ELSEVIER

PII: S0032-3861(97)00403-5

Polymer Vol. 39 No. 5, pp. 1059–1066, 1998

0032-3861/97/$17.00+0.00

Investigation of water dynamic behaviour in poly(HEMA) and poly(HEMA-co-DHPMA) hydrogels by proton T_2 relaxation time and self-diffusion coefficient n.m.r. measurements

R. Barbieri, M. Quaglia, M. Delfini and E. Brosio*
Dipartimento di Chimica – Università di Roma 'La Sapienza', P.le Aldo Moro, 5-00185 Rome, Italy
(Received 7 May 1997)

Proton transverse relaxation time (T_2) and molecular self-diffusion coefficient (D) n.m.r. measurements of water in three hydrogels based on poly(2-hydroxyethyl-methacrylate) (pHEMA) have been performed in order to investigate the state of water and its interaction with the polymer network. Measured T_2 values are discussed and quantitatively interpreted by assuming the chemical exchange process between water protons and hydroxyl protons of polymer chains as the major relaxation source, with no recourse to the bound water concept. The pseudo first-order kinetic constants and the activation energies for the exchange process in the different hydrogels have been calculated. The Pulsed Field Gradient (PFG) spin–echo technique has been used to measure diffusivity of water internal to the hydrogels; for hydrogels surrounded by external water, PFG multiple spin–echo experiments at different echo times and subsequent Diffusion Analysis by Relaxation-Time-Separated (DARTS) have been performed in order to obtain simultaneous determination of both internal and external water diffusion coefficients.

(Keywords: poly(HEMA); hydrogels; n.m.r.)

INTRODUCTION

Hydrogels based on hydroxyethylmethacrylate (HEMA) homo- and copolymers are widely employed in various biomedical fields[1]. Due to their high biocompatibility, they find application as controlled drug delivery systems, prosthetic materials, and both intraocular and contact lenses. Biocompatibility is attributed not only to the low irritant and toxic effect of the monomer and the high resistance of the polymeric chains to degradation, but also to the role played by water in ensuring polymer plasticisation as well as solubilisation and transport of gases and metabolites within the macromolecular matrix[2].

To better understand such properties and to develop suitable biomedical hydrogels, the physico-chemical properties of water inside pHEMA hydrogels and its interaction with the polymeric chains have long been studied by a number of techniques such as differential scanning calorimetry[2–5], dilatometry[4], differential thermal analysis[6–8], adiabatic calorimetry[8] and specific conductivity[4]. The experimental results have been analysed in terms of different classes or types of water molecules, each class being characterised by a different degree of interaction with the macromolecular matrix and hence by a different mobility. This classification appears to be technique-dependent. Thus the relative amounts and relevant properties of the different types of water molecules as determined by one experiment cannot be used to interpret the results obtained by a different technique. Generally speaking, three types of water molecules have been evidenced: 'bound' water, strongly associated with the polymeric chains by means of hydrogen bonds or polar interactions; 'interfacial' water, characterised by hydrophobic interaction with the macromolecule; and 'bulk' water, whose properties are not affected by the presence of the polymeric matrix[9]. However, note that some other thermoanalytical works came to the conclusion that double melting peaks are to be accounted for not by the presence of thermodynamically different classes of water but by the development of metastable non-equilibrium states[8,10]; whereas the lack of freezing behaviour of part of the water within a pHEMA hydrogel can be attributed to the fact that the formation of ice crystals is inhibited by a polymer that has undergone its rubber–glass transition[7].

Proton Nuclear Magnetic Resonance (n.m.r.) experiments have been often associated with the above studies. In particular, n.m.r. relaxation time T_1 and T_2 measurements have been used to investigate the mobilities of water molecules in the hydrogels and their interactions with the gel network. However, such measurements failed to reveal a multiplicity of water types, i.e., no deviations from monoexponentiality were observed for magnetisation decay curves. This has been attributed to molecules belonging to different water classes being either in fast diffusive exchange with respect to the chemical shift or the spin–lattice relaxation time[3,6,11,12], or in the intermediate exchange regime with respect to the spin–spin relaxation time[11]. Based on $H_2^{17}O$ n.m.r. relaxation experiments, Roorda *et al.*[13] confirmed that, if bound water molecules exist, they are to be considered in fast exchange with bulk water molecules on a timescale of less than a

* To whom correspondence should be addressed. Tel: +39-6-49913123; fax: +39-6-490631; e-mail: brosio@chemsig.sci.uniromal.it

millisecond. They also demonstrated that all the water molecules within the gel contributed to the overall signal amplitude. Moreover, doubling the number of potential binding sites for water, as in the case of dihydroxypropylmethacrylate (DHPMA) polymers, the ^{17}O relaxation rate values were the same as in pHEMA hydrogels with the same water content (an observation which is in contrast with the higher amount of free water in these hydrogels reported by Choi *et. al.*[14]). These results cast doubts on the presence of bound water on any timescale. Water dynamics inside hydrogels was also studied by Pulsed Field Gradient (PFG) n.m.r. diffusion measurements[15]. Analysis of the echo attenuation dependence on field gradient amplitude yielded a single diffusion coefficient at the echo times at which measurements were carried out, thus confirming that the exchange among water classes, if present, takes place so as to average the translational velocities of the classes on a timescale of the order of few milliseconds or less. The water self-diffusion coefficient for a completely hydrated pHEMA hydrogel was found to be lower by about one order of magnitude than that of neat water and mainly dependent on the polymer hydration degree rather than on the crosslinker content.

In the present work, transverse n.m.r. relaxation times and self-diffusion measurements have been used to gain further information about the macromolecule and water dynamic behaviour in three hydrogels with different equilibrium water contents (EWCs). The hydrogels, usually employed as contact lens materials, are formed either by a HEMA homopolymer or by HEMA–DHPMA copolymers. Proton T_2 measurements have been interpreted, without postulating the presence of a bound water fraction, in terms of a chemical exchange between water protons and hydrogens on hydroxyl groups of side chains of the monomeric units. The contribution of proton exchange can be calculated in terms of well-defined quantities, i.e., the fraction and the intrinsic relaxation time of polymer exchangeable hydrogens, their mean lifetime, and the chemical shift difference between protons of water and polymer exchangeable groups. This mechanism has been shown to constitute the major relaxation source in high water content polysaccharide and protein solutions and gels[16,17]. Taking it into account, the enhanced water relaxivity observed in the hydrogels was quantitatively accounted for. Furthermore, both the relaxation times of polymeric chains and the kinetic constant of the chemical exchange process itself were calculated through the relevant equations describing the dependence of water T_2 values on parameters characterising the contribution of the exchange process to relaxation.

In addition to relaxation time measurements, PFG–n.m.r. experiments were carried out in order to measure water self-diffusion coefficients which, being related to the mean square random displacement of water molecules, provide information on the material transport properties. Measurements on hydrogels at their equilibrium water content were performed by the PFG Spin–Echo (PFG–SE) sequence, while for polymer samples more hydrated than their EWC, i.e., hydrogels surrounded by external water, water self-diffusion coefficients were measured by means of the recently proposed Diffusion Analysis by Relaxation-Time-Separated PFG–n.m.r. (DARTS PFG–n.m.r.)[18]. This approach is based on a combination of the analysis of Carr–Purcell–Meiboom–Gill (CPMG) decay curves and the measurement of the apparent diffusion coefficient dependence on the echo time as obtained by a PFG Multiple Spin–Echo (PFG–MSE) sequence[19]. The DARTS PFG–n.m.r. technique allows the measurement of molecular self-diffusion coefficients which are about a factor of two or less apart and appears to be particularly important considering that heterogeneous biosystems, like hydrogels when used as biomaterials, are often characterised by several water-containing compartments that differ in both relaxation time and self-diffusion coefficient values. Because of the relatively small differences between these self-diffusion coefficients, a single average value of water diffusivity is generally measured. By combining T_2 and diffusion measurements at various echo times, accurate determinations of self-diffusion coefficients of water in different compartments can be obtained. Up to now, this technique has found application to self-diffusion measurements of water in the vacuole and in the cytoplasm of apple parenchyma tissue[18] and water trapped in and confined out (i.e., residual syneresis water) of casein aggregates in Ricotta cheese[20].

EXPERIMENTAL

Samples

Three HEMA (CH_2=$C(CH_3)CO_2CH_2CH_2OH$) polymers (kindly supplied by Benz Research and Development Corporation, Sarasota, FL, USA) with different percentages of DHPMA (CH_2=$C(CH_3)CO_2CH_2CH(OH)CH_2OH$) as comonomer were analysed: Benz 38 (pure pHEMA), Benz G-45 (DHPMA molar fraction = 0.24) and Benz G-55 (DHPMA molar fraction = 0.48). Compositions were determined by integration of signals corresponding to the two monomers in ^{13}C n.m.r. spectra of polymers dissolved in DMSO–d_6 at 37°C. The equilibrium water contents, as reported by the manufacturer, were 38%, 45% and 55%, respectively, of the total weight; they were assumed not to vary with the temperature*.

Discs of polymeric materials were allowed to completely hydrate in distilled water at 40°C for at least two weeks. Two cylinders per hydrogel were cut by means of an 8 mm cork borer and washed. One cylinder was then gently pressed between two sheets of filter paper and inserted in a 10 mm o.d. n.m.r. tube—'wiped' sample. The other cylinder was inserted in the tube together with a little amount of water in order to obtain completely water-surrounded hydrogel; we have called the latter a 'wet' sample. To prevent water evaporation the hydrogel samples were capped by a Teflon insert and the tubes sealed with a laboratory film.

N.m.r. measurements

Experiments were carried out on a Bruker Minispec PC120 pulsed n.m.r. spectrometer (Bruker Spectrospin Company) with an operating frequency of 20 MHz for protons. The gradient coil-fitted probe was thermostated at the desired temperature (with a stability of ± 1°C) by circulating a completely fluorinated fluid. Before measurements, samples were allowed to rest within the probe, for temperature equilibration, for at least 90 min.

Transverse relaxation measurements were performed on 'wet' samples by means of a CPMG sequence[22]. The pulse spacing τ between two following π pulses was 1 ms and the

* This is, however, an approximation, at least for pHEMA. In fact Refojo and Yasuda[21] reported a decrease of the hydrogel EWC when passing from 10°C to 60°C. Such a variation, some 5% relative to the mean value of 38%, was found not to heavily influence the values of the physico-chemical parameters we have calculated (see 'Results and Discussion').

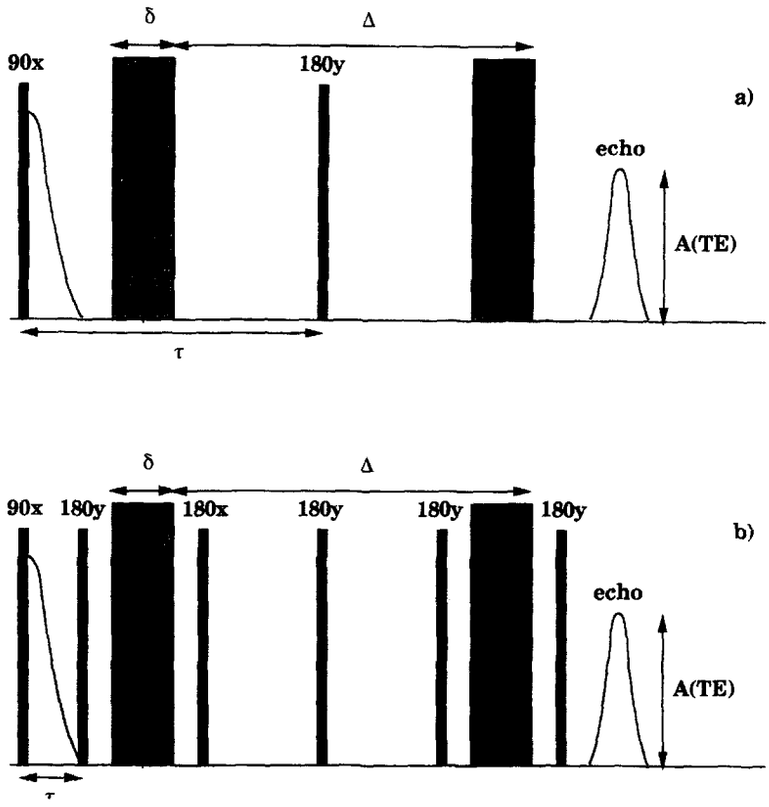

Figure 1 The PFG–SE (a) and –MSE (b) sequences. Grey and black areas represent gradient and radio-frequency pulses, respectively

signal was measured every 2 or 10 echoes. Typically, 49 scans were acquired with a recycle delay of 40 s. The decay of the transverse magnetisation was found to be always biexponential; the amplitudes and relaxation rates of the two components were calculated by means of nonlinear least-squares data fitting with a home-written computer program based on the Marquardt algorithm[23]. The fast-relaxing component was assigned to water inside the hydrogel (internal water), whereas the slow-relaxing component was attributed to water molecules surrounding the hydrogel cylinders (external water).

Self-diffusion measurements of hydrogel water for 'wiped' samples were carried out using a standard Stejskal–Tanner (PFG spin–echo) sequence[24]. The sequence, as reported in *Figure 1*a, is a modification of the spin–echo (90–τ–180–τ–echo) experiment in which two magnetic field gradient pulses of amplitude G and duration δ are applied along the static magnetic field direction z before and after the 180° refocussing pulse. Provided that the time interval between the two gradient pulses Δ (which is usually kept equal to 2τ) is much greater than δ and that the translational motion of the molecule is completely free, the echo attenuation R is:

$$R(TE) = A(TE, G_{on})/A(TE, G_{off}) = \exp(-bD) \quad (1)$$

where $A(TE, G_{on})$ and $A(TE, G_{off})$ are the echo amplitudes as measured with and without the pulsed gradient, respectively, at echo time TE; b is a factor given by $b = (\gamma G\delta)^2(\Delta - \delta/3)$ (γ is the proton magnetogyric ratio), and D is the water self-diffusion coefficient. D values were obtained by a monoexponential fitting of R measured at different b values (the parameter which was varied was Δ, at least 8 values between 5.5 and 22 ms, whereas δ was kept constant at 500 μs). Two gradient amplitudes were used, namely $G = 1.3$ and $G = 2.1$ T m^{-1}. The standard deviations of the Ds are always less than 5% of the fitted value.

Diffusion coefficients for hydrogels surrounded by water were measured by means of a PFG multiple spin–echo sequence[19] as reported in *Figure 1*b. For each echo time at least 8 R values were acquired by varying G between 0.6 and 0.8 T m^{-1}; δ was chosen as 1.732 ms, according to the formula $2\tau^2 = (2/3)\delta^2$, in order to reduce the effect of background or *in situ* gradients, and Δ was 6 ms ($\tau = 2$ ms). From one experiment to another, the echo time TE given by $2n\tau$ (n is the total number of π pulses) was varied by varying the number of π pulses before the first gradient pulse, while keeping Δ constant. Again, the diffusion coefficient D was calculated by a monoexponential fitting of the echo attenuation according to equation (1); in this situation, an apparent value of D (D_{app}) is calculated, because of the contribution of internal and external water to the overall water signal. However, on the basis that not only D but also T_2 values are different for external and internal water, it is possible to demonstrate that at echo time TE the attenuation of the signal is given by[18]

$$\ln R(TE) = f_{ext}(TE)(-bD_{ext}) + f_{int}(TE)(-bD_{int}) = -bD_{app} \quad (3)$$

where the coefficients $f_{ext}(TE)$ and $f_{int}(TE)$ are the fractions of the signal amplitude related to external and internal water, respectively, at a given echo time. For each component the fraction is given by

$$f_i(TE) = A_i(TE)/\Sigma_i A_i(TE). \quad (4)$$

The values $A_i(TE)$ are calculated by the formula

$$A_i(TE) = A_i(0)\exp(-TE/T_{2i}) \quad (5)$$

and the values of $A_i(0)$ and T_{2i} are obtained by a CPMG experiment carried out with the same pulse spacing as in

PFG–MSE. By performing experiments at different *TE*s, while maintaining the same Δ, it is possible to extract the coefficient diffusion of both internal and external water. For this reason van Dusschoten *et al.*[18] named this approach 'Diffusion Analysis by Relaxation-Time-Separated' (DARTS) PFG–n.m.r.

RESULTS AND DISCUSSION

Transverse relaxation measurements

The analysis of the proton transverse magnetisation decay curves of water in pHEMA hydrogel 'wet' samples showed a biexponential behaviour: the slow-relaxing component was attributed to external water, whereas the fast-relaxing one was assigned to water inside the hydrogel. The values of spin–spin relaxation times measured at 25°C for both components in the three types of hydrogel studied are reported in *Table 1*. It is immediately evident that the T_2 values of external water, i.e., water surrounding the hydrogel, are of the same order of magnitude as those of pure water (about 2 s), whilst those of internal water are significantly lower.

This result is often encountered when dealing with water relaxation in gels and other heterogeneous systems. The reason for it is generally explained by a slowing down of the rotational motion of water molecules, the degree of the reduction being related to the strength of the interaction with the macromolecular network. However, Hills *et al.*[17] demonstrated that, for systems in which exchangeable protons (i.e., –OH, $-NH_2$, –SH groups) are present, at pH values close to neutrality, the proton chemical exchange between those groups and water is an important relaxation source. According to this mechanism, when water protons are in exchange with hydroxyls or other exchangeable hydrogens on a macromolecule, the spin–spin relaxation time is lowered by a factor that depends in a complex fashion on a number of parameters, including the pulse spacing τ at which the CPMG experiment has been carried out. This gives rise to the well-known T_2 dispersion curves[16]. At low magnetic fields (as in the present case, where $B_0 = 0.47$ T), when water protons are overexceeding with respect to the exchangeable protons of the macromolecule, the observed T_2 value can be expressed by[25]

$$T_{2obs}^{-1} = T_{2w}^{-1} + P_m/(T_{2m} + k_m^{-1}) \quad (6)$$

where P_m is the fraction of macromolecular exchangeable protons, T_{2w} and T_{2m} are the spin–spin relaxation times of pure water and exchangeable protons, respectively, and k_m is the pseudo first-order kinetic constant of the exchange process of the proton jump from the macromolecule to the water magnetic site. Since T_{2w} is known (~2 s) and it is possible to estimate the parameter P_m from the composition of the sample ($P_m = 0.101, 0.090, 0.070$ for Benz 38, Benz G-45, Benz G-55 hydrogels at their EWC, respectively), the values of either T_{2m} or k_m^{-1} can be determined, provided that the other parameter is known.

Moreover, while a linear relationship of proton spin–spin relaxation time with the reciprocal temperature is expected for water molecules not undergoing proton chemical exchange—the dipolar mechanism being the dominant contribution to relaxation—equation (6) predicts a minimum in a plot of T_{2obs} against $1/T$. Such exchange minima have been reported, for example, for cellulose gels[26] and for cationically charged poly(vinyl alcohol) membranes[27]. Other authors, however, reported a minimum in the temperature dependence of spin–spin relaxation time in similar systems (e.g. in agarose[28,29] and bovine serum albumin gels[30], and in pHEMA hydrogels of different water content[3]), though ascribing it to a diffusive averaging over a distribution of heterogeneous water environments. In correspondence to the minimum, the relationship $T_{2m} = k_m^{-1}$ holds, so that equation (6) simplifies to

$$T_{2obs}^{-1} = T_{2w}^{-1} + P_m/(2T_{2m}^*) \quad (7)$$

or

$$T_{2obs}^{-1} = T_{2w}^{-1} + P_m/(2k_m^{-1*}) \quad (7')$$

Therefore, once the minimum is found, the value of both T_{2m} and k_m at the temperature of the minimum can be easily calculated.

This is exactly what was observed for hydrogels when measurements were carried out at temperatures ranging from 4 to 50°C. In *Figure 2*, the variations of T_{2obs} of the internal water in the above temperature range for Benz 38, Benz G-45 and Benz G-55 hydrogels are reported. The temperature of the minimum for the pure pHEMA hydrogel (about 27°C) is different from the one reported by Smyth *et al.* in the above-mentioned work[3] (about 37°C), a possible explanation being found in a different crosslinking degree or in a different thermal treatment of the sample before n.m.r. measurements (e.g. different hydration temperature or hysteresis effects). The variation of T_2 of external water with the temperature reciprocal is linear, as expected (data not shown), since its transverse relaxation is solely governed

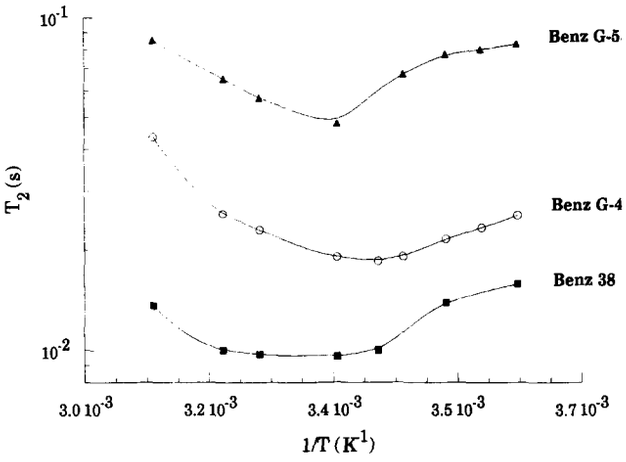

Figure 2 Temperature dependence of transverse relaxation times (T_2) of 'wiped' samples of Benz 38 (filled squares), Benz G-45 (open circles) and Benz G-55 (filled triangles) hydrogels. Solid lines are guides to eyes only

Table 1 Spin–spin relaxation times of external water and internal water at 25°C and of hydroxyl protons in correspondence with the exchange minimum for water-surrounded samples of the three hydrogels

	T_{2ext} (s)	T_{2int} (s)	T_{2m}* (s)
Benz 38	1.56 (± 0.02)	9.6 (± 0.4) × 10^{-3}	4.9 (± 0.2) × 10^{-4}
Benz G-45	1.77 (± 0.02)	19.0 (± 0.4) × 10^{-3}	8.4 (± 0.2) × 10^{-4}
Benz G-55	2.04 (± 0.01)	47.7 (± 0.4) × 10^{-3}	1.73 (± 0.02) × 10^{-3}

by the dipolar contribution. From the T_{2obs} *versus* $1/T$ plots, the temperatures of the minimum, ranging from 21 to 27°C, were determined, and the corresponding $T_{2m}^* = k_m^{-1}*$ values are reported in *Table 1*. The calculated values of spin–spin relaxation times of the exchangeable hydroxyls are related to the mobilities of the macromolecular side chains, clearly indicating that the more hydrated the polymers, the more mobile the gel networks. On the other hand, note that, at a similar temperature, the pseudo first-order exchange rate constant $k_m^* = T_{2m}^{-1}*$ for the proton transfer process becomes higher for systems with a higher fraction of exchangeable protons (Benz 38, $P_m = 0.101$). This may be related to the probability for a water molecule to 'find' a hydroxyl group to exchange the proton with, in the absence of an acid–base catalysis.

It would be interesting to obtain from the above data the temperature dependence of the parameter k_m, but one would have to know the T_{2m} values at the various temperatures. However, for temperatures below the minimum (the right-hand side of the plots reported in *Figure 2*) the exchange process is expected to slow down, so that the k_m^{-1} values become progressively higher than the T_{2m} values, and T_{2obs}, the measured relaxation time in equation (6), is mainly determined by the term k_m^{-1}. Therefore, a good estimate of the temperature dependence of the exchange process kinetic constants can be obtained even when T_{2m} values are not accurately known. In any case, though it should be considered an approximation, we chose to assign to T_{2m} the same relative reduction as was observed for the relaxation time of the external water molecules when decreasing the temperature. Thus, it was possible to estimate T_{2m} values at temperatures below the minimum and hence obtain k_m values in a temperature range of 4–25°C, from which the activation energies for the proton transfer process were derived.

The k_m values obtained at different temperatures are reported in *Figure 3*, together with the best-fit lines from which the activation energies were calculated. These energies, with values of 6.6 ± 1.3, 6.0 ± 0.4 and 4.9 ± 0.9 kcal mol^{-1} for Benz 38, Benz G-45 and Benz G-55 hydrogels, respectively, are of the same order of magnitude as that of a hydrogen bond and in good agreement with those calculated by Watanabe *et al.*[31] in a Sephadex G-25 sample

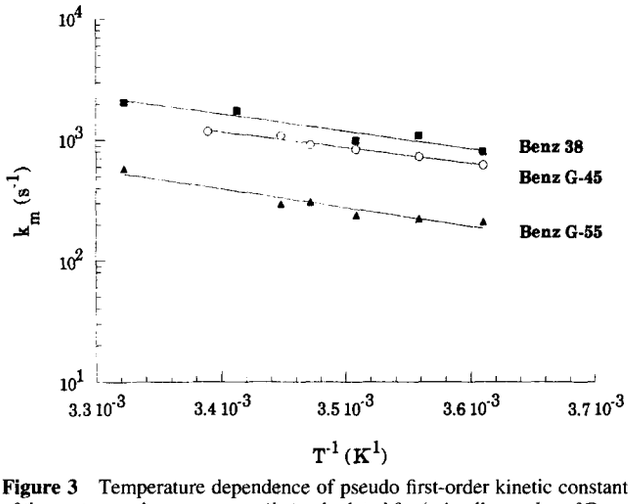

Figure 3 Temperature dependence of pseudo first-order kinetic constant of the proton exchange process (k_m) calculated for 'wiped' samples of Benz 38 (filled squares), Benz G-45 (open circles) and Benz G-55 (filled triangles) hydrogels. Solid lines represent the monoexponential fitting of values from which the activation energies were calculated

(chemically crosslinked dextran) with a 30% water content. As expected, they are therefore likely to be representative of the same chemical process.

Note that, so far, we have not taken into account any contribution from bound water, i.e., water strongly interacting with the polymeric matrix. This class of water, which represents a significant percentage of the total water according to the papers mentioned in 'Introduction', may possibly be present in fast diffusive exchange with molecules in the bulk on a submillisecond timescale[13] (this would explain the reported field dependence for the ^{17}O relaxation showed by ^{17}O-enriched water in pHEMA).

Assuming a fast diffusive exchange between free and bound water and an intermediate chemical exchange between water and polymeric exchangeable protons, in the limit of $P_f + P_b \gg P_m$, the observed relaxation times of water may be accounted for by an equation as follows[32]:

$$T_{2obs}^{-1} = P_f T_{2f}^{-1} + P_b T_{2b}^{-1} + P_m/(T_{2m} + k_m^{-1}) \qquad (8)$$

where P_f and P_b are the proton fractions of free and bound water ($P_f + P_b = P_w \approx 1$) and $T_{2f} = T_{2w}$ and T_{2b} are the relaxation times of free and bound water, respectively. It would be interesting to determine whether the major contribution to relaxation is to be attributed to the second or the third term in the right-hand side of equation (8), i.e., to state whether the observed relaxation time of internal water is dominated by the diffusive exchange phenomenon between free and bound (motionally hindered) water or by the chemical exchange between essentially unperturbed (bulk) water and hydroxyls on the macromolecule.

From a qualitative point of view, note that, according to equations (6) and (8), not taking into account any contribution of bound water would lead to an overestimated k_m value. However, the calculated k_ms (values ranging from 2041 ± 83 to 578 ± 67 s^{-1} at 28°C) are of the same order of magnitude as, or slightly less than, those reported at neutral pH for glucose solutions[33] (1400 s^{-1} at 23°C), methanol–water systems[34] (1800 s^{-1} at 25°C), polysaccharide solutions and gels[16,29] (2200 s^{-1} at 25°C), and for BSA solutions[35] (about 5000 s^{-1} at 23°C). All this seems to support the assumption that such a contribution is negligible.

As previously noted, information on macromolecule hydration, i.e., the presence of bound water molecules, can be gained by performing and analysing ^{17}O relaxation measurements at different magnetic fields[36–38]. Indeed, ^{17}O relaxation is not, or little, affected by the proton exchange process and is mainly caused by the intramolecular rotational modulation of the electric quadrupolar interaction. This accounts for the enhancement of the nuclear relaxation rate (the reciprocal of the relaxation time), to be attributed to the presence of water molecules undergoing slow, possibly anisotropic, reorientation, those molecules being in fast diffusive exchange with the so-called free water. It turns out that, if diffusive exchange between bound and free water is the major relaxation mechanism in such systems, proton and ^{17}O relaxation times would be similarly decreased by the presence of a macromolecule.

To verify this, the excess relaxation rates, R_r, i.e., the enhancement of nuclear relaxation rate relative to pure water, given by the formula[39]

$$R_r = (T_{2obs}^{-1} - T_{2w}^{-1})/T_{2w}^{-1} \qquad (9)$$

were calculated for both ^1H and ^{17}O nuclei, using previously

published data for the latter. The R_r values for water in pHEMA at 25°C are $R_r(^1H) \cong 207$ (present work) and $(^{17}O) \cong 9$ (Roorda *et al.*[13]), thus indicating a major contribution of chemical exchange to proton relaxation. This is suggestive of the possibility for the correlation time values, calculated with no account taken of the chemical exchange process, to be overestimated.

Self-diffusion coefficient measurements

Penetrant diffusion in polymers is a phenomenon of great importance in a variety of processes. For this reason PFG–n.m.r. experiments are of ever-growing relevance in measuring the self-diffusion coefficients of molecules in polymer solutions and gels[26,40–51], allowing the time dependence of the mean square displacement of molecules to be determined and the transport properties of such materials to be investigated.

For 'wiped' samples of the above mentioned pHEMA-based hydrogels, water diffusivity was measured at temperatures of 25°C and 35°C (the latter being the temperature of the corneal surface) by the standard PFG–SE Stejskal–Tanner sequence. The echo attenuation values R, measured either at different diffusion times or gradient amplitudes, lay on the same line on a semilog plot of R *versus* $(\gamma G\delta)^2(\Delta - \delta/3)$ (see example in *Figure 4*). This seems to discard any contribution from other diffusing species, or the presence of barriers to the translational motion of water molecules on a distance scale of a few micrometres (in such conditions, the mean square displacement Δz of the molecules is given by the formula $2Dt = \langle \Delta z \rangle^2$, where $t = (\Delta - \delta/3)$ is the effective diffusion time). The self-diffusion coefficients of water in the different hydrogels, calculated by monoexponential data fitting, are reported in *Table 2*. It is evident that the transport properties of the three hydrogels at their EWC are very different, indicating a faster water diffusion for samples with a higher water percentage, which in turn can be related to the higher DHPMA/HEMA molar ratio of the copolymers. Anyway, self-diffusion in such systems is significantly slower than that of neat water at the same temperature ($D = 2.30 \times 10^{-9}\ m^2\ s^{-1}$)[52]. The absolute value of D for the water contained in pHEMA is somewhat higher than that reported by Wisniewski and Kim[53] and by Peschier *et al.*[15]. This difference can be ascribed either to some differences in the hydrogel sample, as previously discussed, or to a low percentage (2–3%) of bulk water (which is characterised by a higher D value) present on the surface of the samples after their equilibration within the n.m.r. probe, which contributes to the n.m.r. signal, systematically affecting the echo attenuation.

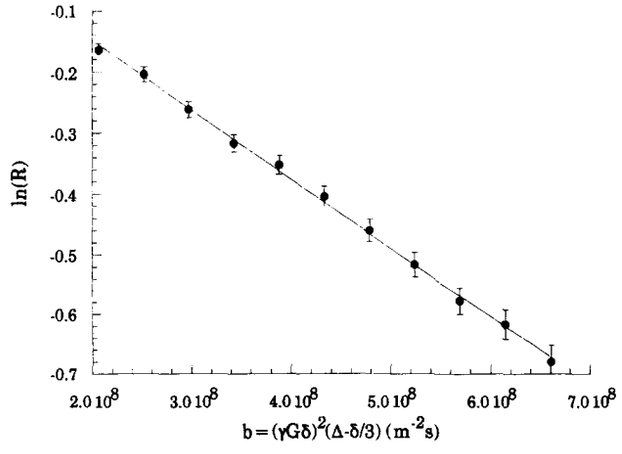

Figure 4 Plot of the echo attenuation ratio (R) *versus* $b = (\gamma G\delta)^2(\Delta - \delta/3)$ of water in a 'wiped' sample of Benz G-55 hydrogel. Values were obtained by a Δ-incremented PFG–SE experiment with $G = 1.3\ T\ m^{-1}$ and $\delta = 500\ \mu s$, at a temperature of 35°C. Solid line represents the monoexponential fitting of the data from which the self-diffusion coefficient was calculated

Table 2 Water self-diffusion coefficients measured at temperatures of 25°C and 35°C for the three hydrogels

	$D_{25°C}$ ($m^2\ s^{-1}$)	$D_{35°C}$ ($m^2\ s^{-1}$)
Benz 38	$0.44\ (\pm 0.01) \times 10^{-9}$	$0.59\ (\pm 0.02) \times 10^{-9}$
Benz G-45	$0.76\ (\pm 0.02) \times 10^{-9}$	$0.91\ (\pm 0.02) \times 10^{-9}$
Benz G-55	$0.93\ (\pm 0.02) \times 10^{-9}$	$1.12\ (\pm 0.02) \times 10^{-9}$

Data are obtained by keeping G constant and by varying Δ in the PFG–SE sequence.

In compartmentalised systems, such as our 'wet' samples (i.e., hydrogels surrounded by substantial amounts of bulk water), the water self-diffusion coefficients cannot be easily measured by PFG–SE or –STE (STimulated Echo) because the n.m.r. signal is the sum of the signals corresponding to water molecules within all compartments. Thus, the measured D is an average value of the different diffusion constants of molecules belonging to the different compartments, weighted by the fraction of water in each compartment. While in a few situations a multiexponential fitting of the echo attenuation curve allows the diffusion coefficients to be calculated, generally speaking, and in our particular case, a biexponential fitting of the attenuation ratio is not possible. This is because the number of the experimental points is limited and self-diffusion coefficients of external water is no more than 5 times greater than that of internal water in the most favourable situation ('wet' Benz 38 sample). In principle, if the two water compartments are well defined and of suitable size, such a problem can be overcome by the acquisition of a series of diffusion-weighted images of a sample suitable slice on an n.m.r.imaging spectrometer and a monoexponential fitting on a pixel-by-pixel basis. However, this approach is made difficult by the high cost of the available n.m.r. equipment.

Therefore, the recently proposed PFG–MSE techniques[19] and the subsequent DARTS analysis[18] were used to measure water diffusivity in water-surrounded hydrogels. Such an approach, described in 'Experimental', takes advantage of the possibility of simply and independently varying the effective diffusion time ($\Delta - \delta/3$) and the echo time (TE) in a CPMG-like sequence such as PFG–MSE. Thus, the overall echo amplitude is made dependent not only on the diffusion constants of the different species, but also on their concentrations and spin–spin relaxation times. Such a dependence causes the apparent diffusion constants (D_{app}) to be affected by the time at which the echo amplitude is sampled, so that the self-diffusion coefficients of the different species can be calculated.

The apparent self-diffusion coefficients D_{app} were measured at 20°C by the PFG–MSE ($2\tau = 1$ ms) sequence at various echo times, whereas the signal fractions and relaxation times of internal and external water were determined by a CPMG sequence at the same pulse spacing. The results are reported in *Figures 5* and *6* (semilog plot of R *versus* $(\gamma G\delta)^2(\Delta - \delta/3)$) and *Tables 3* and *4* (signal fractions) for Benz G-55 and Benz G-45 samples, respectively. *Tables 3 and 4* also report the self-diffusion coefficient values calculated by means of DARTS analysis.

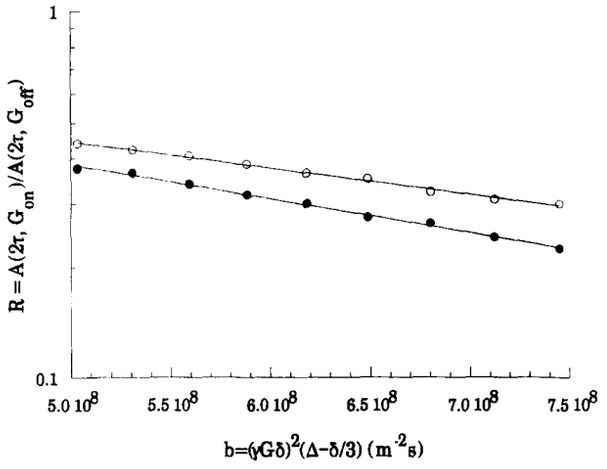

Figure 5 Plot of the echo attenuation ratio (R) *versus* $b = (\gamma G\delta)^2(\Delta - \delta/3)$ of water in a 'wet' sample of Benz G-55 hydrogel. Values were obtained at a temperature of 20°C by a G-incremented PFG–MSE experiment with $\Delta = 6$ ms and $\delta = 1.732$ ms. Open and filled circles refer to values obtained at $TE = 26$ ms and $TE = 190$ ms, respectively. Solid lines represent the monoexponential fittings of the data from which the apparent self-diffusion coefficients (D_{app}) were calculated

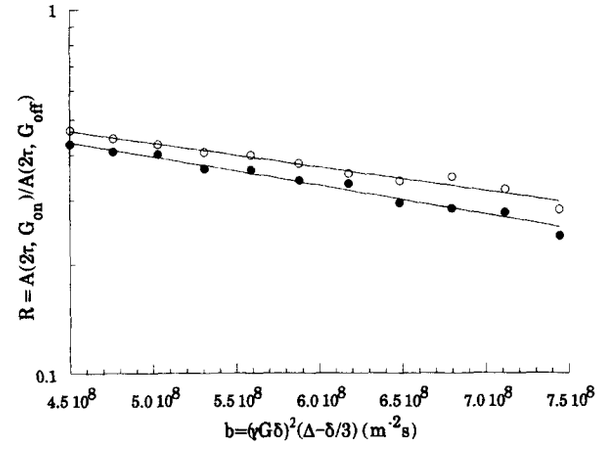

Figure 6 Plot of the echo attenuation ratio (R) *versus* b $(\gamma G\delta)^2(\Delta - \delta/3)$ of water in a 'wet' sample of Benz G-45 hydrogel. Values were obtained at a temperature of 20°C from a G-incremented PFG–MSE experiment with $\Delta = 6$ ms and $\delta = 1.732$ ms. Open and filled circles refer to values obtained at $TE = 18$ ms and $TE = 70$ ms, respectively. Solid lines represent the monoexponential fittings of the data from which the apparent self-diffusion coefficients (D_{app}) were calculated

Measurements on Benz 38 samples were not performed because the relaxation time value of the internal water was too low, leading to a dramatic T_2 attenuation even at the lowest echo time.

Note that, despite the poor precision of D measurements (the relative standard deviation being up to 17% for external water and up to 75% for internal water), the results obtained are in agreement with the known value[52] of self-diffusion coefficient for neat water at 20°C (2.02×10^{-9} m^2 s^{-1}) and with the previously measured D of internal water when a correction factor for the temperature difference is applied (leading to $D_{int} \approx 0.80 \times 10^{-9}$ m^2 s^{-1} for Benz G-55 and $D_{int} \approx 0.66 \times 10^{-9}$ m^2 s^{-1} for Benz G-45). As expected, the relative standard deviation of the measurements is higher for systems with a higher D_{ext}/D_{int} ratio and containing a fast-relaxing component, due to a less favourable signal-to-noise ratio in both CPMG and PFG–MSE techniques and, hence, poorer precision in signal amplitude and D_{app} values.

CONCLUSIONS

From the experimental results of water spin–spin relaxation measurements it can be concluded that, in water-rich hydrogels containing exchangeable protons, the chemical exchange process is to be considered as an important source of transverse relaxation. The appearance of a characteristic T_2 minimum when the sample temperature is varied confirms this assumption. The quantitative analysis of T_2 data, carried out taking into account the chemical exchange process, allows the calculation of the pseudo first-order kinetic constants of the proton jump process between exchangeable groups of a macromolecule and water, as well as the estimation of the relaxation times of the macromolecular hydroxyl-carrying moieties. In this respect, it has been demonstrated that the more hydrated the polymers, the more mobile the side-chains, i.e., the more plasticised are the materials. Conversely, the activation energies for the chemical exchange process have been shown not to vary, their values being of the same order of magnitude as that of a hydrogen bond. The analysis of our proton data and of those reported by Roorda *et al.*[13] for ^{17}O relaxation does not seem to support the conclusion drawn by some authors[6,11] that diffusive exchange between bulk water and more or less strongly bound water pools is responsible for the dramatic lowering of T_2 values, as observed in the hydrogels under study. Note that these authors mostly based their conclusions on spin–lattice relaxation measurements, taking into account neither chemical exchange (which is found to affect the spin–lattice relaxation measurements[54]) nor cross-relaxation, i.e., the transfer of longitudinal magnetisation between the various proton pools in these systems, a phenomenon observed in pHEMA hydrogels[3,55].

PFG–n.m.r. measurements evidenced the different transport properties of the three hydrogels. While we have demonstrated that water possesses faster translational motions in hydrogels with a higher EWC, we did not go into the details of the concentration dependence of the D/D_0 ratio (D_0 is the self-diffusion coefficient of neat water), this being beyond the aim of this work. However, it will be interesting to carry out a study on this matter, using, for instance, either the free-volume theory or the kinetic theory[56]. The PFG–MSE sequence was applied to 'wet' hydrogel samples and the results were analysed by the DARTS approach to simultaneously calculate the self-diffusion coefficients of both external and internal water.

Table 3 Apparent self-diffusion coefficients, signal fractions and calculated diffusion coefficients (by DARTS—see text) of internal and external water of a 'wet' sample of Benz G-55 hydrogel

	D_{app} (m^2 s^{-1})	f_{int}	f_{ext}
TE = 26 ms	1.66 (± 0.04) × 10^{-9}	0.341 (± 0.002)	0.659 (± 0.002)
TE = 190 ms	2.10 (± 0.06) × 10^{-9}	0.002 (± 0.002)	0.998 (± 0.002)
		D_{int} (m^2 s^{-1})	D_{ext} (m^2 s^{-1})
		0.8 (± 0.2) × 10^{-9}	2.1 (± 0.2) × 10^{-9}

Table 4 Apparent self-diffusion coefficients, signal fractions and calculated diffusion coefficients (by DARTS—see text) of internal and external water of a 'wet' sample of Benz G-45 hydrogel

	D_{app} ($m^2 s^{-1}$)	f_{int}	f_{ext}
TE = 18 ms	$1.59 (\pm 0.08) \times 10^{-9}$	0.166 (± 0.003)	0.834 (± 0.003)
TE = 70 ms	$1.82 (\pm 0.09) \times 10^{-9}$	0.001 (± 0.003)	0.999 (± 0.003)
		D_{int} ($m^2 s^{-1}$)	D_{ext} ($m^2 s^{-1}$)
		$0.4 (\pm 0.3) \times 10^{-9}$	$1.8 (\pm 0.3) \times 10^{-9}$

The D values obtained for external water are in agreement with the known self-diffusion coefficients of neat water, while those concerning internal water are consistent, within the experimental errors, with the values previously measured by the PFG–SE technique on 'wiped' samples. The D values calculated by this method are affected by uncertainties far greater than those affecting PFG–SE; nonetheless, this approach overcomes the problems arising with sample heterogeneity and allows water diffusivity to be determined in sample conditions more resembling those of its application as biomaterial.

REFERENCES

1. Montheard, J. P., Chatzopoulos, M. and Chappard, D., *J. Macromol. Sci.—Rev. Macromol. Chem. Phys.*, 1992, **C32**, 1.
2. Corkill, P. H., Jolly, A. M., Ng, C. O. and Tighe, B., *J. Polymer*, 1987, **28**, 1758.
3. Smyth, G., Quinn, F. X. and McBrierty, J., *Macromolecules*, 1988, **21**, 3198.
4. Lee, H. B., Jhon, M. S. and Andrade, J. D., *J. Coll. Interf. Sci.*, 1975, **51**, 225.
5. Allen, P. E. M., Bennett, D. J. and Williams, D. R. G., *Eur. Polym. J.*, 1993, **29**, 231.
6. Sung, Y. K., Gregonis, D. E., Jhon, M. S. and Andrade, J. D., *J. Appl. Polym. Sci.*, 1981, **26**, 3719.
7. Bouwstra, J. A., Salomons-de Vries, M. A. and van Miltenburg, J. C., *Thermochimica Acta*, 1995, **248**, 319.
8. Roorda, W. E., Bouwstra, J. A., de Vries, M. A. and Junginger, H. E., *Biomaterials*, 1988, **9**, 494.
9. Khare, A. R. and Peppas, N. A., *Polymer*, 1993, **34**, 4736.
10. Roorda, W. E., Bouwstra, J. A., de Vries, M. A. and Junginger, H. E., *Pharm. Res.*, 1988, **5**, 722.
11. Yamada-Nosaka, A., Ishikiriyama, K., Todoki, M. and Tanzawa, H., *J. Appl. Polym. Sci.*, 1990, **39**, 2443.
12. Quinn, F. X., McBrierty, J., Wilson, A. C. and Friends, G. D., *Macromolecules*, 1990, **23**, 4576.
13. Roorda, W. E., de Bleyser, J., Junginger, H. E. and Leyte, J. C., *Biomaterials*, 1990, **11**, 17.
14. Choi, S., Jhon, M. S. and Andrade, J. D., *J. Coll. Interf. Sci.*, 1977, **61**, 1.
15. Peschier, L. J. C., Bouwstra, J. A., de Bleyser, J., Junginger, H. E. and Leyte, J. C., *Biomaterials*, 1993, **14**, 945.
16. Hills, B. P., Cano, C. and Belton, P. S., *Macromolecules*, 1991, **24**, 2944.
17. Hills, B. P., Wright, K. M. and Belton, P. S., *Molec. Phys.*, 1989, **67**, 1309.
18. van Dusschoten, D., de Jager, P. A. and Van As, H., *J. Magn. Reson., Ser. A*, 1995, **116**, 22.
19. van Dusschoten, D., de Jager, P. A. and Van As, H., *J. Magn. Reson., Ser. A*, 1995, **112**, 237.
20. Brosio, E. and Barbieri, R., *Rev. Anal. Chem.*, 1996, **15**, 273.
21. Refojo, M. F. and Yasuda, H., *J. Appl. Polym. Sci.*, 1965, **9**, 2425.
22. Meiboom, S. and Gill, D., *Rev. Sci. Instrum.*, 1958, **29**, 688.
23. Marquardt, D. W., *J. Soc. Ind. Appl. Math.*, 1963, **11**, 431.
24. Stejskal, E. O. and Tanner, J. E., *J. Chem. Phys.*, 1965, **42**, 288.
25. Carver, J. P. and Richards, R. E., *J. Magn. Res.*, 1972, **6**, 89.
26. Nyström, B., Moseley, M. E., Brown, W. and Roots, J., *J. Appl. Polym. Sci.*, 1981, **26**, 3385.
27. Fushimi, H., Ando, I. and Iijima, T., *Polymer*, 1991, **32**, 241.
28. Woessner, D. E. and Snowden, J. B. S., *J. Coll. Interf. Sci.*, 1970, **34**, 290.
29. Derbyshire, W. and Duff, I. D., *Faraday Disc. Chem. Soc.*, 1974, **57**, 243.
30. Oakes, J., *J. Chem. Soc. Faraday Trans. I*, 1976, **72**, 228.
31. Watanabe, T., Murase, N., Staemmler, M. and Gersonde, K., *Magn. Reson. Med.*, 1992, **27**, 118.
32. Lewis, G. P. and Derbyshire, W., *Carbohyd. Res.*, 1987, **160**, 397.
33. Hills, B. P., *Mol. Phys.*, 1991, **72**, 1099.
34. Hills, B. P. J., *Chem. Soc. Faraday Trans.*, 1990, **86**, 481.
35. Hills, B. P., *Mol. Phys.*, 1989, **67**, 903.
36. Halle, B., Anderson, T., Forsen, S. and Lindman, B., *J. Am. Chem. Soc.*, 1981, **103**, 500.
37. Belton, P. S., Ring, S. G., Botham, R. L. and Hills, B. P., *Mol. Phys.*, 1991, **72**, 1123.
38. Mulder, C. W. R., Schriever, J., Jesse, W. J. and Leyte, J. C., *J. Phys. Chem.*, 1983, **87**, 2342.
39. Hills, B. P., Takacs, S. F. and Belton, P. S., *Food Chem.*, 1990, **37**, 95.
40. Matsukawa, S. and Ando, I., *Macromolecules*, 1996, **29**, 7136.
41. Ilyina, E. and Daragan, V., *Macromolecules*, 1994, **27**, 3759.
42. Kim, D., Caruthers, J. M., Peppas, N. A. and von Meerwall, E., *Polymer*, 1994, **51**, 661.
43. Korsmeyer, R. W., von Meerwall, E. and Peppas, N. A., *J. Polym. Sci.: Polym. Phys. Ed.*, 1986, **24**, 409.
44. Yasunaga, H. and Ando, I., *Polym. Gels & Networks*, 1993, **1**, 83.
45. Yasunaga, H. and Ando, I., *Polym. Gels & Networks*, 1993, **1**, 267.
46. Blum, F. D., Durairaj, B. and Padmanabhan, A. S., *J. Polym. Sci.: Part B: Polym. Phys.*, 1986, **24**, 493.
47. von Meerwall, E. D., *Adv. Polym. Sci.*, 1984, **54**, 1.
48. Nose, T., *Ann. Rep. NMR Spectr.*, 1993, **27**, 217.
49. Griffiths, L., Horton, R., Parker, I. and Rowe, R. C., *J. Coll. Interf. Sci.*, 1992, **154**, 238.
50. Björling, M., Herslöf-Björling, A. and Stilbs, P., *Macromolecules*, 1995, **28**, 6970.
51. Brosio, E., D'Ubaldo, A. and Verzegnassi, B., *Cell. Mol. Biol.*, 1994, **40**, 569.
52. Mills, R., *J. Phys. Chem.*, 1973, **77**, 685.
53. Wisniewski, S. and Kim, S. W., *J. Membrane Sci.*, 1980, **6**, 309.
54. Hills, B. P., *Mol. Phys.*, 1992, **76**, 489.
55. Peschier, L. J. C., Bouwstra, J. A., de Bleyser, J., Junginger, H. E. and Leyte, J. C., *J. Magn. Res., Ser. B*, 1996, **110**, 150.
56. Waggoner, R. A., Blum, F. D. and MacElroy, J. M. D., *Macromolecules*, 1993, **26**, 6841.