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Preliminary communication

Use of n.m.r. imaging to map the spatial distrubution of structure in polysaccharide gels*

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Investigation of gel formation in such carbohydrate solutions as those of agar and agarose[1–5] has demonstrated that nuclear magnetic resonance (n.m.r.) is an effective tool for investigating these biopolymer matrices[6–11]. Proton relaxation measurements are particularly useful for probing the state of the water in such gels since the spin–spin and spin–lattice relaxation rates are sensitive to molecular motion at specific frequences[12–15]. Thus, a theoretical model based on rapid exchange between the small proportion of relatively immobile polysaccharide protons and those of water predicts a linear relationship between the spin–spin relaxation rate and solute concentration, and this relationship has been confirmed experimentally[9,12,15–18]. It has also been demonstrated that the morphology within a gel affects the spin–spin relaxation processes[12,13].

In this paper, we have measured the n.m.r. image signal intensity of agar gels at different concentrations, and we demonstrate that, by carefully setting the experimental parameters, the n.m.r. image signal intensity is directly related to the solute concentration.

All n.m.r. images were obtained using an Oxford Research Systems Biospec I spectrometer, operating at 84.7 MHz for protons, connected to an Oxford Instrument 31-cm horizontal bore 2T, superconducting magnet. Home-built gradient coils produced three orthogonal linear magnetic field gradients each of 8 $kHz.cm^{-1}$ (ref. 19). The samples were studied in a home-built split-ring resonator[20] with an inner diameter of 6 cm. A slice-selective 90° spin-echo imaging pulse sequence was used[21]. The non-selective 180° pulse length was 100 μs and the frequence-selective 90° pulse excited a horizontal region of 3 mm through the sample. The total echo time (*TE*) was 120 ms and the recycle time (*TR*) was 15 s. The signal was digitised every 32 μs and 128 complex points were acquired. The sequence was repeated using 128 phase encode steps and no signal averaging was required. All experiments were performed at ambient temperature (295 K).

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.

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Agar gels (0.3–6% weight/volume) were made by dissolving agar in hot water in 1-cm diameter vials. Six vials were imaged simultaneously and the images integrated; the total image signal intensity from each sample was normalised with respect to the image intensity of a water standard. Each gel was studied three times and the results were averaged. In order to check that the image intensity observed was not adversely affected by spectrometer hardware, inhomogeneities in the magnetic field, or inhomogeneities in the radiofrequency produced by the probe, six identical vials of water were imaged at the start of each measurement. Their image intensities never varied by more than 6% from the mean value.

The intensity of the n.m.r. signal from each pixel in a spin-echo n.m.r. image is normally dependent upon five parameters: the concentration of mobile protons (ρ), the spin–lattice (T_1) and spin–spin (T_2) relaxation times of the nuclear spins, the echo time (TE) between excitation and detection of magnetisation, and the recycle time delay (TR) between successive pulse sequences during which the magnetisation relaxes back to its equilibrium state. Assuming that there is no mass transport such as diffusion or flow, then the signal intensity M can be expressed as:

$$M = \rho \times \exp(-TE/T_2) \times [1 - \exp(-TR/T_2)]. \quad (1)$$

The T_1 relaxation term in the equation is unity for all the experiments described here, as the recycle time (TR) was always set to be at least five times T_1. Since the agar concentration in the gels is low (below 6% weight/volume), the proton density (ρ) can be assumed, to a first approximation, to be the same for all samples, and Eq. *1* may be simplified to:

$$M = k \times \exp(-TE/T_2) \quad (2)$$

Therefore, only the spin–spin relaxation rate ($1/T_2$) affects the image intensity. Since it has been shown that ($1/T_2$) for water in a gel is linearly dependent on the gel concentration c, except at high concentrations[9,12,15–17], this gives a direct relationship between solute concentration and n.m.r. image intensity.

$$M \propto \exp(-TE \times c) \quad (3)$$

Thus, at low solute concentrations and when the n.m.r. recycle delay is set to be at least five times the T_1 relaxation time, the natural logarithm of the signal intensity M arising from a pixel in the image of water in a gel should be linearly proportional to the solute concentration c.

The images of the six vials of agar gel of different concentrations are displayed in Fig. 1. Clearly, the higher the agar concentration the lower the signal intensity. The graph of "ln (image intensity)" normalised with respect to the water standard plotted against the concentration of agar in the gel is shown in Fig. 2. This has the straight line predicted by Eq. *3*.

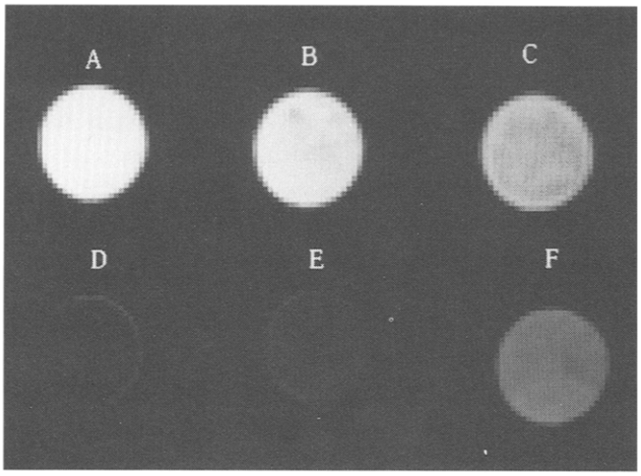

Fig. 1. Spin-echo images (128 × 128) of six vials of agar gel. Agar concentrations (weight/volume): (A) 0.17%, (B) 0.37%, (C) 0.71%, (D) 4%, (E) 2.5%, (F) 1.43%. *TE*, 330 ms; *TR*, 15 s; slice thickness, 3 mm; inplane resolution, 0.25 mm.

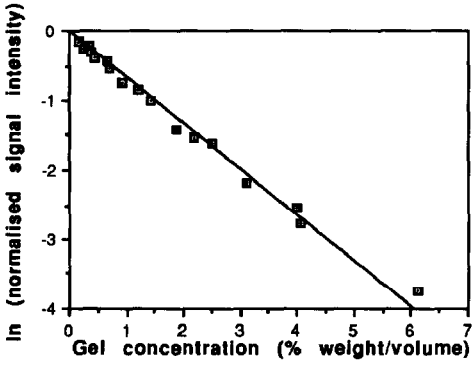

Fig. 2. A plot of ln (image intensity) against agar concentration for images of individual vials of agar gel (normalised with respect to a water standard).

These experiments demonstrate that, when the experimental parameters are carefully set, n.m.r. imaging can be used to map the spatial distribution of solute concentration in polysaccharide solutions. The major advantage of n.m.r. imaging is that different spatial regions of large, intact heterogeneous samples may be studied non-destructively. Thus, changes in gel concentration in composite foods can be monitored as a function of time.

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