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Research Note

Correlation between the Temperatures of Formation/Breakdown of the Gel Network and Conformational Transitions of Agarose Macromolecules

ABSTRACT

The melting temperature of a 1% agarose gel in a solvent consisting of equal weights of water and dimethylsulphoxide, exceeds the temperature of completion of the helix-coil transition by 25°C. It is concluded that the agarose gel network may be retained even when the polysaccharide is in the disordered conformation.

INTRODUCTION

Plashchina *et al.* (1980, 1986), have reported that the formation and melting of κ-carrageenan gels may occur at temperatures exceeding the temperature interval of the helix $\rightleftarrows$ coil transition. Here, we report the results of studies on the relationship between the above transitions for agarose.

MATERIALS AND METHODS

A commercial preparation of agarose ('Serva', FRG) was used in the study. The preparation contained no traces of sulphur (when determined by titration with $BaCO_3$) and therefore contained only negligible amounts of agaropectin. It was also free of Na^+, K^+ and Ca^{2+} ions (as evidenced by X-ray fluorescence analysis).

The polysaccharide was dissolved in a mixture of deionized water and dimethylsulphoxide, at 90°C.

Determination of the temperatures of gelation and melting as well as measurements of the optical rotation of the gels were performed as described in the study of Plashchina *et al.* (1986). The temperature at which the change in optical rotation exceeded the experimental error by a factor of three was considered to be the temperature of the beginning of the coil-to-helix conformational transition (T_0^{c-h}). The temperature of

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the transition end point (T_1^{c-h}) was determined in a similar way. The temperature of the transition midpoint was determined by the graphic differentiation of the temperature curve of the optical rotation. The temperature variation rate was 0·3°C/min.

The gel formation temperature, T_g, was determined with the rotational viscometer VPN-2 produced in the Special Design Bureau of the A.V. Topchiyev Institute of Petrochemical Synthesis, USSR Academy of Sciences. This viscometer permits deformation to be measured at a constant stress. This allows samples of gel to be measured below the rupture point.

The agarose solution was kept in the viscometer cell for 10 min at 80°C, the temperature was then lowered at a rate of 0·3°C/min. A shear stress of 8·5 Pa was periodically applied to the sample and the viscosity was measured. The gel formation point T_g was taken as the temperature at which $\partial\eta/\partial T \to \infty$, i.e. when a yield stress appears. To avoid drying of the sample, a thin layer of silicone oil was applied to the melt surface in the annular gap of the viscometer. The cell temperature was recorded with a thermocouple of ± 0·5°C.

The melting temperature of the gels, T_f, was determined by observing how the melt is displaced by a heavier fluid (perfluorodecalin) predeposited on the gel sample (Braudo *et al.*, 1973). The inner diameter of the test tube was 15 mm. The thickness of the layer of perfluorodecalin was 4 mm. The temperature was increased at a rate of 0·3°C/min.

RESULTS

Introduction of dimethylsulphoxide increases the transparency of the agarose solutions (Ng Ying King & Yaphe, 1972) allowing optical rotation measurements to be made. The temperatures of gelation (T_g) and the coil-to-helix transition midpoint ($T_{1/2}^{c-h}$) as functions of dimethylsulphoxide concentration for a 1% agarose gel are plotted in Fig. 1. As can be seen from Fig. 1, the 30–50% (w/w) concentration of dimethylsulphoxide provides the optimal conditions for gelation and helix formation of agarose. In this composition range dimethylsulphoxide trihydrate predominates (Watase & Nishinari, 1986). Subsequent measurements were made with 50% dimethylsulphoxide.

The concentration dependencies of the temperatures of gelation and of the coil-to-helix transition of agarose are presented in Fig. 2. The curves clearly show that gelation of agarose proceeds in the same temperature range as the coil-to-helix transition.

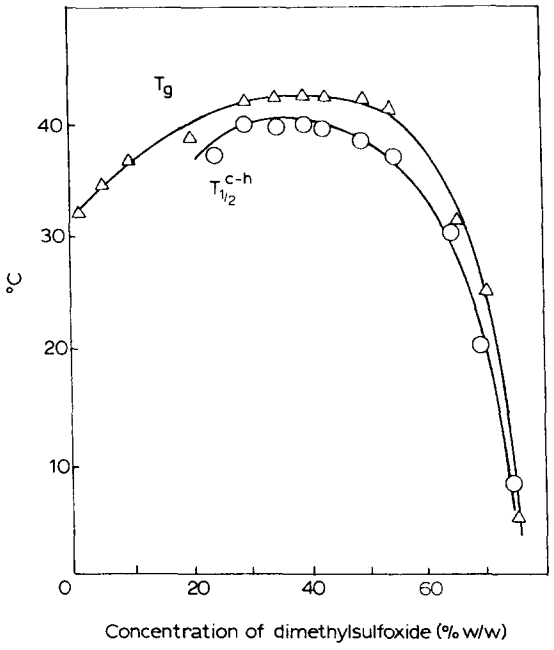

Fig. 1. The temperatures of gelation (T_g) and of the coil-to-helix transition midpoint ($T_{1/2}^{c-h}$) as a function of dimethylsulphoxide concentration for a 1% agarose gel.

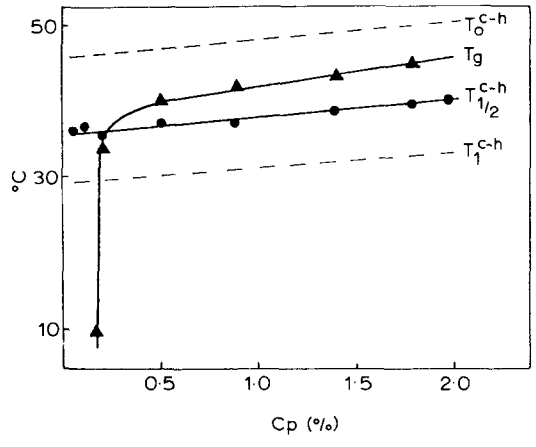

Fig. 2. The temperatures of gelation (T_g) and of the coil-to-helix transition midpoint ($T_{1/2}^{c-h}$) as a function of agarose concentration. The solvent was a 1:1 (w/w) mixture of water and dimethylsulphoxide.

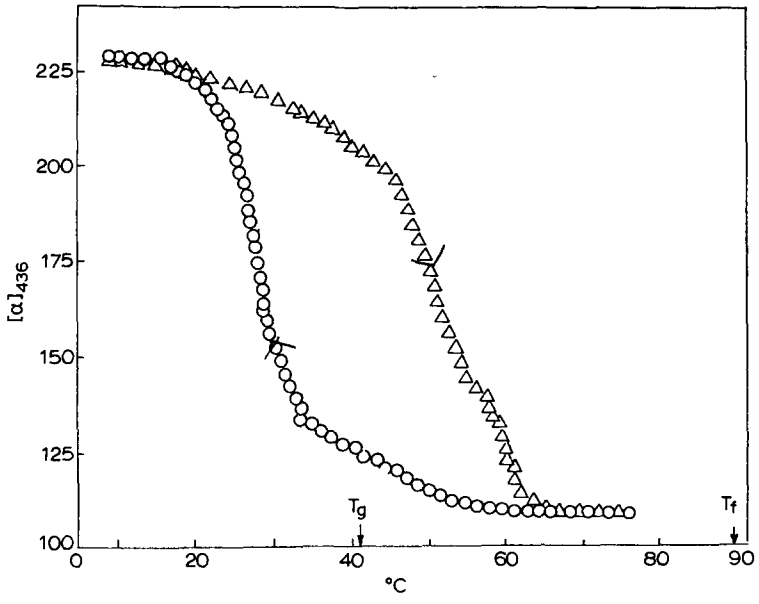

Fig. 3. Temperature dependence of the specific optical rotation for an agarose 1% gel in a 1:1 mixture of water and dimethylsulphoxide. (T_g, the gelation temperature; T_f, the melting temperature. —△—, heating; —○—, cooling.)

It is well known that temperature hysteresis in setting and melting cycles is very pronounced in the case of agarose (Rees, 1969; Dawydoff *et al.*, 1984). As can be seen from Fig. 3, the hysteresis of the formation and melting of the agarose gel network exceeds that of the conformational transitions: the difference in the temperatures of setting and melting is 47°, while the difference in the temperatures of the transition midpoints is only 22°. As a result, the melting temperature of agarose gels is 25° higher than that of the completion of the helix-to-coil transition as measured by optical rotation.

It is worthwhile to note that the results of the present study do not appear to be in agreement with the data of Arnott *et al.* (1974) according to which the melting temperature of agarose gels lies in the interval corresponding to the helix-to-coil conformational transition. The discrepancy with our results may be due to the conditions selected by the above authors that provided greater equilibration of the systems under study (Dea *et al.*, 1972).

In conclusion, our results show that the agarose gel network may be retained even when the agarose macromolecules acquire a disordered conformation.

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