^{1}H MAS n.m.r. and two-dimensional nuclear Overhauser enhancement spectroscopy in hydrogels*

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The combination of ^{1}H magic angle spinning (MAS) n.m.r. and two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY) is shown to be readily applicable to hydrogels. This is demonstrated in the superabsorbing polymer, hydrolysed starch-*g*-poly(acrylonitrile), which readily responds to hydration. Enhanced molecular mobility of the polymer is directly revealed by polymer–polymer NOESY connectivities. The two-dimensional experiment also reveals the presence of an efficient cross-relaxation mechanism between the water of hydration and the polymer.

(Keywords: hydrogel; nuclear magnetic resonance; NOESY)

Introduction

The microdynamic response of polymer networks to macroscopic perturbations has many interesting facets[1–3]. In a polymeric gel, a macroscopic perturbation may be imposed by the stress field created by solvent influx into the polymer matrix[4]. The cooperative solvent movement into the polymer network leads to chain expansion and this manifests as enhanced local segmental mobility. This behaviour is well documented in a number of polymer–solvent systems[5–9].

The enhanced polymer mobility in the gel phase[10] leads to an effective motional averaging of the various spatially dependent n.m.r. spin interactions[11]. As a result, the severe spectral line broadening, experienced for rigid polymers, is greatly reduced in the gel state. Although residual line broadenings ($\sim$1–5 kHz) remain, solid-state n.m.r. techniques employing sample rotation at the 'magic angle' (MAS)[12,13] can be effectively applied by taking advantage of this inherent line narrowing in the polymer system. It has been shown that high resolution proton n.m.r. spectra can be obtained in gel media by employing the MAS technique alone[14–17] without the need to employ the experimentally demanding multiple-pulse homonuclear line narrowing techniques[18]. As discussed elsewhere[17], there is a transformation of the homogeneous dipolar interactions amongst abundant proton spins to an inhomogeneous one, when the polymer is highly swollen. Since an inhomogeneously broadened dipolar line can be narrowed by MAS at moderate spinning speeds (1–4 kHz), well resolved proton spectra are easily obtained in the gel state.

It is the purpose of this communication to show the effectiveness of this method in conjunction with two-dimensional nuclear Overhauser enhancement spectroscopy (2-D NOESY) to glean information about hydration-induced dynamic response in a synthetic polymer. The 2-D exchange spectroscopy[19] correlates n.m.r. resonances, which undergo either a chemical or a magnetic exchange[19–21]. This is readily applicable in its elementary form to hydrogels since MAS resolution enables us to frequency label the distinct proton sites in the polymer during the evolution period (t_1) of the 2-D experiment. The whole experiment benefits from the enormous sensitivity of the observed ^{1}H nucleus and the high resolution afforded by MAS. Even at low spinning speeds (1.5–2 kHz), the resulting 2-D plots map the spin connectivity without interference from spinning side bands.

We report the first direct application of this technique to polymeric hydrogels. The system chosen is a superabsorbing polymer, hydrolysed starch-*g*-poly(acrylonitrile), which has applications in diverse areas[22–27]. This polymer is known to respond readily to hydration and has also been extensively studied by us[11,16,17,28,29].

Experimental

The phase-sensitive 2-D NOESY experiments were performed on a Bruker MSL-300 FT-NMR spectrometer at the proton resonance frequency of 283.7 MHz and at ambient probe temperature (295 K). MAS was kept at 1.8 kHz and a recycle time of 3 s was used. The total time for 2-D experiment was approximately 2 h. The t_1 was incremented in steps of 162 s following the time proportional phase incrementation (TPPI)[30] procedure and 16 transients were accumulated in each of the 64 experiments. A sweep width of 3 kHz and 512 real data points were used. The data were apodized with sine-squared bell window function in both F_1 and F_2. The water resonance at 4.8 ppm reflects residual partially deuterated water (HOD) as well as any initial water in this superabsorbing polymer[31]. A 10% random variation of the mixing time was used to suppress *J* cross-peaks.

Hydrolysed starch-*g*-poly(acrylonitrile) was synthesized by graft copolymerization of acrylonitrile onto gelatinized starch using ceric ammonium nitrate as a redox initiator for the free-radical polymerization. The temperature of the reaction was maintained at 303 K. The graft copolymer was subsequently alkali hydrolysed and isolated in the form of a dry powder. It had an equilibrium water absorption capacity of 170 g water/g of dry polymer.

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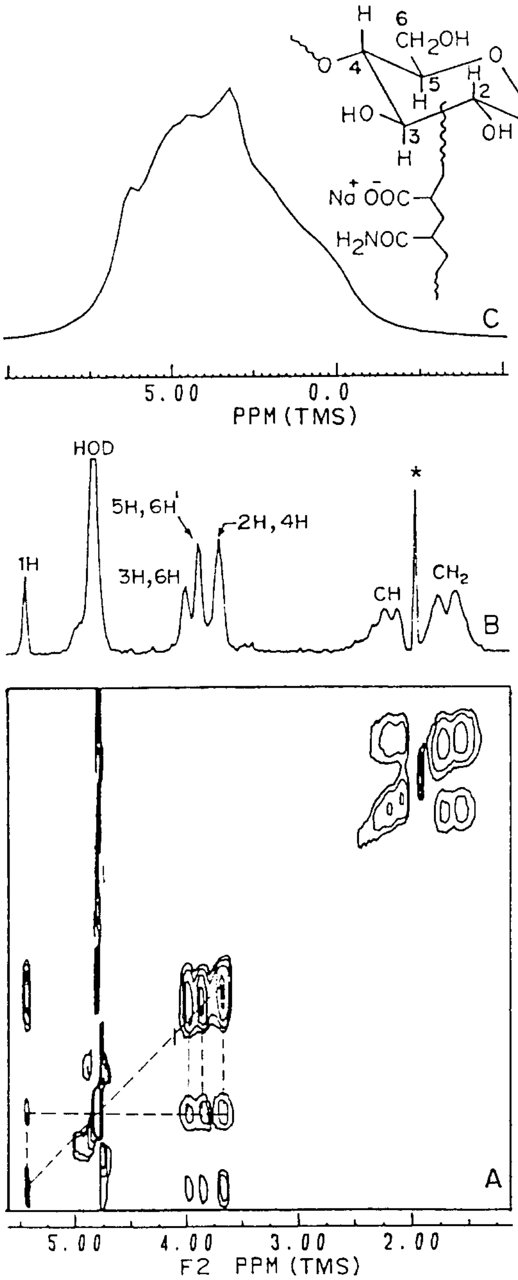

Figure 1 (A) Phase-sensitive homonuclear ^{1}H–^{1}H 2-D NOESY contour plots of hydrolysed starch-*g*-poly(acrylonitrile) gel hydrated to 3.4 g/g (water/polymer) in 99.8% D_2O. $\tau_m = 500$ ms. (B) Projection of 2-D data in (A) along F_2 axis, showing the 1-D MAS spectrum. The signal marked with an asterisk is the impurity signal. (C) Static ^{1}H spectrum of the polymer. The strong water signal gives a ridge parallel to the F_1 axis

Hydrated samples were prepared by adding known amounts of water (99.8% D_2O) directly to the dry polymer and equilibrating the samples for 4 days. Hydration levels were estimated gravimetrically and are expressed as weight ratios (water/polymer).

Results and discussion

Figure 1 shows the 2-D NOESY contour plot of the hydrated polymer (3.4 g/g) taken at a mixing time of 500 ms. At this hydration level, the integrated area of water (HOD) peak was estimated to be 54% of the total integrated area of the 1-D MAS spectrum. Considering the polymer composition and the molecular weight (1.2×10^5), we estimate that at this hydration level all the hydrophilic sites in the pendent chain and starch backbone are hydrated[28]. It may also be noted that to observe cross-peaks between water and the polymer, the water signal should not be very intense, lest the water ridge swamp the cross-peak signals in both F_1 and F_2 directions. The amount of residual H_2O in the 3.4 g/g sample represents an appropriate dilution of H_2O in D_2O required to hydrate all the sites and yet reveal the polymer–water cross-peaks. The 2-D experiment was conducted in the phase-sensitive mode, rather than in the magnitude mode, to reveal the absolute sign of the resulting cross-peaks. The projection of the 2-D NOESY data parallel to the F_2 axis is also shown. This gives rise to the ^{1}H MAS spectrum (*Figure 1B*), where the distinct proton sites in the pendent chain and starch backbone could be identified and assigned. This fine spectral resolution is not attainable in the static gel (*Figure 1C*).

The 2-D cross-peaks develop exclusively within the pendent and starch units. Experiments conducted at various mixing times in the range 50–500 ms confirm that cross-peak intensities follow a transient response characterized by (1) an exponential rise due to cross-relaxation between the interacting spins and (2) a decay due to dissipative effects of spin-lattice relaxation[19]. The dependence of the cross-peak intensities on the mixing time τ_m is shown in *Figure 2* for the proton resonances in the pendent chain and the starch backbone. The behaviour is seen to be markedly different. It is further noticed that the cross-peaks have the same positive sign as the diagonal peaks, showing thereby that the resulting Overhauser enhancements are negative[19,32].

It is clear that we are in the so-called 'spin-diffusion' regime, or the long correlation limit ($\omega\tau_c \gg 1$), in so far as the time-scale of the hydration-induced polymer mobility is concerned[31,33–36]. This is in accordance with our earlier steady-state ^{13}C NOE measurements[29], where it was found that the theoretical maximum ^{13}C NOE of 198.8% could not be observed even at very high hydration levels. The cross-peaks also distinctly demarcate the intramolecular dipolar interactions within a given seg-

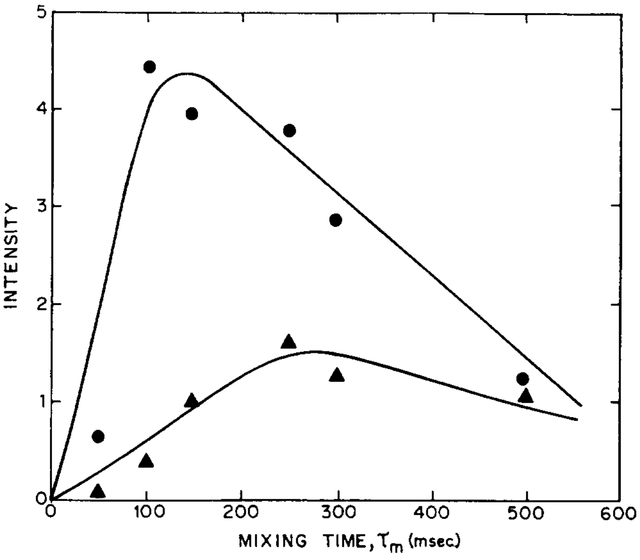

Figure 2 Dependence of the cross-peak intensity on the mixing time τ_m for hydrolysed starch-*g*-poly(acrylonitrile) gel hydrated to 17.0 g/g (water/ polymer). Cross-peak intensities measured from experimental 2-D NOESY data are denoted by: ●, between pendent CH and CH_2; ▲, between starch 1H and 2H. The solid line through the data points has been drawn by hand to show the transient response of the cross-relaxation process

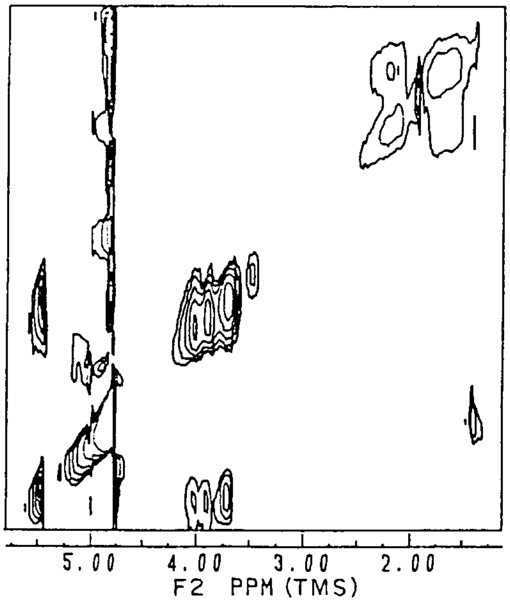

Figure 3 Phase-sensitive $^1H-^1H$ 2-D NOESY contour plots of hydrolysed starch-*g*-poly(acrylonitrile) gel hydrated to 34.0 g/g in 99.8% D_2O, $\tau_m = 500$ ms

ment (pendent chain or starch backbone). In fact, no starch–pendent group 2-D NOESY connectivity could be established, irrespective of the level of hydration of the polymer or the choice of mixing time. This confirms that in this grafted copolymer, the pendent chains are indeed spatially isolated from starch units. The intraunit connectivity is established primarily by dipolar cross-relaxation interaction within the given segment, modulated by chain motions. These are strongly favoured for the CH and CH_2 protons of the pendent chains and the CH_2, anomeric protons of the puckered glucopyranose ring of the starch backbone. The observed difference in the mixing time dependence of the cross-peak intensity for the pendent and starch protons (*Figure 2*) may be attributed to the difference in motional correlation time, molecular geometry or both.

The interaction between the water of hydration and the polymer system is also revealed in the 2-D NOESY experiment (*Figure 1*). There is a uniform migration of water magnetization across the entire starch unit, as revealed by enhanced water–polymer cross-peaks and a decreased water resonance on the diagonal. There is no 2-D connectivity of water with the aliphatic resonances of the pendent unit, however. As shown previously[28], water is easily accessible to the various hydrophilic groups (COO^-, $CONH_2$ of pendent units and OH^- of starch), which act as hydration sites. While the plasticization induced by water to enhance the polymer mobility is revealed by polymer–polymer cross-peaks, the direct molecular association of water with the polymer system is revealed by water–polymer cross-peaks. Thus the 2-D experiment brings about this important feature on the state of water, which has drawn the attention of many researchers investigating biopolymer hydration[35,36], in a single run. The absence of cross-peaks with the pendent protons is likely to be caused by the long H–H distance due to hydrogen bonding of water at the carboxylate and amide sites, or due to very short correlation time for water motion, or both.

For the glucose unit, only hydroxyl sites affect hydrogen-bonding capability for water. At low hydration levels the water molecule is tightly bound, presenting very reduced mobility due to at least two strongly energetic hydrogen bonds with primary absorption sites[37]. This would lead to a significant cross-peak development in the 'spin diffusion' regime ($\omega\tau_c \gg 1$). We observe this in the 3.4 g/g hydrated polymer (*Figure 1*). On the other hand, increased water mobility at higher hydration levels would attenuate or eliminate cross-peaks with starch protons. This is clearly demonstrated in *Figure 3*, where the 2-D NOESY data taken at a constant mixing time of 500 ms on a 34.0 g/g hydrated polymer are shown for comparison. The importance of cross-relaxation between the starch and water protons via the secular 'flip-flop' process, rather than proton chemical exchange, has been suggested recently[38]. Our 2-D NOESY data, especially for the 3.4 g/g hydrated polymer, lend support to this view.

Conclusions

We have shown that useful 2-D NOESY experiments can be conducted on hydrogels, taking advantage of enhanced spectral resolution obtained by sample rotation at the magic angle. The resulting data contain valuable information about the spin connectivity established by dipolar relaxation interactions in the mobile phase of the polymer gel. It appears that a common feature of the gel NOESY behaviour is that the exchange network is largely dominated by cross-relaxation mechanism in the 'spin-diffusion' regime. The importance of dipolar cross-relaxation interactions between the water of hydration and the polymer system is also demonstrated.

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