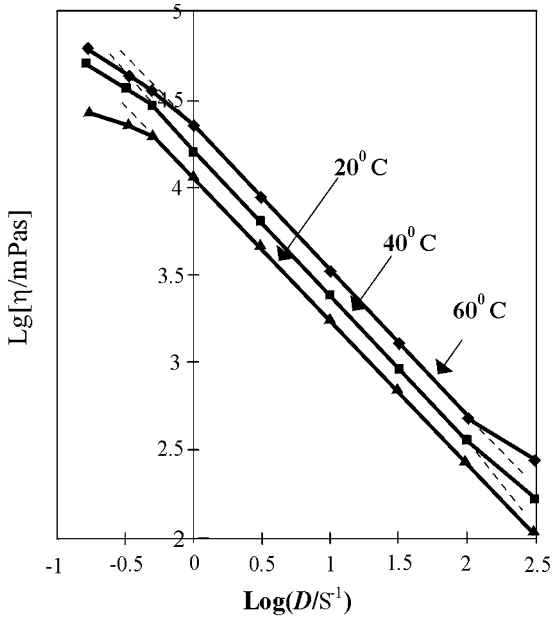

Fig. 6. Dependence of viscosity (η) on shear rate (D) for polymyxan–water system at polymer concentration of $C = 1$ wt% at temperatures shown near curves.

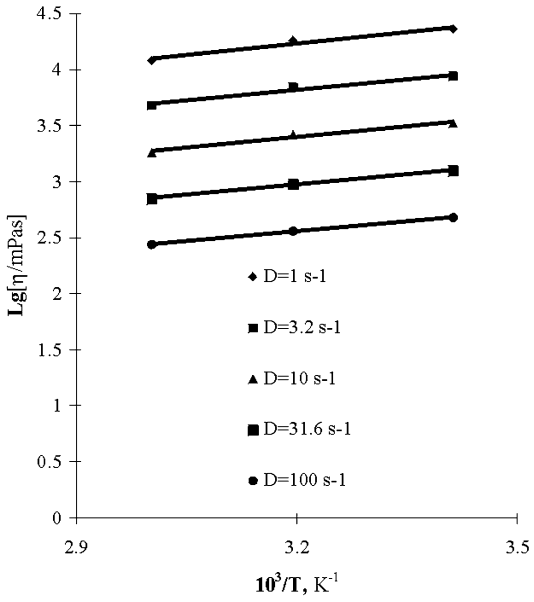

Fig. 7. Temperature-dependence of viscosity for the polymyxan–water system at different values of shear rate (D). Polymer concentration: $C = 1.0$ wt%.

In the case presented in Fig. 7, the system was being cooled with a rate of 2 K/min while in the second case (Fig. 3) the cooling proceeded stepwise with a mean rate of 0.15 K/min. Slower cooling gives rise to deeper structural changes.

In the case presented in Fig. 3, the system had a 18–20 min rest after the mechanical impact and, hence, had enough time to restore, partially or fully. In Fig. 7, the mechanical impact was applied continuously with a rising intensity. This is a destroying mode, which allows no stable structural bonds to be formed. Subjected to such a regime, the system at cooling pays no attention to the boundary curves on the phase diagram—Fig. 7 demonstrates it.

To estimate the activation enthalpy (ΔH) and entropy (ΔS) of viscous flow, the Frenkel–Eyring–Kobeko equation

$$\eta = 10^4 \cdot \exp\left(\frac{\Delta H}{RT} - \frac{\Delta S}{R}\right) \text{ (Pa s)}$$

was employed due to the linearity of the $\log \eta$ vs $1/T$

Table 1
Enthalpy (ΔH) and entropy (ΔS) of viscous flow activation for the polymyxan–water system at polymer concentration of $C = 1$ wt%

Shear rate, s^{-1}	ΔH (kJ/mol)	ΔS (J/mol K)
1.0	13	−59
3.2	12	−54
10.0	12	−46
31.6	12	−39
100.0	11	−32

dependence (Tager, 1978). The calculated values are presented in Table 1. From this table the following conclusions could be drawn:

- The activation enthalpy of viscous flow practically does not change after a 100-fold increase of shear rate, keeping its average value about 12 kJ/mol.
- Within the above range of shear rate, the activation entropy of viscous flow takes on negative values. This means that a more ordered (in comparison with the initial state of the system) structure appears under the influence of shear stress. We may speak about an orienting action of the mechanical stress, this effect frequently being faced in polymer systems (Lapasin & Pricl, 1995). With reference to our system, this phenomenon requires further investigation.

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