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Note

Electron microscopic study of guluronate-rich alginate

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Alginic acid, a polysaccharide extracted from brown algae, is used extensively in industry as a stabiliser for emulsions and foams, and interacts with cations to form gels and viscous aqueous solutions. It is made up of (1→4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G), which occur[1,2] in blocks of $(M)_n$, $(G)_n$, and $(MG)_n$. The block composition is a function of the location of the alginic acid within the plant, the species, season, and geographical location. The samples studied contained ~75% of guluronic acid.

The electron microscopic study of sodium alginate described used heavy metal replication after oven-drying of aqueous solutions. When dispersed polymer or polymer aggregates are visualised in the electron microscope, it is important to ensure that the features seen are not only representative of the substance under investigation but also that some other method is used to confirm their genuineness, since contaminants can be incorporated inadvertently when small quantities are being used. Thus, selected area electron diffraction was performed and the results were compared with those of X-ray diffraction of bulk-orientated samples.

The recorded electron diffraction pattern (Fig. 1) shows an oriented pattern with a sharp meridional diffraction signal at a spacing of 0.44 ±0.01 nm and broader, more diffuse equatorial arcs, indicating poorer lateral packing of the chains, at a spacing centred on 0.60 ±0.01 nm. The X-ray diffraction patterns of oriented fibres of alginates containing >70% of guluronate have been reported[3,4]. A two-fold helical conformation was proposed for the guluronic acid chain, with the molecules packed in an orthorhombic unit cell with dimensions $a = 0.77 \pm 0.01$, $b = 1.06 \pm 0.01$, and c (fibre axis) $= 0.87 \pm 0.01$ nm for the dry fibre. At high relative humidity, the a and b dimensions increased to 0.87 and 1.07 nm, respectively, and the c dimension remained constant[4]. The prominent X-ray diffraction signals are the 002 meridional, which occurs at a spacing of 0.44 nm, and the 110 equatorial at a spacing of 0.62 nm. The X-ray diffraction pattern of the sodium salt form[5] is similar to that of polyguluronic acid. The basic electron diffraction pattern

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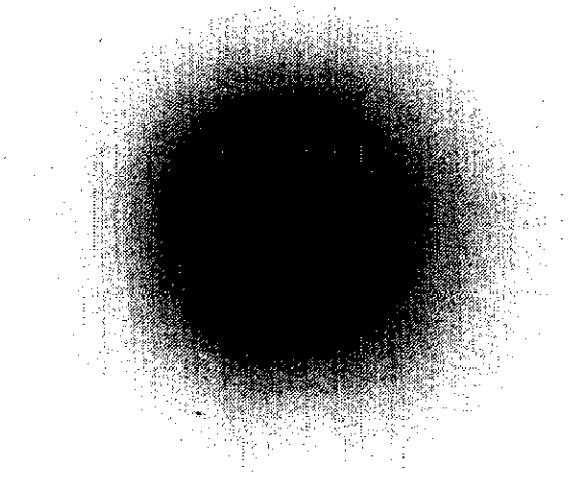

Fig. 1. Electron diffraction of guluronate-rich sodium alginate, obtained using the low dose beam method[7]. The pattern shows a prominent sharp meridional arc at spacing 0.44 nm, which represents the projected axial advance of the guluronate monomer and an equatorial broad diffraction signal at spacing 0.60 nm.

compares favourably with the X-ray diffraction patterns obtained from less crystalline polyguluronates[3]. The slightly smaller value of 0.60 nm (compared with 0.62 nm) for the equatorial electron diffraction signal is understandable in terms of the drying effect of the high vacuum conditions in the electron diffraction experiments. Scanning the sample with the electron beam shows that the direction of orientation changes over size ranges of a few microns. The electron diffraction pattern was obtained over large areas of the film and is therefore representative of the material deposited. The diffraction pattern, which is sensitive to electron beam damage and fades away after ~10 s, confirms that the substance examined is the guluronate-rich part of the alginate preparation and exists with a semi-crystalline fibrous texture which changes in direction over the surface of the film, presumably due to orienting forces caused by surface tension during the drying process.

A replica electron micrograph obtained from the sodium alginate acid solution is illustrated in Fig. 2a. A network of fibrous strands is seen, which is typical of large areas on the electron microscope grid. Fig. 2b shows a region where the network is more dispersed, and Fig. 2c is a higher magnification of a selected region. Measurements of these electron micrographs show values for the thicknesses of the strands in the range 13–26 nm. Lengths between junction points are in the range 200–400 nm.

Oriented bundles of guluronate-rich sodium alginate acid are formed during the concentration to dryness of aqueous solutions. The bundles contain $\geqslant 10^2$ chains and have a semi-crystalline fibrous texture with good local orientation. The connecting elements in the network are relatively straight (see Fig. 2b).

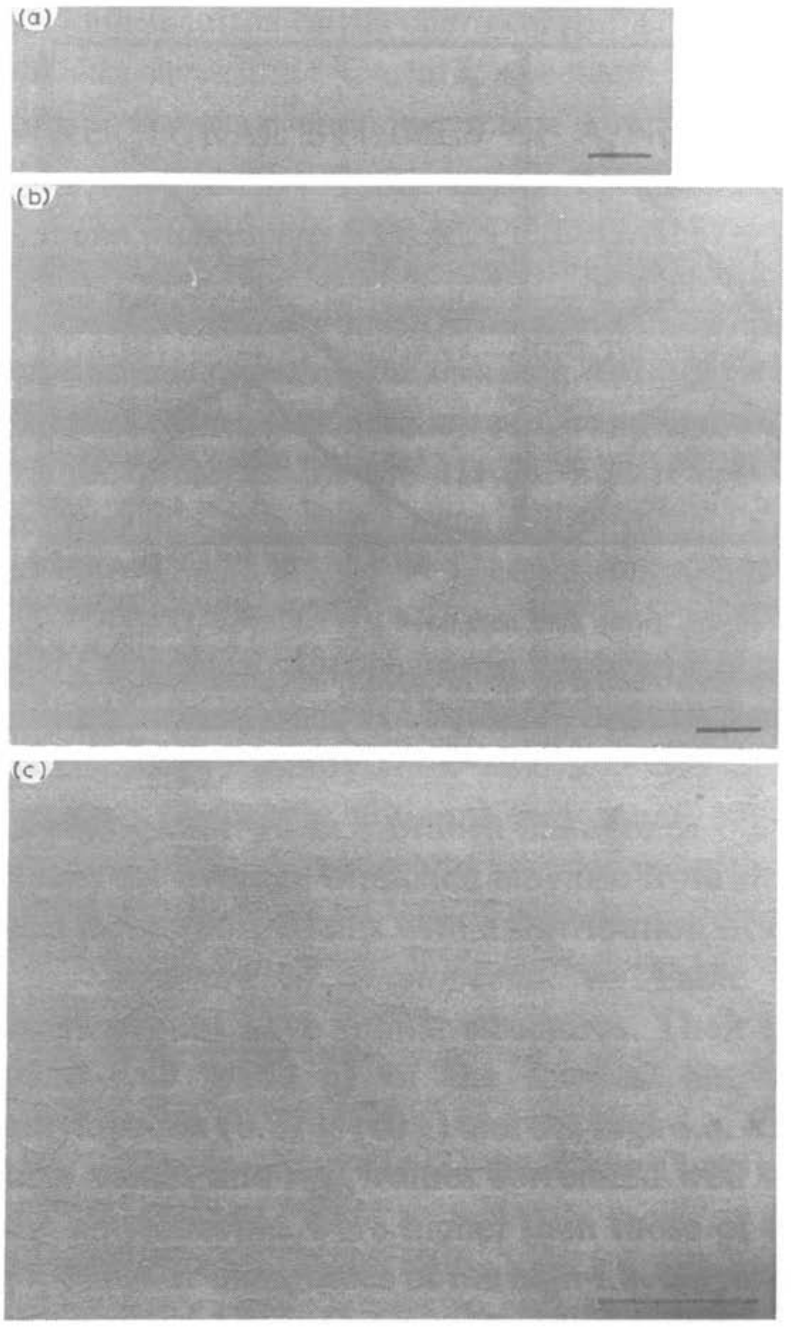

Fig. 2. Representative electron micrographs of guluronate-rich sodium alginate. Shadow replicated at 10^{-5} Torr with a mixture of tungsten and tantalum; bars represent 500 nm; (*a*) a relatively concentrated region, (*b*) a more dispersed region, and (*c*) a higher magnification of a selected area.

Visualisation of individual mannurate-rich (up to 90%) alginate chains in the Me_4N^+ salt form have been reported[6]. At the low concentrations used, the sodium salt form gave too low a contrast for easy identification.

At the much higher concentrations used here and with a high percentage of guluronate, a clear and reproducible visualisation of the network formation of the sodium alginate was obtained. The network is composed of oriented fibrillar elements, containing some hundreds of polymer chains with diameters typically in the range 13–26 nm. The molecular orientation and degree of crystallinity can be assessed from electron diffraction patterns from selected areas. The electron diffraction results confirm that the network is the guluronate-rich part of the alginate and show the ability of the guluronate polymer to aggregate into semi-crystalline stiff fibrils and that the local interaction of these fibrils leads to the formation of the network.

EXPERIMENTAL

Preparation of alginate solutions. — A freeze-dried sample of the sodium alginic acid (75% of guluronic acid), obtained[3] from *Laminaria hyperborea*, was kindly supplied by Dr. W. Mackie (University of Leeds). A 0.1% solution in distilled water was used.

Grazing-incidence shadowing electron microscopy. — Fine droplets of the above aqueous solution were sprayed onto a freshly cleaved mica surface, using an atomiser. The droplets were dried for 1 h at 80°. A mixture of tungsten and tantalum was used to shadow the sample at angles $<10°$ to the surface, using a Balzers electron beam evaporator operating at 10^{-5} Torr. Replicas were obtained by evaporating carbon normal to the surface. The carbon replica was floated off in distilled water and collected onto an electron microscope grid.

Electron diffraction. — A drop of the 0.1% solution was placed on an electron microscope grid of mesh size 400 per sq. in. and dried for 1 h at 80°. An appropriately thin film (<100 nm) was obtained. A low dosage beam technique was used to record electron diffraction on selected areas (~1 μm diameter)[7]. Gold coating was used as an internal calibration for the interplanar spacings. A Philips EM301 instrument operating at 100 keV was used for both electron diffraction and imaging experiments.

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