diffusion-fundamentals

The Open-Access Journal for the Basic Principles of Diffusion Theory, Experiment and Application

NMR measurement of small-molecule diffusion in PVA hydrogels: a comparison of CONVEX and standard PGSE methods

*[§]Regan, D. G., [†⊗]Momot, K. I., *Martens, P. J., [†]Kuchel, P. W., *Poole-Warren, L. A.

*Graduate School of Biomedical Engineering, University of New South Wales, NSW 2052, Australia

[†]School of Molecular and Microbial Biosciences, University of Sydney, NSW2006, Australia

Keywords: NMR solvent suppression; phenylalanine; poly(vinyl alcohol); Stejskal-Tanner plot; diffusion in a polymer network; metabolite transport in tissue scaffolds

[§]Current Address and address for correspondence:

David Regan

National Centre in HIV Epidemiology and Clinical Research

The University of New South Wales

376 Victoria Street, Darlinghurst

NSW 2010, Australia

Email dregan@nchecr.unsw.edu.au

[⊗]Current address:

Konstantin Momot, School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane, Qld 4001, Australia

Abstract

Hydrogels are biocompatible polymeric materials that are becoming increasingly important in biomedical applications, such as drug delivery and tissue engineering. Understanding of small-molecule diffusion in these systems is important in the contexts of controlled drug release; transport of nutrients (e.g., O_2 and growth factors) into the gel; and transport of cellular waste out of the gel. In this work, the diffusion coefficient of the aromatic amino acid phenylalanine (Phe) in non-crosslinked and crosslinked poly(vinyl alcohol) (PVA) hydrogels was measured using two NMR diffusion methods, CONVEX and the standard pulsed-gradient spin-echo (PGSE). Pulsed field-gradient (PFG) NMR measurements provide the advantage of measuring the molecular self-diffusion coefficient directly and without having to rely on the physical release of the solute, but are often difficult to perform in tissues and hydrated polymers due to a large water signal. CONVEX is a recently proposed diffusion method that alleviates this problem by means of NMR excitation-sculpting water suppression. In the measurements presented here, CONVEX results were superior to those from PGSE measurements with respect to every test applied, and enabled a reliable comparison of the diffusion coefficients of Phe in crosslinked and non-crosslinked hydrogels. The value of D(Phe) was smaller in the non-crosslinked hydrogel than in the crosslinked gel; this finding is discussed in the paper.

Diffusion Fundamentals 4 (2006) 1.1 - 1.18

Introduction

Hydrogels are insoluble, water-swellable networks that can be made from a variety of hydrophilic polymers. Poly(vinyl alcohol) (PVA) hydrogels are becoming increasingly important in a wide range of biomedical applications due to their "tissue-like" properties, high hydrophilicity, the ease of controlled crosslinking, and the absence of low molecular-weight post-production by-products [1-5]. An understanding of solute and water transport within these materials is crucial for the rational design of successful candidates for practical applications such as drug delivery and tissue engineering [6-8].

NMR enables the non-invasive measurement of molecular diffusion over a wide range of time scales (from milliseconds to seconds) [9-11]. For heterogeneous materials, microstructural information can often be obtained by studying the dependence of the apparent diffusion coefficient on the observation time [12-14].

Proton-based diffusion measurements are the most common, due to the natural abundance and high NMR receptivity of the ^{1}H nucleus. However, in systems with high water content (such as hydrogels), a number of obstacles render ^{1}H NMR diffusion measurements of low-concentration solutes technically complicated. The large water signal can produce baseline distortions or ghost images, can limit digitisation of the signal of interest, and can result in significant NMR radiation damping effects [15]. These factors can severely limit the ability to measure accurately diffusion coefficients of low-concentration solutes in non-deuterated aqueous systems. Substitution of H_2O with D_2O is a commonly used approach for reducing the adverse effects of the dominant water signal, but in biological or biocompatible systems, solvent replacement can be either expensive or undesirable. In this case, NMR solvent suppression is often required

for quantitative measurements of small solute peaks [15]. While crude techniques such as presaturation tend to cause baseline and phase distortions, a number of other techniques have been proposed that enable the suppression of the solvent peak without introducing artifacts in the acquired spectra [16-21]. CONVEX [20] (see Fig. 1) is a recently proposed NMR diffusion experiment incorporating double-echo excitation sculpting [22]. It is a particularly effective technique for solvent suppression that produces spectra with pure phase and undistorted baseline.

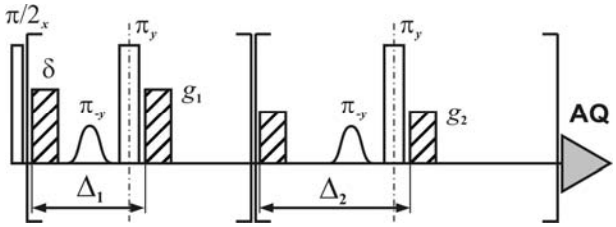

Figure 1. CONVEX pulse sequence [20]. The two bracketed intervals represent two spin-echo blocks. Non-selective π pulses (open rectangles) are centered within the spin-echo blocks. Selective π pulses (open curves) are applied at the resonance frequency of the water signal, and their power is calibrated for optimal water suppression at a small (but non-zero) gradient amplitude. The amplitudes of the gradient pulse pairs (hatched rectangles) are inversely related to the respective echo times: $g_2:g_1 = \Delta_1:\Delta_2 = C$.

An understanding of the diffusion behaviour of amino acids in hydrogels is important in a number of contexts. In solid state protein synthesis, for example, the diffusion of functionalised amino acids is often the rate-limiting step [23]. The swelling behaviour of hydrogels has been shown to be influenced, in a pH-dependent manner, by some aromatic amino acids including phenylalanine [24]. The effective diffusion coefficient of a wide range of amino acids has been used as a measure of the strength of interaction between polymer networks and the guest molecules [25]. The permeability of hydrogel membranes to small molecules, proteins and water has been measured in the context of artificial skin and corneal prostheses [26].

In this study we measure the diffusion of phenylalanine (Phe) in non-crosslinked and crosslinked PVA hydrogels. Phenylalanine was selected as it is an amino acid with an aromatic side-chain which gives rise to a well-resolved signal envelope in the NMR spectrum that is separated from that of the hydrogel (see Fig. 2). We show that CONVEX is effective for measuring small-molecule diffusion in hydrogels; it produces undistorted diffusion spectra and yields diffusion plots that are linear over a greater attenuation range than the standard pulsed-gradient spin-echo (PGSE) technique.

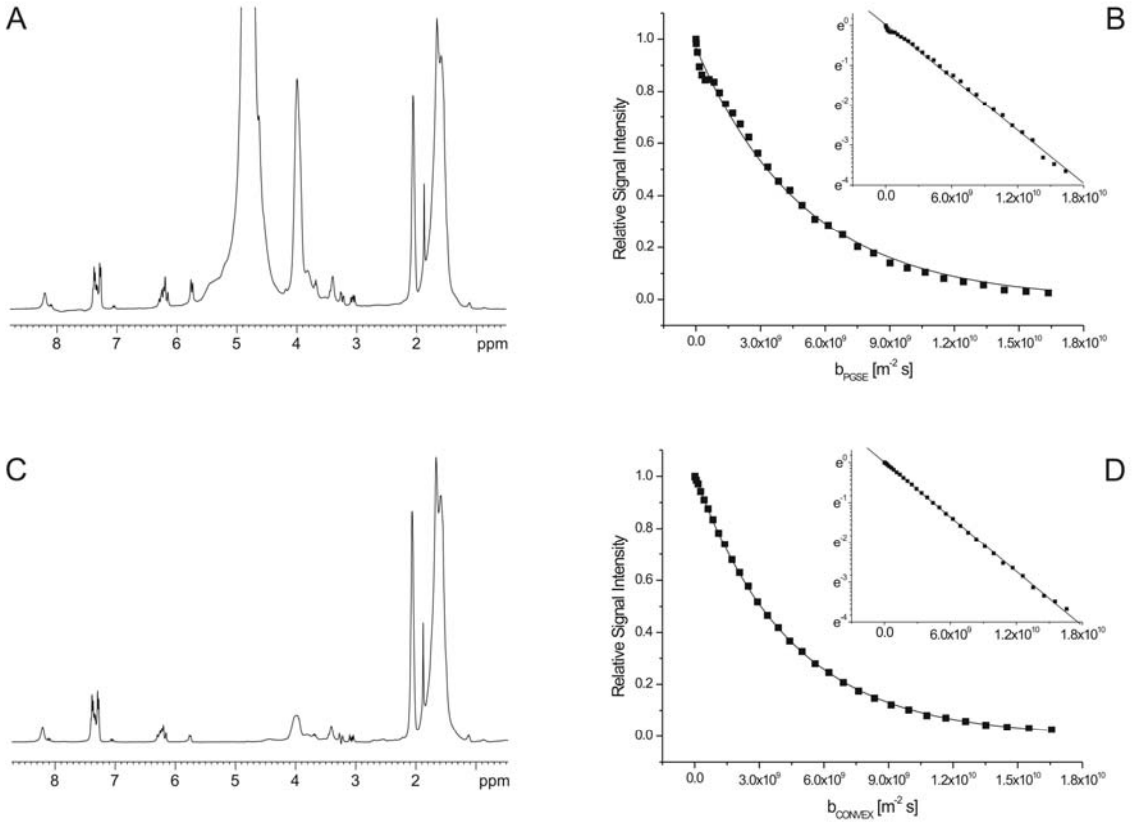

Figure 2. Sample spectra and the results of data fitting for the non-crosslinked sample: (A) sample PGSE spectrum; (B) curvilinear fit and (inset) linearised Stejskal–Tanner fit of the PGSE data; (C) and (D), same as (A) and (B), but for the CONVEX data. The phenylalanine resonance envelope is centered at ~7.3 ppm, water is at 4.7 ppm, and the main PVA resonances are centered at ~3.9 (C*H*-OH) and 1.5 ppm (CH_2). Other resonances are due to the photoinitiators, acrylate functional groups, and impurities.

Overview of PGSE and CONVEX

PGSE has long been the standard NMR method for measuring molecular diffusion [27-30]. The method is based on sensitising the sample to molecular translational displacement by the application of magnetic field-gradient pulses. Molecular diffusion results in the attenuation of the refocused echo signal whose relative amplitude in isotropic solution is given by [28]:

$$S(g) = S(0)e^{-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)}, \quad (1)$$

where D is the diffusion coefficient, γ is the magnetogyric ratio, g is the applied magnetic field-gradient strength, Δ is the time interval separating the gradient pulses (the effective diffusion interval) and δ is the gradient pulse duration. For isotropic diffusion, the self-diffusion coefficient can be obtained by non-linear fitting of Eq. (1) to the data, or by calculating the negative slope of the regression line of the Stejskal-Tanner plot, which is obtained by plotting the natural logarithm of the relative signal intensities as a function of $b_{PGSE} = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$.

The CONVEX pulse sequence is shown in Fig. 1. Each bracketed interval is a spin-echo block that includes two gradient pulses (δ), a hard 180° pulse (π_y), and a soft 180° pulse (π_{-y}). The water peak is placed on-resonance and experiences both the hard and the soft 180° pulses, with the net rotation angle being zero. Off-resonance peaks experience only the hard 180° pulses. The on-resonance water signal is therefore not refocused by the spin-echo block. As shown by Hwang and Shaka [22], a double application of this block (double-echo excitation sculpting) results in a suppression efficiency that is the square of that of a single spin-echo. Even more importantly, it produces a NMR spectrum that

is free of phase or baseline distortions. The duration of the soft 180^o pulse is usually selected to minimize the spectral width of the suppressed region, and its power level is optimized to produce the largest attenuation of the suppressed signal.

In CONVEX, double-echo excitation sculpting water suppression is combined with an NMR diffusion measurement. The two functions rely on the same spin-echoes; therefore, the same pulse sequence is used to provide water suppression and to measure the diffusion attenuation of off-resonance signals [20]. The amplitudes of the gradient pairs g_1, g_2 and the diffusion intervals Δ_1, Δ_2, which appear in Fig. 1, are related as g_2:g_1 = Δ_1:Δ_2 = C. In a multi-FID diffusion measurement, the time intervals and C are kept constant; g_1 is incremented; and g_2 is incremented as Cg_1. The diffusion attenuation of off-resonance peaks is then given by

$$S(g) = S(0)e^{-D\gamma^2 g_1^2 \delta^2 \left[\Delta_1(1+C) - \frac{\delta(1+C^2)}{3}\right]}. \qquad (2)$$

The diffusion coefficient is determined from the non-linear fit of Eq. (2) to the data, or as the negative slope of the Stejskal-Tanner plot where $b_{CONVEX} = \gamma^2 g_1^2 \delta^2 [\Delta_1(1+C) - \delta(1+C^2)/3]$.

Materials and Methods

PVA preparation

PVA with a weight-average MW of 14,000 g/mol and 83% hydrolysis was used as supplied by Clariant. Hydrochloric acid and sodium hydroxide (BDH Chemicals, Kilsyth, Victoria, Australia) were also used without further purification. The photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone,

(Irgacure 2959, Ciba Specialty Chemicals, Melbourne, Victoria, Australia) was used as supplied at a 0.1 wt% concentration in all formulations. N-(2,2-dimethoxyethyl) acrylamide was synthesised in house [31].

An acrylamide-modified PVA was synthesized by the reaction of the pendant alcohol groups on the PVA with N-(2,2-dimethoxyethyl) acrylamide in an acidic aqueous environment [32]. A 40 wt% solution of N-(2,2-dimethoxyethyl) acrylamide in water (calculated to yield 7 acrylamides per PVA macromer) was added to a 20 wt% solution of PVA in water with stirring. Hydrochloric acid (37%) was added and the reaction allowed to proceed for 22 hours at room temperature. The solution was adjusted to pH 7 with sodium hydroxide and the excess salts were removed by ultrafiltration (Millipore, Amicon 10,000 MW cutoff filter, Bedford, MA). The mixture was freeze-dried and stored at room temperature in the dried state.

Sample preparation

Lyophilised Phe (BDH Chemicals Ltd, Poole, England) was dissolved in distilled H_2O to yield a 1 wt% solution. Functionalized PVA was re-dissolved in this solution, at 80 ^oC, to yield a 20 wt% PVA solution. Upon cooling, the photoinitiator, Irgacure 2959, was dissolved in the solution at a concentration of 0.1 wt%.

The final solution was loaded into 5mm NMR tubes, with magnetic susceptibility matched to that of H_2O (Shigemi, Allison Park, PA), to a sample height of 1 cm. Photopolymerisation (crosslinking) was achieved by exposing the sample-containing region of the tubes to an ultraviolet light source (Green Spot UV, UV Source, Torrance, CA) for 90 sec on two sides.

NMR

All experiments were conducted on a Bruker DRX-400 NMR spectrometer equipped with a single-axis (z) diffusion probe and gradient amplifier capable of delivering a maximum field-gradient of 9.8 T m^{-1}. Experiments were conducted at 20 ^{o}C, calibrated using the chemical shift of the ^{1}H ethylene-glycol doublet [33]. Gradients were calibrated using the known diffusion coefficient of water at 20 ^{o}C [34].

Diffusion measurements were made using PGSE and CONVEX pulse sequences as previously described [20]. For PGSE experiments, trapezoidal gradient pulses with ramp times τ = 0.1 ms and duration δ = 1 ms were used. For CONVEX experiments, selective Gaussian soft-π pulses of 2 ms duration, with power optimised for maximum water suppression, were used. For all experiments, rf pulse separation was Δ = 5 ms, and gradient strength was varied between 0 and 5 T m^{-1} in 32 steps with 4 transients per spectrum and a 15 ms repetition delay.

Data analysis

Integration of spectral peaks was performed using the Bruker-supplied XWin-NMR software. Data were plotted and diffusion coefficients calculated by both non-linear and linearised fitting according to Eq. (1) for PGSE and Eq. (2) for CONVEX. The fitting was done using Origin Pro software (OriginLab, Northampton, MA); the standard errors and the residuals were obtained from the fitting routines.

For each set of results, the accuracy of integration of the NMR signal, Δy, was assumed to be uniform, i.e., independent of the amplitude of the signal. This accuracy was determined for each data set from the respective histograms of the residuals of the curvilinear fits of the non-transformed data (signal *vs* gradient strength). For the

linearised Stejskal–Tanner plots, the error envelopes for the logarithmic data were calculated as $\Delta(\ln y) = \Delta y/y$, in accordance with the standard expression for the propagation of random errors [35].

Results

Diffusion coefficients of Phe were measured in non-crosslinked and crosslinked PVA hydrogel samples using both standard spin-echo (PGSE) and CONVEX techniques. Representative results are shown graphically in Figs. 2 and 3; a comprehensive quantitative summary is presented in Table 1.

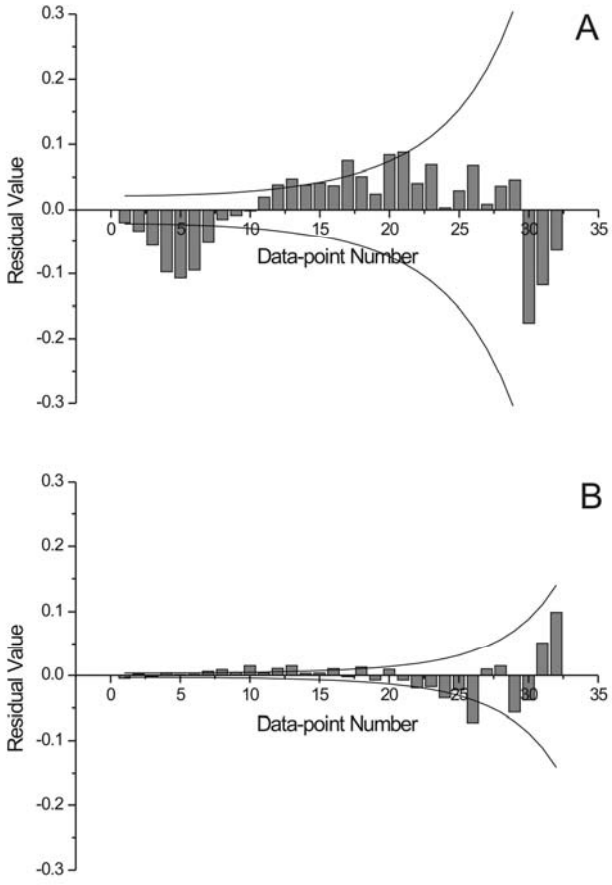

Figure 3. Fitting residuals and error envelopes for the linearised fit of the PGSE data (A) and CONVEX data (B).

Table 1. Measured diffusion coefficients of phenylalanine in non-crosslinked (NCL) and crosslinked (CL) PVA, calculated from untransformed ($D_{U)}$ and linearised (D_L) data, together with the standard error (SE) and sum of squared residuals (SSR) associated with the fits.

Sample	**Pulse Sequence**	$\boldsymbol{D}_U \times 10^{10}$ $m^2\ s^{-1}$	**SE** $\times 10^{12}$	**SSR**	$\boldsymbol{D}_L \times 10^{10}$ $m^2\ s^{-1}$	**SE** $\times 10^{12}$	**SSR**
NCL	PGSE	2.00	4.0	0.015	2.20	2.3	0.13
	CONVEX	2.27	0.8	4.2×10^{-4}	2.28	1.0	0.026
CL	PGSE	2.13	3.9	0.012	2.30	1.6	0.064
	CONVEX	2.45	1.1	7.4×10^{-4}	2.44	0.7	0.013

The diffusion coefficient of Phe in pure water at a concentration of 1 wt % was measured using CONVEX, and a value of $(5.70 \pm 0.02) \times 10^{-10}$ $m^2\ s^{-1}$ was obtained.

The diffusion coefficient of water was measured in the non-crosslinked and crosslinked hydrogels using PGSE, and values of $(1.23 \pm 0.01) \times 10^{-9}$ $m^2\ s^{-1}$ and $(1.16 \pm 0.01) \times 10^{-9}$ $m^2\ s^{-1}$ were obtained, respectively.

Figure 2 shows representative NMR spectra and the fits of the echo attenuation for Phe, from both untransformed and linearised data, for the non-crosslinked hydrogel. Figure 3 shows the distribution of the linearised fit residuals of the PGSE and CONVEX results for the non-crosslinked sample.

Table 1 shows the measured diffusion coefficients of Phe, their respective standard errors, and the sums of squared residuals obtained for the non-crosslinked and crosslinked hydrogel samples using both standard PGSE and CONVEX. From the Table it is evident that: 1) there was a closer agreement between diffusion coefficients of Phe

obtained using non-linear and linear fitting from CONVEX than from PGSE, in both non-crosslinked and crosslinked samples; 2) the standard errors were more than a factor of 2 (linear fit) and 3 (non-linear fit) larger for PGSE than for CONVEX, in both samples; 3) the sums of the squared residuals for the PGSE data were larger by a factor of approximately 5 (linear fit) and 20 (non-linear fit) than for CONVEX, in both samples.

It can also be seen from Table 1 that the diffusion coefficients obtained using CONVEX were approximately 5% (linear fit) and 15 % (non-linear) larger than those obtained using spin-echo, for both samples. However, independent of the measuring method, the diffusion coefficients of Phe were 5-10% larger in the crosslinked sample than in the non-crosslinked sample.

Longitudinal relaxation of hydrogel protons was measured in both samples from the peaks at 3.9 ppm (assigned as tertiary PVA protons adjacent to hydroxyl/ester oxygens); 2 ppm (assigned as methyl groups of the acetate); and 1.5 ppm (assigned as secondary protons of the PVA chains) [36,37]. In both samples, the longitudinal relaxation of each of the three signals deviated markedly from single-exponential behaviour, but there was no significant dependence on the crosslinking state of the hydrogel.

Discussion

Figure 2 provides a qualitative comparison of the results obtained from PGSE and CONVEX. Table 1 provides a direct comparison of the values obtained for the diffusion coefficients and the errors associated with the fits used to calculate these values. CONVEX spectra (Fig. 2C) had flatter baselines and less phase distortion than PGSE

spectra (Fig. 2A). The improvement afforded by CONVEX is also qualitatively apparent from the non-linear and linear regression plots; in the latter, the linearised CONVEX data display visual linearity over a greater attenuation range than the PGSE data. It should be noted, however, that CONVEX solvent suppression is frequency-selective and has the capacity to suppress peaks lying very close to, or overlapping with, the solvent peak. In systems where this is the case, alternative water suppression approaches in diffusion measurements include frequency-independent techniques such as double-quantum filtering [21].

Table 1 shows that while diffusion coefficients calculated from CONVEX data were independent of the fitting method (non-linear or linear), those calculated from PGSE data differed significantly. The errors associated with the fits were also significantly larger for PGSE than for CONVEX. Several observations can be made from the plots of residuals of linearised fits shown in Fig. 3. The normalized standard error of signal integration, determined as the standard deviation of the histogram of the residuals of the curvilinear fit, was 0.021 for the PGSE data and 0.0037 for the CONVEX data. (These errors are given relative to the amplitude of the signal at $g \rightarrow 0$.) Therefore, the standard error associated with the integration of Phe peaks was approximately a factor of 5 smaller in CONVEX spectra than in those from PGSE. This is reflected in the relative spread of the error envelopes shown in panels A and B.

Secondly, the magnitudes of the residuals of the linearised PGSE fit are markedly non-random, and their magnitudes do not behave as predicted by the respective calculated error envelope. The Wald-Wolfowitz runs test [35] placed the observed number of positive/negative runs ($r = 3$) at 4.8 standard deviations from the expected number of runs if the errors were random. Therefore, the linearized PGSE plot systematically

deviates from a straight line. The residuals in the CONVEX plot appear to be less strongly correlated; the magnitudes of their residuals are in good agreement with the calculated error envelope; and the observed number of runs (r = 12) is only 1.3 standard deviations away from that expected for randomly distributed errors. The distribution of errors was similar for the crosslinked hydrogel sample. Therefore, it can be concluded that the CONVEX diffusion decay curves were subject to significantly smaller systematic deviations than the PGSE decay curves. It can be inferred from this analysis that PGSE provided underestimated values of the diffusion coefficients of Phe in non-crosslinked and crosslinked hydrogels.

Diffusion coefficients obtained by both PGSE and CONVEX were higher in the crosslinked sample than in the non-crosslinked sample. From geometric considerations alone this results appears counterintuitive. Moreover, the apparent macroscopic viscosity of the crosslinked hydrogel was greater than that of the non-crosslinked sample. In particular, the non-crosslinked hydrogel flowed as a viscous liquid, while the crosslinked hydrogel possessed some degree of elasticity. Contrary to the results observed for Phe, the diffusion coefficient of water was smaller in the crosslinked sample than in the non-crosslinked one. This phenomenon could be due to a stacking interaction between the phenyl ring of the amino acid and the aromatic acetophenone groups of the photoinitiator. Another mechanism could be a hydrophobic association between the carboxyl groups of phenylalanine and the side-chain acrylamide groups. In the crosslinked polymer, the carbon–carbon double bonds of the acrylamide groups are replaced with single bonds, which would result in the acrylamide–phenylalanine association being diminished. However, these explanations should be treated as working

hypotheses, and further experiments will be necessary in order to determine conclusively the physical mechanism.

Conclusions

The ability to measure accurately the diffusion coefficients of small molecules in hydrogels is important for many practical applications of these materials. We have applied two diffusion techniques, CONVEX and PGSE, to the measurement of the diffusion coefficient of the aromatic amino acid phenylalanine in non-crosslinked and crosslinked PVA hydrogels. CONVEX addresses several of the shortcomings of standard PGSE methods and provides a flatter baseline, less phase distortion, and more linear Stejskal-Tanner plots than PGSE. This enables the determination of the diffusion coefficient of phenylalanine with relative accuracy better than 1%, yielding a simple, rapid and accurate method for measuring small-molecule diffusion in hydrogels. In the context of biomedically relevant hydrogels, this method is particularly applicable to aromatic low molecular-weight compounds but could also be used for measuring diffusion of large, aromatic-rich molecules.

References

[1] A.S. Hoffman, Hydrogels for biomedical applications, Adv. Drug Deliv. Rev. 43 (2002) 3-12.

[2] H. Nordman, J.R. Davies, A. Herrmann, N.G. Karlsson, G.C. Hansson, I. Carlstedt, Mucus glycoproteins from pig gastric mucose: Identification of different mucin populations from the surface epithelium, Biochem. J. 326 (1997) 903-910.

[3] N.A. Peppas, Y. Huang, M. Torres-Lugo, J.H. Ward, J. Zhang, Physicochemical foundations and structural design of hydrogels in medicine and biology, Annu. Rev. Biomed. Eng. 2 (2000) 9-29.

[4] T.A. Waigh, A. Papagiannopoulos, A. Voice, R. Bansil, A.P. Unwin, C.D. Dewhurst, B. Turner, N. Afdhal, Entanglement coupling in porcine stomach mucin, Langmuir 18 (2002) 7188-7195.

[5] K.S. Masters, S.J. Leibovich, P. Belem, J.L. West, L.A. Poole-Warren, The effects of nitric oxide releasing poly(vinyl alcohol) hydrogel dressings on dermal wound healing in diabetic mice, Wound Repair Regen. 10 (2002) 286-294.

[6] P. Martens, S. Bryant, K.S. Anseth, Tailoring the degradation of hydrogels formed from multivinyl poly(ethylene glycol) and poly(vinyl alcohol) macromers for cartilage tissue engineering, Biomacromolecules 4 (2003) 282-292.

[7] P. McConville, J.M. Pope, A comparison of water binding and mobility in contact lens hydrogels from NMR measurements of the water self-diffusion coefficient, Polymer 41 (2000) 9081-9088.

[8] S.L. Bourke, M. Al-Khalili, T. Briggs, B.B. Michniak, J. Kohn, L.A. Poole-Warren, A photo-crosslinked poly(vinyl alcohol) hydrogel growth factor release vehicle for wound healing applications, AAPS Pharm. Sci. 5 (2003) 1-11.

[9] C.S. Johnson, Diffusion ordered nuclear magnetic resonance spectroscopy: Principles and applications, Prog. Nucl. Magn. Reson. Spectrosc. 34 (1999) 203-256.

[10] K.I. Momot, P.W. Kuchel, Pulsed field gradient nuclear magnetic resonance as a tool for studying drug delivery systems, Concepts Magn. Reson. 19A (2003) 51-64.

[11] O. Söderman, P. Stilbs, NMR studies of complex surfactant systems, Prog. Nucl. Magn. Reson. Spectrosc. 26 (1994) 445-482.

[12] A. Coy, P.T. Callaghan, Pulsed gradient spin-echo NMR diffusive diffraction experiments on water surrounding close-packed polymer spheres, J. Colloid Interface Sci. 168 (1994) 373-379.

[13] D.G. Regan, P.W. Kuchel, Simulations of molecular diffusion in lattices of cells: Insights for NMR of red blood cells, Biophys. J. 83 (2002) 161-171.

[14] D.G. Regan, P.W. Kuchel, Simulations of NMR-detected diffusion in suspensions of red cells: The effects of variation in membrane permeability and observation time, Eur. Biophys. J. 32 (2003) 671-675.

[15] W.S. Price, Water signal suppression in NMR spectroscopy, Annu. Rep. NMR Spectrosc. 38 (1999) 289-354.

[16] C. Dalvit, J.M. Bohlen, Analysis of biofluids and chemical mixtures in non-deuterated solvents with ^{1}H diffusion-weighted PFG phase-sensitive double-quantum NMR spectroscopy, NMR Biomed. 10 (1997) 285-291.

[17] W.S. Price, F. Elwinger, C. Vigouroux, P. Stilbs, PGSE-WATERGATE, a new tool for NMR diffusion-based studies of ligand-macromolecule binding, Magn. Reson. Chem. 40 (2002) 391-395.

[18] W.S. Price, M. Walchli, NMR diffusion measurements of strong signals: The PGSE-Q-switch experiment, Magn. Reson. Chem. 40 (2002) S128-S132.

[19] A.K. Simorellis, P.F. Flynn, A PFG NMR experiment for translational diffusion measurements in low-viscosity solvents containing multiple resonances, J. Magn. Reson. 170 (2004) 322-328.

[20] K.I. Momot, P.W. Kuchel, Convection-compensating PGSE experiment incorporating excitation-sculpting water suppression (CONVEX), J. Magn. Reson. 169 (2004) 92-101.

[21] K.I. Momot, P.W. Kuchel, Convection-compensating diffusion experiments with phase-sensitive double-quantum filtering, J. Magn. Reson. 174 (2005) 229-236.

[22] T.L. Hwang, A.J. Shaka, Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients, J. Magn. Reson. A 112 (1995) 275-279.

[23] Y. Yamane, M. Matsui, H. Kimura, S. Kuroki, I. Ando, Diffusional inhomogeneity of probe molecules in chemically cross-linked polymer gels as studied by time-dependent diffusion NMR, Macromolecules 36 (2003) 5655-5660.

[24] H.N. Öztop, D. Saraydin, E. Karadağ, Y. Çaldiran, O. Güven, Influence of some aromatic amino acids on the swelling behavior of acrylamide/maleic acid hydrogel, Polym. Bull. 40 (1998) 575-581.

[25] R. Wimmer, F.L. Aachmann, K.L. Larsen, S.B. Petersen, NMR diffusion as a novel tool for measuring the association constant between cyclodextrin and guest molecules, Carbohydr. Res. 337 (2002) 841-849.

[26] H. Matsuyama, M. Teramoto, H. Urano, Analysis of solute diffusion in poly(vinyl alcohol) hydrogel membrane, J. Membr. Sci. 126 (1997) 151-160.

[27] W.S. Price, Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion: Part i. Basic theory, Concepts Magn. Reson. 9 (1997) 299-336.

[28] E.O. Stejskal, J.E. Tanner, Spin diffusion measurements: Spin echoes in the presence of a time-dependent field gradient, J. Chem. Phys. 42 (1965) 288-292.

[29] J. Kärger, H. Pfeifer, W. Heink, Principles and application of self-diffusion measurements by nuclear magnetic resonance, Adv. Magn. Reson. 12 (1988) 1-89.

[30] P. Stilbs, Fourier transform pulsed-gradient spin-echo studies of molecular diffusion, Prog. Nucl. Magn. Reson. Spectrosc. 19 (1987) 1-45.

[31] J. Zabransky, M. Houska, J. Kalal, Aldehydes and acetals based on acrylamides and methacrylamides as potential monomers, Makromol. Chem. 186 (1985) 223-229.

[32] B. Muller, Photocrosslinked polymers, U.S. Patent 5,508,317, 1996.

[33] D.S. Raiford, C.L. Fisk, E.D. Becker, Calibration of methanol and ethylene glycol nuclear magnetic resonance thermometers, Anal. Chem. 51 (1979) 2050-2051.

[34] R. Mills, Self-diffusion in normal and heavy water in the range 1-45°, J. Phys. Chem. 77 (1973) 685-688.

[35] S. Siegel, N.J. Castellan, Nonparametric statistics for the behavioral sciences, McGraw-Hill, New York, 1988.

[36] S.G. Gholap, J.P. Jog, M.V. Badiger, Synthesis and characterization of hydrophobically modified poly(vinyl alcohol) hydrogel membrane, Polymer 45 (2004) 5863-5873.

[37] P. Martens, K.S. Anseth, Characterization of hydrogels formed from acrylate modified poly(vinyl alcohol) macromers, Polymer 41 (2000) 7715-7722.