

# A Method of Studying the Competitive Binding of Small Molecules to Macromolecules in the Gel State by High Resolution $^1\text{H}$ NMR Spectroscopy. Solvent Effects in Polyuronide Gels

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It has been known for some time that it is possible to prepare stable, rigid, voluminous, and highly transparent gels of calcium alginate in organic solvents.<sup>1,2</sup> The state of solvation of the macromolecules in these gel systems, as well as of macromolecular gels in general, is far from completely understood. The main object here is to describe a method by which high resolution  $^1\text{H}$  NMR-spectroscopy can be used for studying the solvation of macromolecules bound in gel matrices.

Gels were prepared by dialysing solutions of sodium alginate (*Laminaria hyperborea*, stipe, M/G=0.57) contained in standard 5 mm thin wall NMR-tubes against 0.1 M aqueous  $\text{Ca}(\text{NO}_3)_2$ , and then against a large volume of water to remove excess  $\text{Ca}(\text{NO}_3)_2$ . The gels formed had a regular cylindrical shape with a diameter about 3 mm and could be removed from the NMR-tube and placed in large volumes of different liquids for solvent exchange, and then reinserted into a heavy wall NMR-tube (inner diameter  $\sim 3$  mm) for recording of spectra (VARIAN A-60A at about 39 °C).

The exchange of  $\text{H}_2\text{O}$  for EtOH occurred with a permanent slight contraction of the gels, which for a 5 % w/v water gel was found to be 8 % (v/v) and 13 % at 95 % and  $\sim 100$  % EtOH, respectively, after 2 d of dialysis, after which time no further volume contraction occurred. The significant shrinkage observed at very low water content suggests that the macromolecules are preferably associated with water molecules.

The  $^1\text{H}$  NMR spectra of ethanol in the gels were found to be highly dependent on the content of water in the ethanol. The spectra from gels containing 95 % EtOH had almost as narrow lines as in pure ethanol solution, while the spectra from gels containing  $\sim 100$  % EtOH had very broad lines, suggesting more interaction between ethanol and the polyuronide chains in the latter case. Since the small amount of water in 95 % EtOH had such an immense effect on the ethanol spectrum, it was thought that a more systematic perturbation of the ethanol spectrum by dialyzing the gels against EtOH containing different solutes might yield

information on their solvation relative to ethanol.

In order to determine the time needed for a complete exchange of solvent in each case some information on the mobility of the solvent in the gels was needed. The mobility of solvents in macromolecular gels is in general fairly high as evidence by self-diffusion coefficient measurements.<sup>3-5</sup> The time for the EtOH/ $\text{H}_2\text{O}$  solvent exchange in the gels used here was found to be about 3 min for hydroxyl and water protons and about 6 min for ethanol molecules as reflected by  $^1\text{H}$  NMR line intensities in the external solution. The exchange times were almost independent of the alginate concentration in the range up to 10 % and comparable to the self-diffusion times in free solvents.<sup>6</sup> This is also to be expected because of the relative wide pores in alginate gels (mean diameter 200–500 Å as judged from electron micrographs).<sup>7-8</sup> Based upon these experiments a standard time of 10 min was used for equilibration of the gels against large excess of solvent mixtures. Attempts were also made to determine the corresponding equilibrium composition in the gels for the EtOH/ $\text{H}_2\text{O}$  system by measuring the release of water by dialysis against  $\sim 100$  % EtOH. It was found that the gels in general contained more water than the external solution. A gel equilibrated against 98 % EtOH had for example about 20 % higher  $\text{H}_2\text{O}/\text{EtOH}$ -ratio than the external solution. Due to the

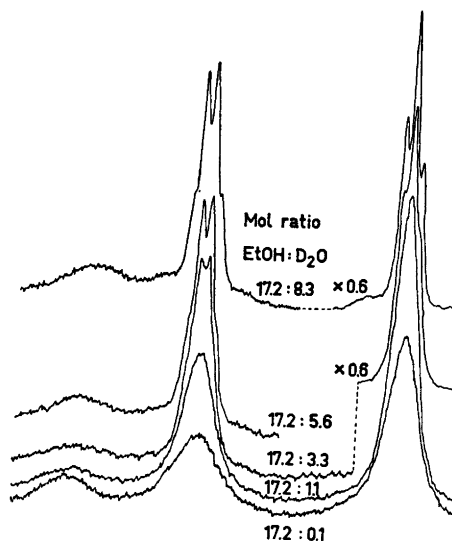


Fig. 1.  $^1\text{H}$  NMR spectra of EtOH in a 10 % w/v calcium alginate gel at  $\sim 39$  °C with successive addition of  $\text{D}_2\text{O}$ . The "100 % EtOH" used in the experiment was found by analysis by gas chromatography to contain less than 0.2 % water.

small volume of the gels, ca. 0.15 cm<sup>3</sup>, this ratio could not be determined with high accuracy, and we will at this stage relate the spectra to the composition of the external solutions.

The striking effect of water in narrowing the ethyl lines of EtOH in a 10% alginate gel is demonstrated in Fig. 1. The behaviour can be interpreted in terms of a two-phase model for the binding of ethanol to alginate.<sup>9</sup> In pure ethanol a minor fraction of the solvent molecules may be restricted in their movements by interaction with the macromolecules leading to increased relaxation rates and broad <sup>1</sup>H NMR lines for their protons. If a rapid molecular exchange occurs between the interacting molecules and the bulk solvent, population-weighted average line widths are obtained. In support of this model is the observations that in pure ethanol the line widths increase with increasing concentration of alginate in the gels, and an observed difference in the line width of the CH<sub>2</sub> and the CH<sub>3</sub> lines in the ethanol-signal. In a 10% gel in pure EtOH the CH<sub>2</sub> lines were 35 Hz wide and the CH<sub>3</sub> lines about 20 Hz wide. This difference is expected if some ethanol molecules are bound by hydrogen bonds to the alginate chains, because the CH<sub>3</sub>-group then will be less restricted in its movement through rotation around one extra bond. The high resolution spectra obtained after addition of relative small amounts of water (corresponding to about 4 water molecules per monomer residue) indicate that no appreciable solvation of EtOH occurs under these conditions. This result strongly indicate that the water molecules bind more strongly to the alginate chains, and thereby hinder their solvation by ethanol.

In Fig. 2 the effect of water is compared with that of methanol, acetone, and cyclohexane. By assuming that the spin-spin coupling in the -CH<sub>2</sub>-CH<sub>3</sub> group was unaffected by the broadening mechanism, the full line width at half maximum could be obtained by computer simulation, and the width of the CH<sub>3</sub> lines is plotted against the mol fraction of ethanol in the external solution. The results indicate a very marked difference in the solvation of the four solutes relative to ethanol with decreasing solvation in the order water > methanol > ethanol > acetone > cyclohexane. This was also supported by the relative line widths of the solutes at a mol fraction of 0.3. They decreased in the order water > MeOH > acetone (10 Hz) > cyclohexane (4 Hz). The series are expected except for the lacking effect of acetone in perturbing the ethanol spectrum. Acetone could be thought to bind to the hydroxyl groups of the alginate, and it is most interesting that this seems not to be the case. This result suggests that alginate is more effective in forming hydrogen bonds as a hydrogen acceptor than as a hydrogen donor, which agrees with current ideas concerning the solvation of carbohydrates in general.<sup>10</sup>

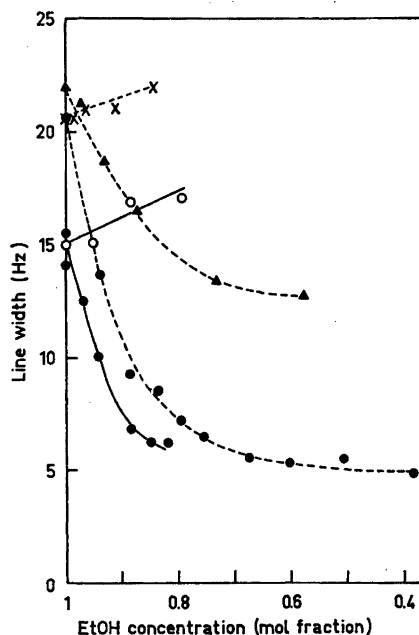


Fig. 2. The width of EtOH methyl <sup>1</sup>H NMR lines in calcium alginate gels at ~39 °C as a function of mol fraction of EtOH in the solvent. ---, 10% gel; —, 5% gel; ●, EtOH/H<sub>2</sub>O; ▲, EtOH/MeOH; ○, EtOH/cyclohexane; ×, EtOH/acetone.

Whatever the molecular mechanism for the differences are, the effects shown in Fig. 2 are large and easy to detect, indicating that the technique may prove valuable in solvation studies on macromolecular gels. A more detailed study involving more model solutes will now be undertaken in our laboratories.

Another interesting aspect of the method is that the solvent binding capacity of the macromolecules must depend on the degree of junction formation of the chains. Crosslinking will probably reduce the macromolecular surface available for solvent interaction. Evidence for this is seen in Fig. 2 where the CH<sub>3</sub> lines in dry EtOH gels increase somewhat less than proportional to the concentration of alginate. This is also the case for the H<sub>2</sub>O line in pure water gels. A study involving alginate gels of different composition and concentration may therefore shed more light on the chain structure of these gels.

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