Polymer Bulletin 32, 111–116 (1994)

Polymer Bulletin

Mechanical and morphological features of agarose-guar gum gels

R. B. Garcia[1], M. De Boinis[2], and C. T. Andrade[1,*]

[1]Instituto de Macromoleculas, Universidade Federal do Rio de Janeiro, PO Box 68525, 21945-970 Rio de Janeiro, Brazil
[2]Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, 21941-970 Rio de Janeiro, Brazil

Summary

Young' s modulus, maximum rupture stress and compression energy to break values were determined for pure agarose (5g/l and 10g/l) and 1:1 agarose-guar gum mixed gels of 10g/l total polymer concentration. The enhancing effect of guar gum was noticed particularly on the tenacity parameter. Transmission electron microscopy, TEM, was used to image the supramolecular structure of 1:1 agarose-guar gum gels. Before sample preparation for TEM, the hot aqueous solutions have been submitted to slow cooling until a determined temperature was reached, and then quenched to -78°C. When this temperature was higher than the critical temperature of gelation, agarose-guar gum mixed samples formed aggregated structures, promoted by freezing. Below gelation, although guar gum continued producing aggregates, agarose appeared as continuous network structures. This result was related to the stabilizing role of guar gum.

Introduction

Certain polysaccharide mixtures show enhanced rheological properties with special applications by the food industry. In general, galactomannans are one of the components of such mixtures. The galactomannans are neutral and non-gelling polysaccharides, extracted from the endosperm of some Leguminosae, and characterized by repeating units of (1→4)-β-D-mannan, to which single residues of (1→6)-α-D-galactose are linked. The content of D-galactose depends on the galactomannan source and has been associated to the degree of the interaction with gelling (1, 2) or non-gelling polysaccharides (3).

Locust bean gum, the galactomannan from ***Ceratonia siliqua***, has a ratio of D-mannose to D-galactose (4) of around 3,5:1 and has been used in almost all synergistic mixtures (5). By the usually accepted model, the unsubstituted D-mannan segments would interact with the helical structures of polysaccharides, such as *k*-carrageenan (1) or xanthan (6).

Guar gum, from ***Cyamopsis tetragonolobus***, is a highly substituted galactomannan. Although no significant enhancement of properties has been observed in its mixtures with *k*-

*Corresponding author

carrageenan (7), some attention has been paid to xanthan-guar synergistic mixtures (8,9).

In this work, some results from compression experiments of agarose-guar gum mixed gels have suggested a contribution of guar gum for enhanced mechanical properties of these gels. Transmission electron microscopy has been used to investigate the supramolecular structure of such mixtures.

Experimental

Agarose hydrogels of 5 and 10g/l concentrations, and agarose-guar mixed gels of 10g/l total polymer concentration were prepared by tranferring the corresponding solutions to glass tubes of 17mm diameter and 150mm height. After gelation, 5 specimens of 17mm height were cut and immersed in water at 25°C overnight. The perfect cylindrical samples were submitted to uniaxial compression tests in a Instron TM-M machine, equipped with a CTM compression cell, at 0,5cm/min and 25°C.

The critical temperatures of gelation were determined by measuring the apparent viscosity during the slow cooling (0.4°C/min) of the solutions with a Brookfield Synchro-Lectric LVT, equipped with a Small Sample Adapter SC-31/13R and a spindle number 31, at $0.1s^{-1}$ shear rate.

A Philips EM 301 transmission electron microscope was used to examine the 1:1 agarose-guar gum mixtures. Agarose-guar gum (1:1) hot solutions were filtered through 5.0µm membranes, maintained at 50°C during 15 minutes and then cooled at 0.4°C/min. When the solutions reached 45, 39 and 23°C, they were quenched to -78°C and freeze-dried. The procedure normally used to prepare biological materials for TEM analysis has been followed (10). The samples were immersed for 2h in 25g/l glutaric aldehyde buffered in 0.1mol/l sodium cacodilate aqueous solution containing 68g/l saccharose, and washed 3 times in 0.1mol/l sodium cacodilate solution over 30min. Sufficient contrast for TEM was obtained by treating the samples for 5 days with 10g/l osmium tetroxide and 1.5g/l ruthenium red in 0.1mol/l sodium cacodilate and re-washing. After drying with acetone, the samples were embedded in Polilyte resin, cut to 600-900A thickness with an LKB-Ultratome V 2088 ultramicrotome, then supported on copper grids and stained with uranyl acetate and lead citrate. An accelerating voltage of 100kV was used for the examinations.

Results and discussion

Table 1 shows the values of Young's modulus, E_{max}, maximum rupture stress, τ_{max}, and compression energy to break, E_n, obtained for agarose hydrogels of 5 and 10g/l, and for 1:1 agarose-guar gum mixed gels of 10g/l total polymer concentration. The comparison of E_{max}, τ_{max} and E_n values in Table 1 reveals that agarose may be partially substituted for guar gum. The critical temperatures of gelation, T_c, are also included in Table 1, as well as the "enhancing factor", *f*, determined as the ratio between the experimental value for the mixed gel and the corresponding one for the 5g/l pure agarose

Table 1: Values of E_{max}, τ_{max}, E_n and T_c for agarose and agarose-guar gum gels, and the "enhancing factors" *f*.

Gel type	E x 10^{-4} (Pa)	τ_{max} x 10^{-4} (Pa)	E_n x 10^{-4} (J x m^{-3})	T_c (°C)
Agarose c = 5g/l	1.0	1.1	2.2	35.0
Agarose c = 10g/l	3.6	2.9	10.1	39.0
Agarose-guar (1:1) c_t = 10g/l	1.4 *f* = 1.4	2.9 *f* = 2.6	9.0 *f* = 3.1	39.0

gel. This "enhancing factor" may be considered as a quantitative means of evaluating the enhancing effect of guar. The higher T_c value determined for the mixed gel indicates that guar gum contributes to stabilize gel formation.

Transmission electron microscopy, TEM, was used to examine the supramolecular structure of 1:1 agarose-guar gum mixtures. Figure 1 shows the micrograph of the sample prepared from

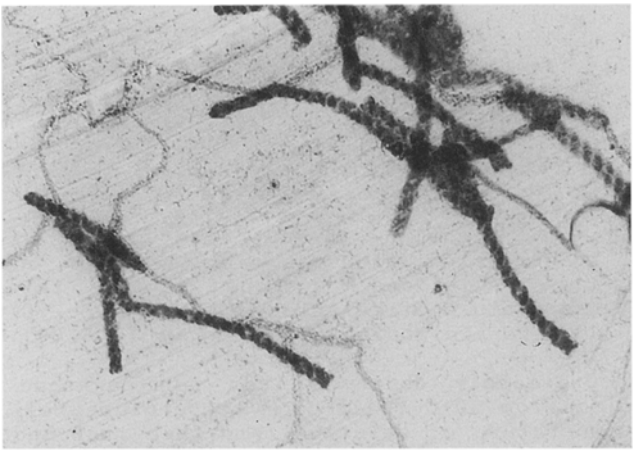

Figure 1: Transmission electron micrograph of agarose-guar gum solutions quenched from 45°C to -78°C (x 5800).

agarose-guar gum solution of 10g/l total polymer concentration, which had been cooled at 0.4°C/min from 50°C to 45°C and then quenched to -78°C. At 45°C, according to Table 1, the solution has not yet formed a gel. Although no evidence of phase separation had been observed for agarose-guar solutions (12), two kinds of aggregates are noticed in Figure 1. By comparison with the micrographs of the pure samples (13), the fibrous dark-coloured aggregates are attributed to agarose, and the more flexible light-coloured aggregates are attributed to guar gum.

Agarose-guar gum solution was quenched from 39°C, the critical temperature of gelation, to -78°C , and gave rise to the sample shown in Figure 2. In this figure, guar gum remains as relatively flexible aggregates, transversely disposed in two of the corners of the micrograph. Agarose no more appears as a fibrous structure, but small domains, distributed in the center of the micrograph, have come out.

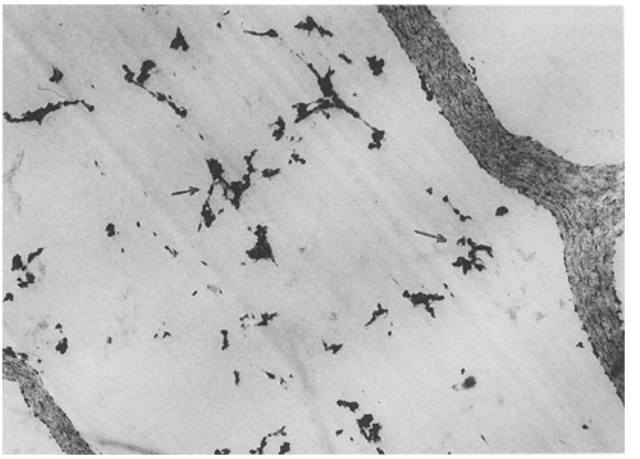

Figure 2: Transmission electron micrograph of agarose-guar gum solutions quenched from the critical temperature of gelation, T_c=39°C, to -78°C; agarose domains are indicated by arrows (x 8200).

It was previously observed that pure agarose solutions slowly cooled from 50°C to 15°C, a temperature well below the critical temperature of gelation, and then quenched to -78°C, showed also networks collapsed into aggregates (14). Although the crystallization of water (15) has to be considered in the formation of the artefacts of Figure 1, the micrograph shown in Figure 3 indicates that the gelled agarose network was

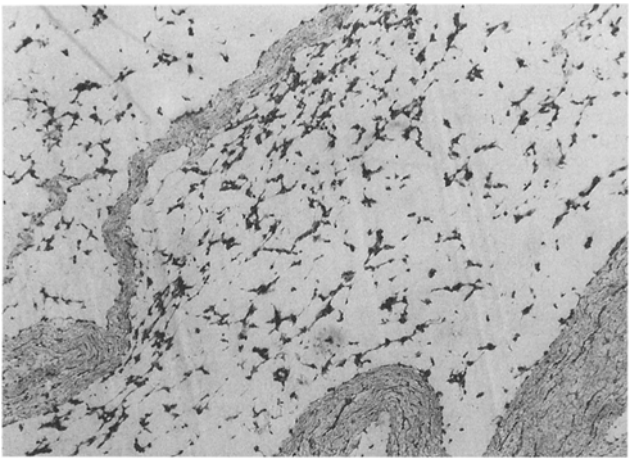

Figure 3: Transmission eletron micrograph of agarose-guar gum gels quenched from 23°C to -78°C (x 7900).

maintained in the agarose-guar gum system. The supramolecular structure expected for a gel (16) may be observed in this figure, obtained when the gelled mixture was slowly cooled from 50°C to 23°C, and then quenched -78°C. In this preparation, the aging period was sufficient to allow the system to achieve a higher degree of physical crosslink. The small domains of agarose, observed in Figure 2, gave rise to a continuous structure, also spread through guar gum aggregates.

Conclusion

The critical temperatures of gelation determined for agarose and agarose-guar gum solutions suggest that this galactomannan contributes to the stability of the mixed gels. The mechanical test results indicate the enhancing effect of guar gum, particularly on the tenacity of the mixed gel. Transmission electron micrographs of agarose-guar gum samples, quenched to -78°C from different temperatures, showed the formation of artefacts, probably due to water crystallization. Nevertheless, when the gelled mixture was aged longer, it was possible to observe a continuous and non-collapsed agarose phase. The maintenance of the agarose continuous phase in agarose-guar gum mixtures may be due to stabilization by guar gum.

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Accepted December 1, 1993 K