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Preliminary communication

Nitroxide spin-labels as molecular probes of the microviscosity of aqueous solutions of carbohydrates: alginate, other polysaccharides, and sucrose*

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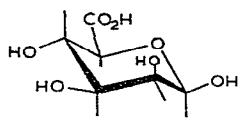
Considerable interest has been aroused in recent years by the remarkable rheological properties that many polysaccharides impart to aqueous media[2], and some structural models have been proposed that involve various types of intermolecular interactions[3]. These models have usually been based on X-ray diffraction data[4]; solution studies have been limited to such relatively restricted techniques as optical rotation[5] and n.m.r. studies of solvent resonances[6]. Nitroxide spin-labels have been widely used to probe molecular interactions[7] and microviscosity[8] in many polymeric systems, especially those of biochemical importance[9], but almost no such studies have been made of polysaccharides[10]. At the risk of introducing a perturbation into the system, and being mindful of the possibility that the information relayed may reflect only local conditions, we have nevertheless begun to explore systematically the usefulness of spin labelling in the elaboration of the solution properties of carbohydrate systems.

The present communication deals principally with alginate, a linear polymer comprising[11] β-D-mannopyranosyluronic (M) and α-L-gulopyranosyluronic (G) acid residues linked 1→4 and organized into different regions of primary structure: $[M]_n$, $[G]_n$, and a region containing both monosaccharides in approximately equal amounts. The proportions of these regions vary, depending on the source of the alginate[12]. In its acidic form (alginic acid), this material is insoluble, but it dissolves in neutral solutions containing alkali-metal ions. Upon addition of Ca^{2+}, an opaque gel is formed; for this an "eggbox" structure has been proposed[13] in which regions of $[G]_n$ from different polymers are linked by Ca^{2+} chelation, forming an infinite, three-dimensional structure.

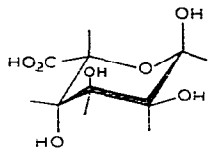
The behavior of this system is initially contrasted with other carbohydrate solutions. Fig. 1 shows the dependence of microviscosity as expressed by a rotational correlation-time (τ) derived from the electron paramagnetic resonance (e.p.r.) lineshape** of 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (**1**) (obtained from Eastman Kodak, Rochester,

*For a previous communication on a related topic, see ref. 1.

**Calculated by the method given in ref. 14.



β-D-Mannopyranuronic acid



α-L-Gulopyranuronic acid

N.Y.) on macroscopic viscosity (measured with a Brookfield Viscometer type LV, from Brookfield, Stoughton, MA) for a series of aqueous solutions of sucrose (α-D-glucopyranosyl β-D-fructofuranoside). Increase of the macroscopic viscosity results in slowing of the rotational reorientation of the label, and a concomitant broadening of the e.p.r. line.

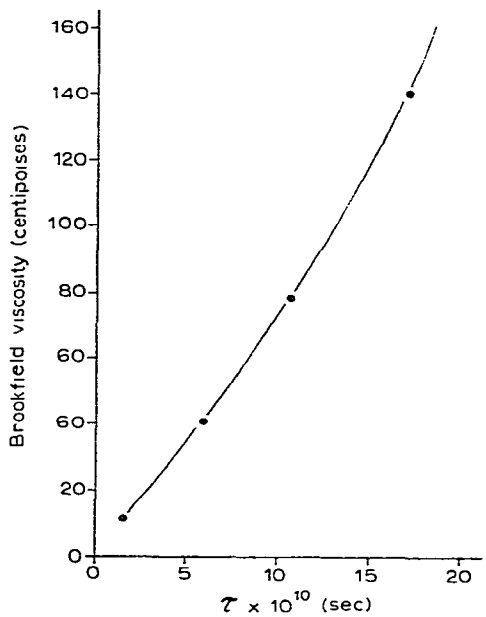
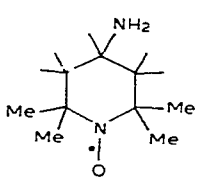
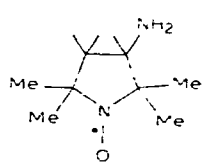


Fig. 1. Dependence of motional correlation time[14] of 3-amino-2,2,5,5-tetramethylpyrrolidin-1-oxyl (2, 0.5mM) upon Brookfield viscosity for increasing concentrations [ranging from ~100% (w/v) to 240% (w/v)] of sucrose in water at 28°.



1



2

No such relationship was found between micro- and macro-viscosities in sodium alginate solutions, or, indeed, in any of a number of solutions of different polysaccharides from plant and bacterial sources (starch, xanthan gum, locust-bean gum*, or mixtures of the last two). Indeed, little or no broadening of the e.p.r. lines of **1** is seen, even in solutions (such as 3% sodium alginate) of much higher macroscopic viscosity than any of the sucrose solutions used (see Fig. 2). Furthermore, even when Ca^{2+} ions are added to a sodium alginate solution, thus forming a gel, the same result obtains. Here, therefore, the label reports only local conditions; the rapid reorientation of the label may arise from its localization in solvent "pockets", the interiors of which are not significantly affected by the interaction (between the macromolecules) that is responsible for the macroscopic viscosity of the system.

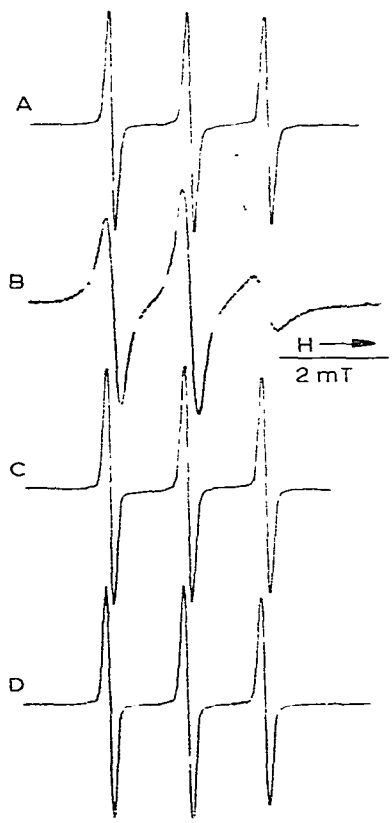


Fig. 2. E.p.r. spectra of 4-amino-2,2,6,6-tetramethyl-piperidin-1-oxyl(**1**; 0.5 mM) dissolved in: (A) water, (B) aqueous sucrose [285% (w/v)], (C) aqueous sodium alginate [1% (w/v)], and (D) aqueous calcium alginate (prepared from C); recorded at 28° with a Varian E-3 Spectrometer.

*With the exception of soluble starch, which was purchased from Allied Chemical Co., the polysaccharides were gifts from Kelco, San Diego, California. The source of the alginate was algal, not microbial.

The foregoing experiments were extended by covalently labelling the hydroxyl groups of alginate by means of the nucleophilic attack of the amine group of 3-amino-2,2,5,5-tetramethylpyrrolidin-1-oxyl (2) on cyanogen bromide-activated alginic acid. The spectrum of this randomly labelled material in solution as its sodium salt is shown in Fig. 3(A); the considerably lower speed of rotational motion now reflects the motions of the polymer chains in solution superimposed upon the rotation of the label about the bonds joining it to the macromolecule. No intimation of the existence of solvent "pockets" is now found. No appreciable change in the e.p.r. lineshape resulted from the dissolution of unlabelled sodium alginate in a solution of the labelled material, or from an increase in the concentration of the latter. This result may, however, accrue from the lower molecular weight of the labelled material, some depolymerization apparently having occurred during reaction with the spin label.

The labelled material, like its native counterpart, forms an opaque gel upon addition of calcium ions; this may be taken as a sign that chemical perturbation by the label of the native system is small at this level of labelling (roughly estimated to be less than one label per 100 monosaccharide units). The spectrum of the labelled calcium alginate gel is shown in Fig. 3(B); it indicates a further lowered rate of rotational reorientation, and the appearance of some rotational anisotropy (that may arise as a result of the greater rapidity of motion about the bonds joining the label to the polysaccharide than of motion normal to this[15]). Furthermore, not all of the alginate has been equally immobilized.

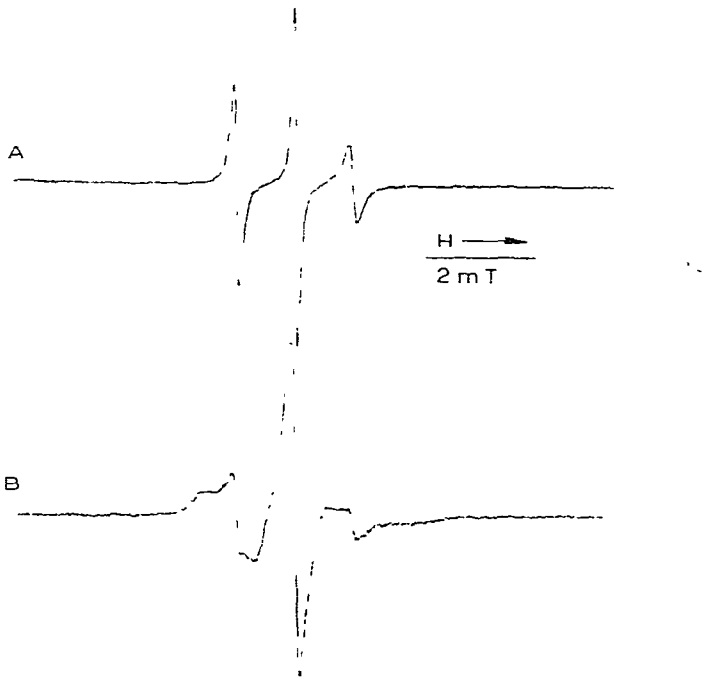


Fig. 3. E.p.r. spectra of: (A) spin-labelled alginate [~3% (w/v)] in aqueous solution; (B) as in A, after addition of $CaCl_2$ solution.

This series of results indicates that the microscopic, motional changes that arise in carbohydrate solutions as a result of intermolecular interactions are reflected by changes in e.p.r. lineshapes, and provides evidence that the drawbacks inherent in the spin-labelling technique are not sufficiently great to preclude the obtaining of useful information.

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