Carbohydrate Polymers 3 (1983) 39–52

Effect of Alkali Pretreatment on the Rheological Properties of Concentrated Agar-Agar Gels

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(Received: 9 May 1982)

SUMMARY

Differential scanning calorimetry and thermogravimetry in the solid state and dynamic mechanical measurements of gels have been carried out for agar-agars of Chilean and Argentinian origin in order to elucidate the rheological changes in the gel as a result of alkali pretreatment. The elastic modulus of the gel prepared from Chilean agar-agar increased with increasing sodium hydroxide concentration up to 10%, while that of Argentinian agar-agar increased with increasing sodium hydroxide concentration up to 7%, and then began to decrease at higher concentrations. The increase in elastic modulus has been attributed to the structural stabilisation induced by the formation of 3,6-anhydro-L-galactose, while the decrease in elastic modulus in Argentinian agar-agar has been ascribed to chain breakage.

1. INTRODUCTION

Alkali treatment of carrageenan eliminates sulphate residues and forms 3,6-anhydro-L-galactose. Since this process is well known and used in the food industry (Glicksman, 1969), the effect of sodium hydroxide

Carbohydrate Polymers 0144-8167/83/0003-0039/$03.00 –

pretreatment on the relaxation spectrum of agar-agar gels has recently been examined in detail (Watase, 1975*a*,*b*; Watase *et al.*, 1975; Watase and Nishinari, 1981). In order to clarify the relationship between the rheological properties and the structural change caused by the alkali pretreatment, differential scanning calorimetry (DSC), thermogravimetry (TG), infrared spectroscopy (IR), intrinsic viscosity and dynamic mechanical measurements have been carried out for agar-agar.

2. EXPERIMENTAL

2.1 Material

100 g samples of the seaweed *Gracilariopsis chorda* (agar-agar), collected in Chile and Argentina in 1980, were suspended in 1·5 litres of 0, 3, 4, 7 and 10% sodium hydroxide aqueous solutions. The suspensions were stirred at 80–85°C for 2 h. After alkali pretreatment, the algae were washed in running water for 24 h to remove excess sodium hydroxide. The algae were then heated in 1·5 litres of water, and extracted by autoclaving at 135°C. After gelation of the extract, it was cut into strings and kept frozen for about 15 h. The frozen preparations were thawed in running water and dried in a thermostatically controlled oven.

The content of 3,6-anhydro-L-galactose in each specimen was determined by the quantitative colorimetric method using fructose as the standard material.

The quantity of sulphur was determined by elemental analysis of powder specimens. From the weight percent of sulphur, the quantity of sulphate ester was evaluated as sulphate ester (mol)/$C_6H_{10}O_5$ (galactose). The sulphate ester and 3,6-anhydro-L-galactose contents are shown in Table 1.

The dried powders were used for DSC and TG measurements.

The cylindrical gels used for viscoelastic measurements were prepared as follows: the dried powders were swollen at 40°C overnight, and preheated at 70°C for 1 h, and then heated at 100°C for 30 min. The solution obtained in this way was moulded into cylindrical gels of 20 mm diameter and 30 mm height using teflon moulds. The gels were kept at 4°C for a week and then at the measurement temperature for 1 h.

TABLE 1
Effect of Concentration of Pretreatment Alkali on the Content of Sulphate Esters and 3,6-Anhydro-L-galactose

NaOH (concentrated)	*Sulphate ester (mol/$C_6H_{10}O_5$)*		*3,6-Anhydro-L-galactose (%)*	
	Sample 1	*Sample 2*	*Sample 1*	*Sample 2*
0	0·052	0·056	27·1	34·0
4	0·036	0·050	32·6	35·1
7	0·030	0·040	36·6	33·8
10	0·024	0·036	41·0	32·4

Sample 1, Chilean; sample 2, Argentinian.

2.2 Methods

2.2.1 IR measurement in the solid state

Infrared absorption spectra of agar-agar films were measured by the Hitachi EPI-2 IR spectrometer.

2.2.2 Differential scanning calorimetry and thermogravimetry in the solid state

The apparatus used in this work was a Rigakudenki Standard Type CN 8085 E1, which permits the simultaneous measurement of DSC and TG. In order to eliminate traces of water and to get the same thermal history, each powder sample was heated at 105°C for 60 min and then allowed to cool to 45°C in a dry nitrogen atmosphere. Heating rate was fixed as 10°C/min. A Dainiseikosha DSC apparatus, SSC/560S, was also used for obtaining the DSC curves using α-alumina as the reference material.

2.2.3 Intrinsic viscosity

The intrinsic viscosity of solutions of Argentinian agar-agar in 0·42 mol/litre sodium salicylate was measured at a temperature of 35°C using an Ubbelohde viscometer. The temperature was controlled within ± 0·02°C.

2.2.4 Viscoelastic measurements on gels

The complex Young's modulus was measured using a Rheolograph CV-100 of Toyoseiki Co. in the temperature range 25–75°C. The cylindrical gel was subjected to sinusoidal oscillation at a fixed frequency of 2 Hz in a silicone oil bath. It has previously been shown that the elastic modulus of gels of this type is almost independent of frequency over a wide range (Nishinari, 1976; Nishinari & Horiuchi, 1977). Details of the apparatus have been described previously (Nishinari *et al.*, 1980).

3. RESULTS AND DISCUSSION

Figure 1(a) shows the IR spectra of agar-agar films. The absorption at 1250 cm^{-1}, observed in specimens prepared from both Chilean and Argentinian seaweed is due to the S=O stretching vibration and is ascribed to the absorption of total sulphuric residues (Lloyd *et al.*, 1961; Onodera *et al.*, 1965; Spedding, 1965; Watase, 1975*a*,*b*; Watase *et al.*, 1975; Watase & Nishinari, 1981). The absorption at 850 cm^{-1} in the Chilean sample is due to C—O—S vibration and is ascribed to the absorption of sulphuric residues. This latter absorption was eliminated by alkali pretreatment. Hence the sulphate residues are considered to be axially located at the C_6 position in 1,4-linked L-galactose and therefore to be unstable energetically. Elimination of this sulphate residue gives rise to the more stable 3,6-anhydro-L-galactose (Watase & Nishinari, 1981).

In contrast the absorption at 850 cm^{-1} in the Argentinian sample was not eliminated by alkali pretreatment, so there appear to be no sulphate residues at the C_6 position of the 1,4-linked L-galactose residues in the Argentinian sample. The suggested structures for Chilean and Argentinian agar-agar are shown in Fig. 1(b). These are consistent with the other experimental results to be discussed below.

Powder specimens were stable up to the temperature range 200–250°C as can be seen from the thermogravimetric curves shown in Fig. 2. The temperature for the commencement of degradation of the Chilean agar-agar was about 200°C, while that for the Argentinian agar-agar was about 250°C. For both Chilean and Argentinian agar-agar, the specimen without treatment showed a more rapid weight decrease than the specimen treated by sodium hydroxide. This suggests that the

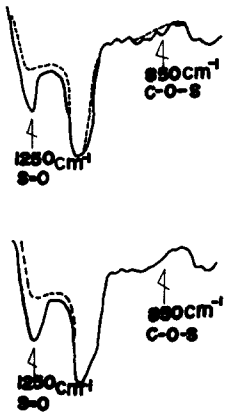

Fig. 1(a). The IR absorption spectra of Chilean (upper) and Argentinian agar-agar films. The solid curves represent the spectra for the samples without alkali pretreatment, and the broken curves those pretreated with 10% NaOH.

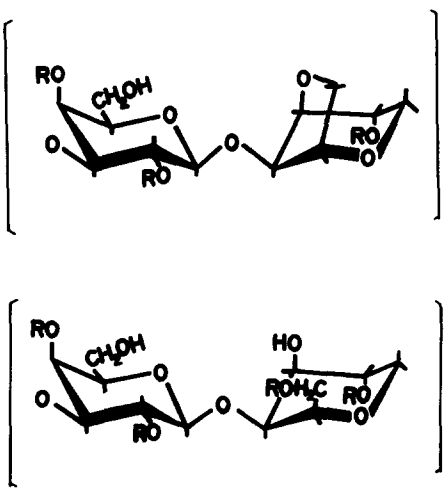

Fig. 1(b). The proposed structure of Chilean (top) and Argentinian (bottom) agar-agar. *R* represents a sulphate residue.

sulphate residues in 1,4-linked L-galactose were de-esterified by the alkali pretreatment (Watase, 1975*a*,*b*; Watase *et al.*, 1975; Watase & Nishinari, 1981). Thus, the difference in the weight loss between the specimens with and without alkali pretreatment was attributed to de-

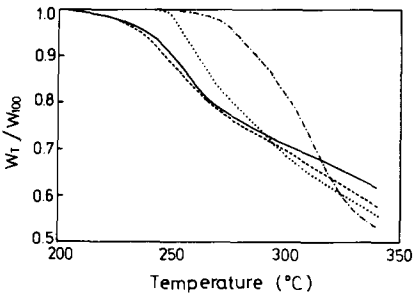

Fig. 2. Thermogravimetric curves of Chilean agar-agar without treatment (– – –), Chilean agar-agar treated with 10% NaOH (——), Argentinian agar-agar without treatment (·····) and Argentinian agar-agar treated with 10% NaOH (– · – · –) W_T, weight at the temperature T (°C); W_{100}, weight at 100°C.

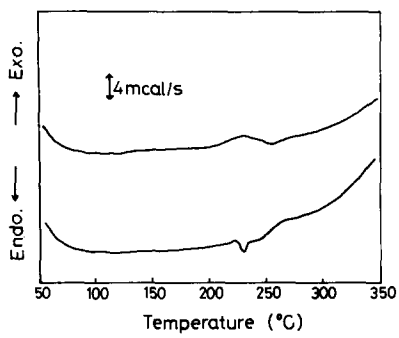

Fig. 3. DSC curves of Chilean agar-agar powder pretreated with 10% NaOH (upper curve) and without pretreatment (lower curve).

esterification during the heating. The further progress of weight loss suggests that another form of chemical degradation, such as main chain scission, occurs simultaneously or just after de-esterification (Shafizadeh *et al.*, 1978; Furneaux & Shafizadeh, 1979).

Figure 3 shows the DSC curves of the Chilean agar-agar with and without alkali pretreatment. The broad endothermic deviation about 100°C observed for both samples was attributed to the evaporation of residual water molecules. Since the weight loss involved was not detected by TG it must be very small. Just after the small exothermic peak at about 210°C, an endothermic peak was seen at about 230°C for the

sample without alkali pretreatment. This endothermic peak was attributed to the de-esterification of the unstable sulphate residues linked axially to the C_6 atom in the 1,4-linked L-galactose ring. The specimen without alkali pretreatment after initial heating to 270°C did not then show this endothermic peak at about 230°C, confirming that it can be attributed to the presence of unstable sulphate residues.

When the specimen was treated by 10% NaOH, the endothermic peak at about 230°C observed in the DSC curve for the specimen without all alkali pretreatment disappeared completely, and the exothermic peak appeared. After alkali pretreatment, the sulphate groups are de-esterified and some 3,6-anhydro-L-galactoses are formed. However, the molecular arrangement remains in a loosely packed state. With increasing temperature, the molecules become more densely packed as in the case of cellulose triacetate (Kimura *et al.*, 1974; Hatakeyama *et al.*, 1976). The exothermic peak was attributed to this increase in packing density and the endothermic peak following it is believed to be associated with degradation.

The DSC curves of the Argentinian agar-agar with and without alkali pretreatment are shown in Fig. 4. The endothermic peak at about 255°C was attributed to the presence of sulphate residues, but since this peak was not eliminated by alkali pretreatment but was shifted to slightly higher temperature (about 280°C), the position of the sulphate residues in this agar-agar was considered to be different from that in the Chilean agar-agar. Perhaps they are located at the C_2 position in the 1,4-linked L-galactose as is shown in Fig. 1(b). These residues are in the

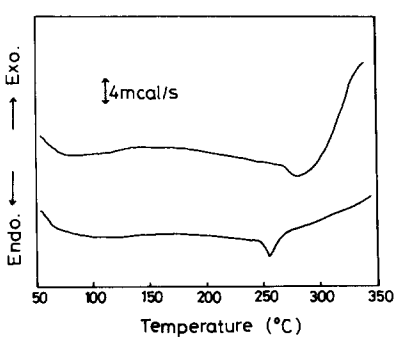

Fig. 4. DSC curves of Argentinian agar-agar powder pretreated with 10% NaOH (upper curve) and without pretreatment (lower curve).

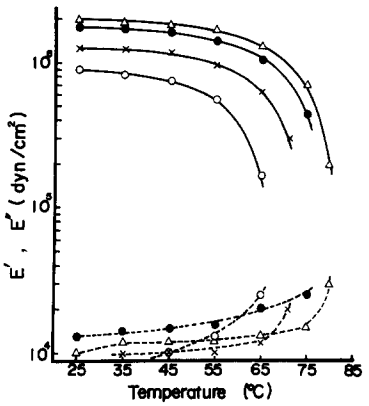

Fig. 5. Temperature dependence of E' and E'' of Chilean agar-agar gel prepared from material pretreated with alkali of various concentrations of NaOH: 3% (○); 4% (×); 7% (⊗); 10% (△). (——), E'; (– – –), E''.

more stable equatorial position and will be more difficult to remove (Anderson *et al.*, 1968).

The temperature dependence of the complex Young's modulus for the 4% w/w Chilean agar-agar gel, prepared from materials pretreated with various concentrations of sodium hydroxide, is shown in Fig. 5. In all cases, E' decreased gradually up to 45–55°C, and beyond this temperature began to decrease rapidly, while E'' increased slightly with increasing temperature. Thus, the mechanical loss tangent E''/E' increased rather rapidly at the inflexion temperature beyond which E' began to decrease rapidly. It is well known that when a sol transforms to a gel, E' is initially smaller than E'' and then becomes larger than E'' at some point during the gelling process (Fukada & Dintenfass, 1971; Kaibara, 1973). Thus the observed experimental results suggest that the gel–sol transition has begun partly at the upper end of the investigated temperature range. The gel elasticity is considered to be essentially entropic, but since the junction points in the so-called thermoreversible gel are formed by secondary weak cross-linkages such as hydrogen bonds, the elastic modulus decreases with increasing temperature. The decrease in E' due to breaking of the hydrogen bonds overwhelms the increase in E' by entropic effect with increasing temperature. With increasing concentrations of sodium hydroxide used in pretreatment, the dynamic Young's modulus E' increased markedly, and the inflexion

temperature beyond which E' began to decrease rapidly shifted to higher temperatures. These changes can be attributed to increased structural stabilisation, because of de-esterification and the formation of 3,6-anhydro-L-galactose, on alkali pretreatment as shown in Table 1. The role of alternating D-galactose and 3,6-anhydro-L-galactose residues in the stabilisation of the double helical structure in agarose gels has also been emphasised by Rees (1969).

The gel forming power of aqueous solutions of agar-agar is determined by the balance between solubility and crystallinity (Kaibara, 1973). Solubility is influenced by the concentration of ionised groups. When the agar-agar is de-esterified by alkali pretreatment, 3,6-anhydro-L-galactose is formed and the number of hydrogen bonds between extra hydroxyl groups and the oxygen atom which bridges the 3rd and 6th carbon atoms in 1,4-linked L-galactose increases. Thus, the microcrystalline structure of agar-agar becomes more stable. On the other hand, specimens without alkali pretreatment contain more sulphate groups in the molecule. The sulphate groups in the neighbourhood of water molecules play a role as a structure breaker (Suzuki & Uedaira, 1974) resulting in an increase in water activity and polysaccharide solubility. Therefore, the gel prepared from the alkali treated material shows a larger viscoelastic constant than that of the specimen without treatment.

A similar situation was observed for the gel prepared from Argentinian agar-agar as is seen in Fig. 6. However, E' began to decrease at concentrations of NaOH used in pretreatment above 7%. Beyond this concentration alkali pretreatment results in chain breakage, as will be discussed later in relation to the intrinsic viscosity data.

Figures 7 and 8 show the alkali pretreatment concentration dependence and temperature dependence of E' for 2% w/w and 4% w/w gels prepared from agar-agars from both sources. The Chilean agar-agar, without alkali pretreatment, could not be moulded into gels, even at quite high concentrations. From the viewpoint of industrial application, the alkali pretreatment is very effective at increasing the gel forming ability of Chilean agar-agar. E' increased more rapidly with the concentration of the sodium hydroxide used in pretreatment for Chilean agar-agar than for Argentinian. As is stated above, it is considered that the sulphate residues are axially linked to the C_6 carbon in the 1,4-linked galactose residues in Chilean agar-agar, while these residues are equatorially linked to other carbon atoms in Argentinian agar-agar. In the case

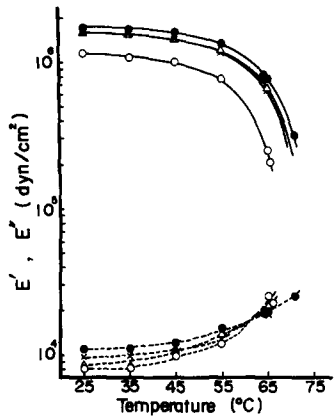

Fig. 6. Temperature dependence of E' and E'' of Argentinian agar-agar gel prepared from material pretreated with alkali at various concentrations of NaOH: 3% (○); 4% (×); 7% (●); 10% (△). (——), E'; (– – –), E''.

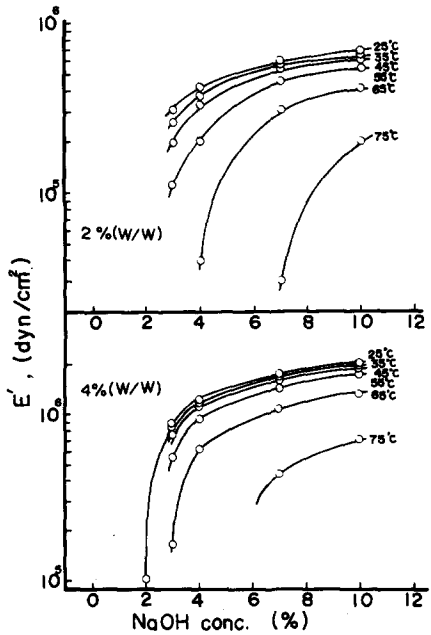

Fig. 7. E' of 2% (w/w) and 4% (w/w) Chilean agar-agar gel prepared from material pretreated with NaOH solutions of various concentrations.

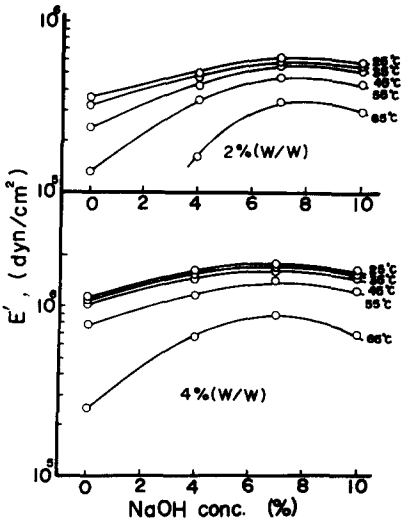

Fig. 8. E' of 2% (w/w) and 4% (w/w) Argentinian agar-agar gel prepared from material pretreated with NaOH solutions of various concentrations.

of agar-agar studied previously (Watase & Nishinari, 1981), the viscoelastic parameters began to increase rapidly when the 3,6-anhydro-L-galactose content exceeded 30%. As can be seen from Table 1, the 3,6-anhydro-L-galactose content increased continuously with increasing sodium hydroxide concentration in the case of Chilean agar-agar, while it decreased above 7% sodium hydroxide in the case of Argentinian agar-agar. For this material at higher sodium hydroxide concentrations alkali pretreatment causes chain scission rather than formation of 3,6-anhydro-L-galactose; this is confirmed by the decrease in intrinsic viscosity of aqueous solutions of Argentinian agar-agar at concentrations of sodium hydroxide used in pretreatment of 7% and above (Fig. 9).

Figures 10 and 11 show the concentration dependence of E' for gels formed from Chilean and Argentinian agar-agars pre-treated with various concentrations of alkali. The value of E' was proportional to about the 4th power of the concentration of the gel in the lower concentration range, and to the 2nd power at higher concentrations. A similar concentration dependence has been reported previously for agar-agar (Watase & Arakawa, 1967, 1968; Nishinari & Horiuchi, 1977) and konjak mannan (Watase, 1975*a*, *b*) gels.

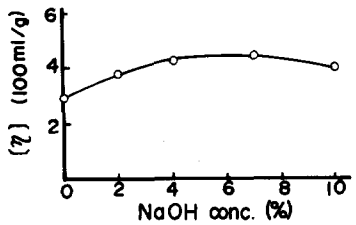

Fig. 9. Effect of alkali pretreatment on the intrinsic viscosity of Argentinian agar-agar in 0·42 mol/litre sodium salicylate solution at 35°C.

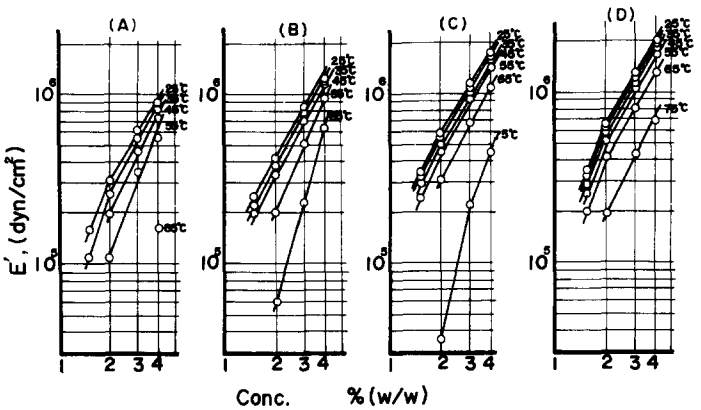

Fig. 10. Concentration dependence of E' of Chilean agar-agar gel for various concentrations of alkali pretreatment: (A), 3% NaOH; (B), 4% NaOH; (C), 7% NaOH; (D), 10% NaOH.

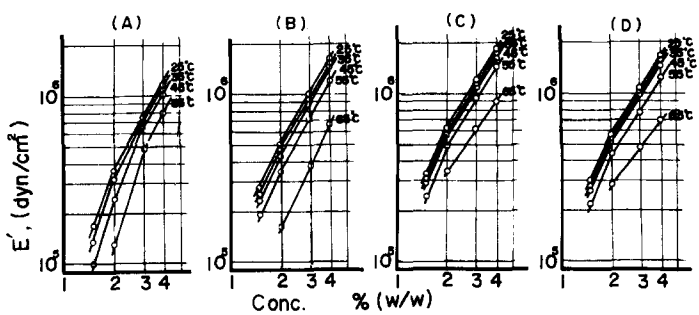

Fig. 11. Concentration dependence of E' of Argentinian agar-agar gel for various concentrations of alkali pretreatment: (A), without pretreatment; (B), 4% NaOH; (C), 7% NaOH; (D), 10% NaOH.

The possible structural changes on alkali pretreatment are as follows: (i) the sulphate residues linked axially to 1,4-linked L-galactose are de-esterified; (ii) the sulphate residues attached equatorially begin to be de-esterified; (iii) chain breakage occurs on further alkali pretreatment. The first process gives rise to the formation of 3,6-anhydro-L-galactose, and as a result of this the extent of the alternating of D-galactose and 3,6-anhydro-L-galactose regions increases; hence the structure of the gel is stabilised. The effect clearly appears in both thermal and viscoelastic measurements. The second process does not seem to have a great effect on the rheological properties. The value of E' for the gels is reduced by chain breakage.

ACKNOWLEDGEMENT

The authors wish to thank Dr T. Hatakeyama of the Research Institute for Polymers and Textiles for her guidance relating to the TG and DSC measurement and for valuable discussions. They also thank Miss K. Oshiro of Dainiseikosha for her cooperation carrying out the DSC measurements.

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