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Note

Selective area electron diffraction from the galactomannan of guar gum

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Guar gum is a member of the galactomannan family and has extensive industrial applications because of its hydrorheological properties. It is a linear (1→4)-β-D-mannan substituted in a non-regular fashion[1] at positions 6 with α-D-galactosyl residues[2]. The ratio of galactose to mannose varies within the galactomannan family, with guar gum having a relatively high ratio of substitution (up to ~50%)[1,3].

X-Ray diffraction of oriented fibres has been used to investigate the chain conformation and three-dimensional packing schemes for the galactomannans[3–6]. The reported unit cells are orthorhombic with two chain segments of opposite polarity per unit cell. Each chain exists in an extended two-fold helix of pitch (fibre repeat *c*) of 1.04 nm. Thus, the backbone resembles a gently rippling, ribbon-like conformation with repeat dimensions very similar to both mannan and cellulose[7]. The *b* dimension of the unit cell is ~0.89 nm and remains approximately constant within the galactomannan family[3], whereas the *a* dimension is a function of galactose content and relative humidity with values reported[3] in the range 1.35–1.66 nm (sometimes this *a* dimension appears doubled[3]). In these X-ray diffraction experiments, the molecular orientation was achieved by stressing films at high relative humidity or immersion in water–ethanol mixtures[8].

In the present work, dried ultra-thin films of guar gum have been prepared suitable for transmission electron microscopy and the electron diffraction patterns recorded from selected areas.

The electron diffraction pattern obtained from the solution-dried thin film of guar gum is shown in Fig. 1. Discrete layer lines are seen, which are orders of a 1.05 ±0.05 nm spacing with meridional signals on even layer lines. Sharp equatorial peaks are seen, the most prominent of which (marked A) occurs at a spacing of 0.44 ±0.02 nm and which matches the strong (020) equatorial peak seen in the

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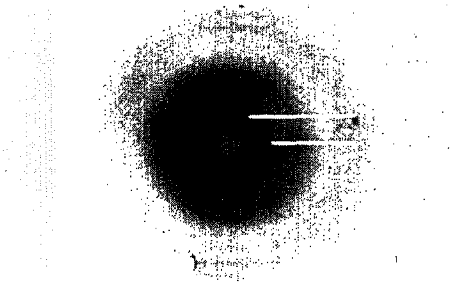

Fig. 1. Electron diffraction from an oriented thin film of guar gum. The prominent reflection on the second layer line is marked as B to compare with X-ray diffraction.

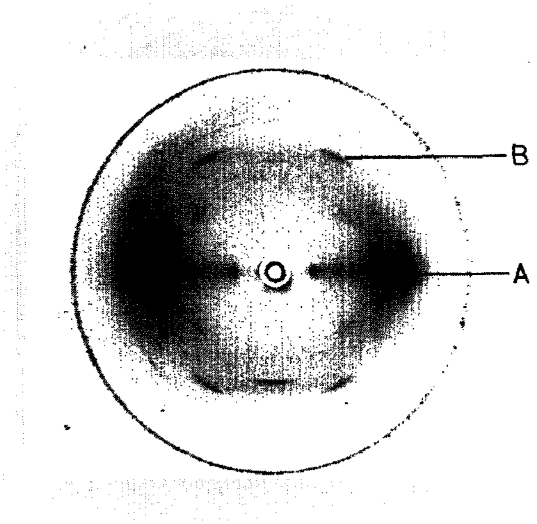

Fig. 2. X-Ray fibre diffraction of oriented guar gum recorded at 0% relative humidity. The prominent reflection on the second layer line is marked as B to compare with electron diffraction.

X-ray diffraction pattern (Fig. 2). Comparison of the electron and X-ray diffraction patterns shows that the degree of orientation is similar in both, but that the X-ray pattern indicates high crystallinity. The electron diffraction pattern, in fact, exhibits features similar to an oriented nematic liquid crystalline texture with discrete equatorial signals but with streaking along the layer lines.

The quality of the electron diffraction pattern diminishes rapidly with time (~10 s) because the crystallinity is destroyed by the electron beam. Moving the electron beam over the surface of the films leads to changes in the orientation direction of the microcrystallites. The similarity between the electron and X-ray diffraction patterns shows that molecular orientation can be achieved in the thin film on drying, whereas the X-ray samples need to be stretched to induce orientation. We believe that the orientation arises in the thin films because of surface tension forces operating during the drying process. Diffraction signals are seen at much wider angles in the electron diffraction pattern and layer lines are easily seen out to the ninth order (~0.1 nm). At present, the number of recorded reflections on the electron diffraction pattern is less compared with the more crystalline X-ray pattern.

The following conclusions are made. (*a*) Oriented thin films of guar gum galactomannan can be prepared from a single drop of dilute solution. (*b*) Electron diffraction patterns exhibiting both good orientation and crystallinity can be achieved from these thin films and which are similar to X-ray fibre diffraction patterns obtained from bulk samples oriented by stretching. (*c*) The direction of the oriented crystalline domains ($\geqslant 10^6$ nm^2) are randomly distributed over the surface area of the thin film. (*d*) The surface tension forces operating on such thin films produce local orientation, and the crystallinity can be improved by subsequent annealing at elevated temperatures (~80°). (*e*) It is possible to collect data to higher resolution (~0.1 nm) in a straightforward manner. (*f*) This technique needs only a minute amount of guar gum polysaccharide (~1 mg) and should be applicable to other polysaccharides. In cases where only such minute amounts of purified material are available, X-ray diffraction will not be possible. (*g*) Care is necessary to avoid the rapid deterioration of crystallinity by the electron beam.

EXPERIMENTAL

An aqueous 0.1% solution of guar gum (Sigma) was centrifuged for 2 h to remove impurities. A drop of the supernatant solution was placed on an electron microscope grid with a mesh size of 400 per sq. in. and dried in an oven at 80° for 1 h, which gave films thin enough ($\leqslant$100 nm) for transmission electron microscopy.

Electron diffraction patterns were recorded with a Philips EM301 electron microscope operating at 100 kV. A low dosage beam technique was used in order to avoid destruction of the molecular organization. The d-spacings in the diffraction patterns were calibrated by evaporating gold onto some of the samples.

Thicker oriented samples of the guar gum were also prepared, in order to record X-ray diffraction following the method reported by Atkins *et al.*[8], for comparison with the electron diffraction results. An Elliott rotating target GX20 generator was used as the source for a flat plate camera with pinhole collimation. Samples were dried over silica gel for $\geqslant$2 days and maintained dry by passing helium through silica gel during exposure.

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