Prochiral and Chiral Resolution in ^{2}H NMR Spectra: Solutes in Stretched and Compressed Gelatin Gels

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We demonstrate prochiral and chiral spectral resolution using residual ^{2}H NMR quadrupolar splittings over a wide range of anisotropic conditions in liquid samples. We use a reversible gel-stretching/compressing device in a conventional high-field NMR spectrometer. We show the stability of gelatin gels as well as their unique ability to switch between multiple stretched and compressed states, thus also changing the sign of residual dipolar couplings in ^{1}H and ^{13}C NMR. This flexibility will be important for resolving spectra of mixtures of other chiral compounds and for structure determination of selected peptides.

Introduction

Aqueous gelatin gels as a chiral alignment medium were recently used to differentiate alanine enantiomers in ^{1}H and ^{2}H NMR liquid-state spectroscopy.[1,2] The method is based on gelatin carrying polar guest molecules in an elastic silicone rubber tube, and this allows the rapid and reversible adjustment of the degree of nuclear alignment inside NMR samples by merely stretching the silicone rubber tube.[2,3] Alanine enantiomers can be differentiated in ^{2}H NMR spectra by the different strengths of the quadrupolar interaction of each deuterium site within the chiral medium.[2] Each sample can be easily and quickly adjusted several times, leading to spectra with the guest molecule exposed to different degrees of anisotropy. This was an important advance on previous work in liquid crystals[4] and stressed polyacrylamide gels[5] because generally not all degrees of alignment resolve all mixtures. Instead of having to prepare a new sample, simply stretching more or less may then lead to NMR resolution for the analysis of many complex samples. In this paper we report in detail on the capabilities of the technique using ^{2}H NMR spectroscopy.

Deuterium NMR produces relatively sharp signals due to its small electric quadrupole moment, and the order parameters for each C−D bond are readily obtained from the measured corresponding quadrupolar splitting under anisotropic conditions.[6] The low natural abundance of deuterium necessitates labeling in most cases,[7] an inconvenience that is lessening with the advent of triply labeled peptides. The small chemical shift difference between ^{2}H spins can be overcome by a combination of deuterium−deuterium[7] and deuterium−carbon NMR correlation spectroscopy[8] and changing the quadrupolar splittings for each deuteron by adjusting the anisotropy of the medium.

The chirality of gelatin means that not only enantiomers but also prochiral sites can be differentiated once gelatin is stretched or compressed. One example we repeatedly report on in this paper is deuterated dimethyl sulfoxide (DMSO-d_6). The central sulfur atom is tetrahedral (the lone pair of electrons acts as a fourth substituent), and its two attached methyl groups are therefore spatially inequivalent when the molecule is placed in the anisotropic and chiral environment of stretched gelatin. Therefore, in ^{2}H NMR spectra two sets of quadrupolar splittings are observed.

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Experimental Methods

Sample Preparation. Figure 1 shows schematically how the alignment apparatus is operated. After the liquid gelatin containing the guest is drawn into the silicone-rubber tubing (for 5 mm NMR tubes, Silastic laboratory tubing by Dow Corning Corp., catalog no. 508-009, 1.98 mm i.d., 3.18 mm o.d.), a plastic plug is inserted. The sample is cooled in a refrigerator to bring about gelation, and the tubing is inserted into a close-fitting open-bottom, 5 mm outer diameter, thick-walled glass tube (Figure 1A).

The ^{2}H NMR spectrum (Figure 1A, right) showed a singlet due to the gelatin being nonaligned (isotropic). If the silicone tubing is stretched, the gelatin partially aligns with the magnetic field $\mathbf{B}_0$, and quadrupolar splittings can be observed, in this case 95 Hz for one CD_3 group and 10 Hz for the other (Figure 1B). The length of stretching divided by the height of the NMR tube yields the extension factor, here 1.0, but in practice it can readily be varied between 0.1 and 2.0. Measurements of small extension factors (around 0.1) become less accurate due to uncertainty brought about by the pinching effect of the thumbscrew on the tubing and enclosed gel. Preparing a sample with a reduced gelatin concentration may then be preferable. The upper limit on the extension factor is due to the plug holding the silicone tubing in place being unable to withstand the high tension. In addition, the effective sample size between the NMR-receiver coils decreases with increased stretching due to the smaller cross-sectional area of the stretched sample.

When the stretched sample was heated to 37 °C inside the spectrometer for ∼10 min, all anisotropy was lost and was not recovered upon cooling (Figure 1C). This "melting of the anisotropy" does not imply that the gel had been denatured (liquefied). Once the gelatin had set again at lower temperatures, releasing the stretched tubing resulted in a compressed state (Figure 1D). The ^{2}H NMR quadrupolar splittings were between 40% and 70% of those of the stretched state, most commonly 50−60%. The gels in both the stretched and melted states increased their quadrupolar splittings with time of storage at 4 °C; e.g., the gel used for Figure 2 was stretched to an extension factor of 0.69, yielding a quadrupolar splitting of 275.8 Hz between the outer DMSO peaks. After the gel was melted at 37 °C, the tubing was fully released, going back to an extension factor of 0.06. The quadrupolar splitting was 160.2 Hz,

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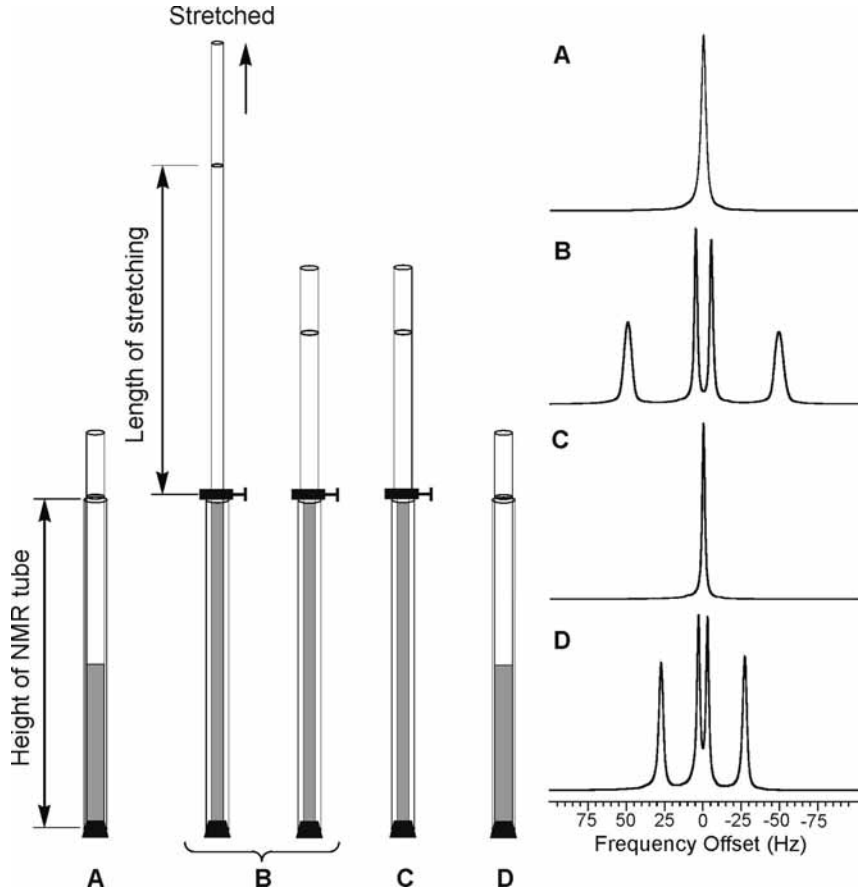

Figure 1. Schematic representation of stretched and compressed gelatin gels in the recently developed apparatus. (A) Insertion of a plugged silicone tube filled with gelatin gel into an open-bottom, 5 mm outer diameter, thick-walled glass tube. The gel was 42% (w/v) gelatin containing 10% DMSO-d_6, 10% DMSO, and 80% H_2O. The unstretched gelatin was in an isotropic state as seen by the singlet in the ^{2}H NMR spectrum (A, right; one signal for DMSO-d_6; all spectra were recorded at 15 °C and 61.4 MHz). (B) The tubing was stretched with an extension factor of 1.0 (extension factor = increase in length relative to the original length). The extension factor can typically be any value between 0.1 and 2.0. A plastic thumbscrew holds the silicone tube in the stretched state. ^{2}H NMR of the anisotropic stretched gelatin (B, right) now shows four lines (two sets of quadrupolar doublets) due to the methyl groups of DMSO-d_6 being prochiral and interacting differently with the chiral medium. (C) Heating the stretched gel to 37 °C for 10 min led to the total loss of anisotropy; upon cooling, a ^{2}H NMR singlet was returned for DMSO-d_6. (D) Releasing the stretched gel (after melting and cooling) by removing the plastic thumbscrew compressed the gel. Its anisotropy was ~50% of that of the corresponding stretched state (D, right; two sets of quadrupolar doublets). Melting and cooling this compressed gel returned the original unstretched and isotropic gel, respectively: each step on this stretching and compressing cycle was reversible. All NMR spectra were scaled to the same peak height.

corresponding to 58.1% of the previous stretched-state value. After 5 days at 4 °C the sample had an extension factor of 0.03 and a quadrupolar splitting of 194.5 Hz. (Figure S1 in the Supporting Information contains more details of this experiment.) This is a general observation applying to both stretched and compressed gels; the anisotropy increased slowly over several days. If the fully compressed gel was heated to 37 °C, the original isotropic and unstretched gel was restored, and the stretching and compressing cycle could be repeated as required.

NMR Measurements. ^{2}H NMR spectra were recorded at 61.422 MHz on a Bruker DRX 400 spectrometer (Bruker Karlsruhe, Germany) with an Oxford Instruments (Oxford, U.K.) 9.4 T, vertical, wide-bore magnet. For COSY spectra we used the Bruker pulse sequence cosydftp (2D homonuclear shift correlation phase sensitive using TPPI with a double-quantum-filter phase cycle), delay$-\pi/2-t_1-\pi/2-$d13$-\pi/2-$acquire; a short delay of 3 μs was used for d13. All signals were in the pure absorption mode for the diagonal and cross-peaks in the two dimensions. We did not observe any scalar couplings in ^{2}H NMR spectra. All spectra were run at 15 °C unless otherwise noted.

Results

Compressed and Stretched Gels. It is possible to prepare gel samples that contain a range of extents of compression and stretching (Figure 2). Melting a stretched gel at a medium extent of stretching allowed a transition from a compressed to a stretched gel and vice versa. We predict that this will become useful for the measurement of residual dipolar couplings for spin 1/2 nuclei. In ^{1}H NMR and ^{13}C NMR we observed that the sign of the residual dipolar coupling changed by switching from a stretched to a compressed gel.[2] This is illustrated in Figure 2. We used a 100% gelatin gel (w/v; note this refers to 1 g of gelatin/mL of solvent) that was prepared with an aqueous solution containing 10% (v/v) DMSO-d_6 and 1 M glycine-*2*-^{13}*C*. The addition of the ^{13}C-glycine did not noticeably influence the deuterium quadrupolar splitting of DMSO-d_6; the same gel without glycine yielded very similar spectral-splitting patterns (see the Supporting Information). The gel was stretched to an extension factor of 0.69 (quadrupolar splittings of 275.8 and 18.2 Hz), melted, and then released (quadrupolar splittings 160.2 and 11.8 Hz). After 3 days at 4 °C the sample had slightly tightened and disproportionately (compared with the decrease

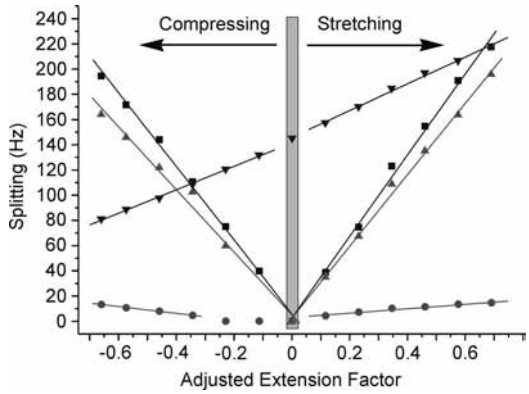

Figure 2. Linear relationship between the ^{2}H NMR quadrupolar splitting of DMSO-d_6 at 61.4 MHz and the extent of stretching and compression of a gelatin gel: the gel was 100% (w/v) gelatin, containing 10% DMSO-d_6, 90% H_2O, and 1 M glycine-*2*-^{13}*C* in the original solute. It was stretched to an extension factor of 0.69, giving ^{2}H quadrupolar splittings of 275.8 and 18.2 Hz, respectively, for the two prochiral methyl groups (not shown in the graph, 15 °C, 61.4 MHz). Melting and cooling refocused the splitting to 0.0 Hz. The gel was able to be reversibly moved to compressed and stretched anisotropic states by adjusting the tension of the silicone tubing; thus, both releasing and tightening the tubing yielded anisotropic ^{2}H NMR spectra in linear relationships. Squares represent the splittings for the larger quadrupolar doublet, circles those for the smaller one. Up-facing triangles represent the largest residual dipolar splitting for the methylene protons of glycine (extracted from ^{1}H NMR spectra at 400.1 MHz), whereas down-facing triangles correspond to the largest ^{13}C NMR residual dipolar splitting ($J_{AX} + 2D_{AX}$) data of glycine-*2*-^{13}*C* (100.6 MHz). The adjusted extension factor was derived from the extension factor by subtracting the extension factor at the melting of the gel in the stretched state (here 0.69), thus separating compressed from stretched states (within the same gel).

in the extension coefficient) increased its ^{2}H splittings to 194.5 and 13.3 Hz. We then stretched the sample in 12 successive steps, going from a compressed state to a stretched state (Table 1, Figure 2, and the Supporting Information). At an intermediate point of stretching (the extent of stretching that was used when the sample was originally melted) there was no anisotropy, and the quadrupolar splitting for DMSO-d_6 and the dipolar splitting for ^{13}C-labeled glycine were 0.0. In ^{13}C NMR spectra only the isotropic scalar coupling J_{AX} was observed. The graphs of splitting versus extent of stretching were generally linear, with the compressed state having a 10−15% smaller slope than that for the stretched state.

Influence of the Gelatin Concentration. The major range of anisotropies available for each sample was determined by the gelatin concentration. When samples having identical concentrations of guest molecule but varying gelatin concentrations were stretched to a constant length, the quadrupolar splitting in ^{2}H NMR spectra showed a clear linear relationship (Figure 3A). However, each individual sample also showed linearity between the value of the quadrupolar splitting and the extension factor (Figure 3B).

The gelatin concentration and length of stretching are the major determining factors of the alignment of molecules in gelatin. This point is graphically illustrated in Figure 4. ^{2}H NMR of ethanol-d_5 in unstretched gelatin showed two resonances for the CD_2 and CD_3 groups (Figure 4A). Stretching a 15% (w/v) gelatin 1.4-fold showed six signals: the methylene group is prochiral, and each ^{2}H atom has a different quadrupolar splitting (Figure 4B). However, to achieve a comparable (slightly larger) alignment in 30% (w/v) gelatin, only a 0.5-fold stretching was needed (Figure 4C), and in a 50% (w/v) gel, even less so, only 0.2-fold (Figure 4D). The same 50% (w/v) gel, when stretched 1.4-fold (Figure 4E) showed much more peak separation, almost overlapping one of the methylene signals with that of the CD_3 group.

Influence of the Solvent and Salt Concentration on Anisotropies in Stretched Gels. There is a solvent dependency on the quadrupolar splitting pattern, but it is not a large effect. If the total amount of DMSO-d_6 was varied between D_2O-containing gels of the same gelatin concentration to up to 50% (of the total volume added to the dry gelatin), the main effect was to change the ratio of the quadrupolar splittings for the enantiotopic methyl groups: more DMSO increased that ratio, and the inner signals moved closer together. The quadrupolar splittings for D_2O and the outer methyl group were barely affected. The total amount of a particular solvent (e.g., DMSO or DMSO-d_6) determined this outcome, not the amount of deuterated isomer. Higher DMSO concentrations yielded reduced quadrupolar splittings (whereas gels with 60% DMSO-d_6 had ∼70% of the ^{2}H splittings measured in gels with a low DMSO concentration; gels with 70% DMSO had ∼40% and gels with 80% DMSO had only 26% of the anisotropy remaining. A gel prepared with 90% DMSO did not show any quadrupolar splittings in the ^{2}H NMR spectrum of DMSO-d_6).

Increasing the salt concentration did not have any detectable influence on the quadrupolar splitting, up to 1 M. Higher salt concentrations decreased the anisotropy to zero. This was observed for the series of alkali-metal chlorides Li^+, Na^+, K^+, and Cs^+, and NH_4^+.

Influence of pH. Breaking down collagen under acidic and basic conditions is how gelatin is produced commercially. Thus, it is not surprising that gelatin is very stable to extremes of pH values. The same appeared to be true for the ability of the collagen helices to be mechanically aligned by stretching the gel in the NMR magnet, but the highest anisotropy for comparable gels was recorded for a gel at pH 7.4. Stretched alkaline gel (pH 11.6) had reduced quadrupolar splittings of the D_2O−DMSO-d_6 sample that was ∼40% of the values recorded for a gel at neutral pH. There was more tolerance to acidic conditions: stretching an acidic gel (pH 1.0) yielded ^{2}H NMR splittings that were 70% of those measured for the neutral gel and increased the quadrupolar splitting ratio between the two methyl groups.

Temperature Influence. We explored whether it would be possible to align gelatin by stretching it at higher temperatures than 15−25 °C. A glycerinated 100% (w/v; 1 g of gelatin/mL of solute) gelatin was prepared that was so viscous that it required heating to above 80 °C to be sufficiently liquid for transfer into the silicone tubing; however, as the graph in Figure 5 shows, there was no anisotropy evident at 37 °C. At this temperature, the collagen helices most likely have too much thermal motion to maintain any average alignment. Another experiment in which gelatin was cross-linked with glutaraldehyde[9] showed that the resulting gel was more thermostable, but the anisotropy when stretched was still lost at ∼30 °C, like an untreated gel.

Resolving Spectra of Complex Mixtures. Compounds that contain overlapping resonances can often have these resolved stereoselectively by stretching in a few steps (e.g., ^{2}H NMR spectra of L- and DL-serine-*1*,*2*,*2*-d_3 in Figure 6). More complicated mixtures can be assigned by using double-quantum-filtered COSY spectra (see the Supporting Information). This experiment correlates each quadrupolar pair.[7] A related alternative experiment is to measure a 2D EXSY experiment using a small mixing time. Deuterium−carbon NMR correlation spec-

TABLE 1: Summary of NMR Data (at 15 °C, 400.1 MHz for ^{1}H NMR, 61.4 MHz for ^{2}H NMR, 100.6 MHz for ^{13}C NMR) Recorded for a Typical Sample[a]

			DMSO-d_6		glycine-2-^{13}C		
			^{2}H quadrupolar splitting (Hz)		^{1}H splitting (Hz)[b]		^{13}C splitting (Hz)[b]
sample age (h)	extension factor	adjusted extension factor	outer	inner	$C\underline{H}_2$	^{13}C-$C\underline{H}_2$ (largest)	$^{13}\underline{C}$-CH_2 (largest)
0	0.69	0	275.8	18.2	241.6	241.6	239.4
1.1	0.69	0	0	0	0	142.8	144.8
1.6	0.06	−0.63	160.2	11.8	91.9	139.8	92.8
70.4	0.03	−0.66	194.5	13.3	164.1	81.0	81.1
70.8	0.11	−0.57	171.7	10.8	145.7	89.9	88.8
71.0	0.23	−0.46	144	8	121.8	98	97.6
71.3	0.34	−0.34	110.7	4.7	102.4	102.4	108.5
71.5	0.46	−0.23	74.9	0	59.7	121.7	120.4
71.8	0.57	−0.11	39.7	0		144.1	131.8
72.0	0.69	0	0	0	0	144.3	145.1
72.3	0.8	0.11	38.6	4.2	34.6	157.4	157.3
72.5	0.92	0.23	74.5	7	67.1	170.6	167.0
72.8	1.03	0.34	122.7	9.9	108.5	185.4	184.4
73.0	1.15	0.46	154.5	11.2	134.8	194.7	196.8
73.4	1.26	0.57	190.6	13.2	163.3	204.9	206.5
73.5	1.38	0.69	217.2	14.2	195.5	215.4	218.2
167.2	1.38	0.69	228.4	14.4	206.8	206.8	217.0
167.7	0.1	−0.59	151.5	10.5			95.2
190.3	0.05	−0.64	174.6	12.2	148.1	88.4	87.4

[a] The gel was 100% (w/v) gelatin containing 10% DMSO-d_6, 90% H_2O, and 1 M glycine-*2*-^{13}*C* in the original solvent. [b] The ^{1}H and ^{13}C NMR spectra contain minor transitions that are to be discussed elsewhere.

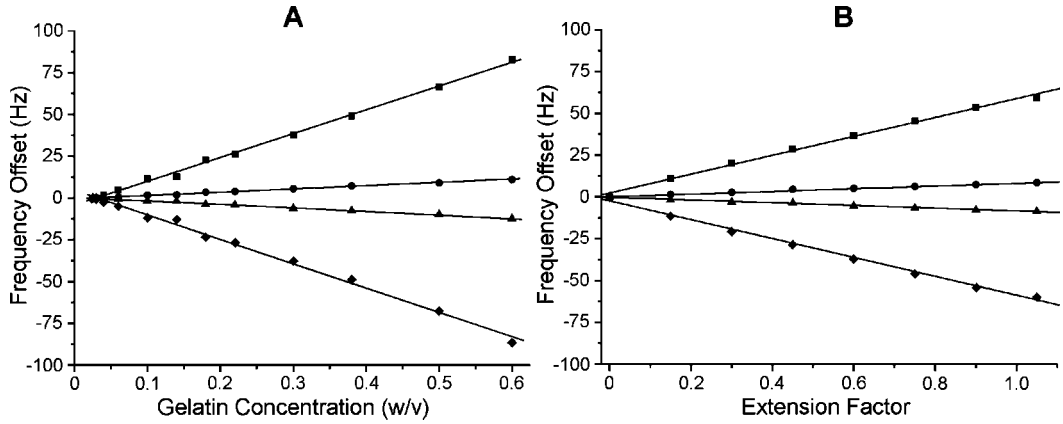

Figure 3. Major adjustable anisotropic properties of gelatin gels in ^{2}H NMR: linear relationships existed between the quadrupolar splitting observed for gels containing DMSO-d_6 and the gelatin concentrations at constant extension factor (A) as well as between the quadrupolar splitting and the extension factor at constant gelatin concentration (B) (A, 5% (v/v) DMSO-d_6 at an extent of stretching of 1.4; B, 5% (v/v) DMSO-d_6 in 60% (w/v) gelatin). All spectra were recorded at 15 °C and 61.4 MHz. Squares and tilted squares represent the larger quadrupolar doublet; circles and triangles the smaller one.

troscopy[8] has previously been reported for liquid crystals and should also work for stretched and compressed gelatin gels.

Incorporation of Multimolecular Complexes into Gelatin. Gelatin showed a remarkable tolerance to incorporated objects such as red blood cells (RBCs) and cross-linked dextran beads (Sephadex).[10] Unstretched, there was no visible ^{2}H NMR difference in the presence of deuterated molecules, but when stretched, anisotropic (gelatin) and isotropic (interiors of erythrocytes or beads) phases coexisted. Deuterons in the gelatin showed the quadrupolar splittings, whereas guests inside the RBCs were not affected by the stretching (Figure 7). Thus, stretched gelatin acts as a shift reagent.[10] Equilibration of guest molecules between the two phases occurred more slowly, within days.

Spin−Lattice Relaxation Times. Deuteration of proteins is used in NMR structural studies to increase the relaxation times of the adjacent ^{13}C and ^{1}H nuclei, to enhance spectral resolution.[11] In gelatin gels, the spin−lattice relaxation time (T_1) of guests is generally shortened, and the extent depends on the gelatin concentration.[10] We obtained an estimate of how much the increase in the relaxation rate of ^{13}C nuclei was counteracted by the decrease caused by the gelatin medium and measured the ^{13}C T_1 values of sodium acetate-*2*-^{13}*C*-d_3 and sodium acetate-*2*-^{13}*C* in solution and in 60% (w/v) unstretched gelatin gels. In a solution of 480 mM sodium acetate at 15 °C, the carbon spin−lattice relaxation time increased from 10.4 ± 0.2 s for the CH_3 group to 30.5 ± 0.4 s for the CD_3 group. Under the same conditions in gelatin, the CH_3 group had a T_1 value of 3.5 ± 0.2 s and the CD_3 group a value of 11.2 ± 0.5 s.

Discussion

Gelatin is a versatile and readily available material for the simple and rapid preparation of chiral anisotropic gels. Chiral and prochiral compounds incorporated into gelatin can be subjected to a wide range of adjustable anisotropies, depending on how much the sample is stretched or compressed. By choosing an appropriate concentration of gelatin and then

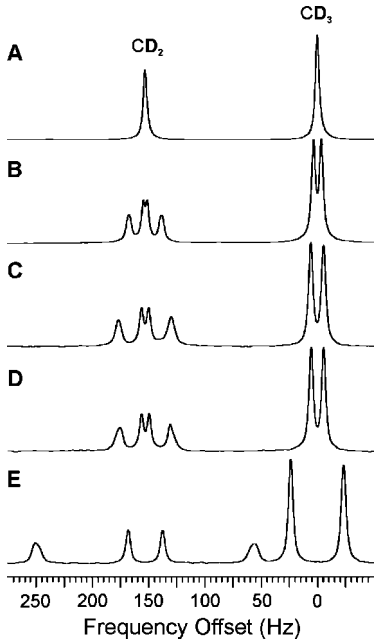

Figure 4. ^{2}H NMR (61.4 MHz) spectra of deuterated ethanol-d_5 (10%, v/v) at different gelatin concentrations: (A) 15% (w/v) gelatin, unstretched, (B) 15% (w/v) gelatin, extension factor 1.4, (C) 30% (w/v) gelatin, extension factor 0.5, (D) 50% (w/v) gelatin, extension factor 0.2. (E) 50% (w/v) gelatin, extension factor 1.4.

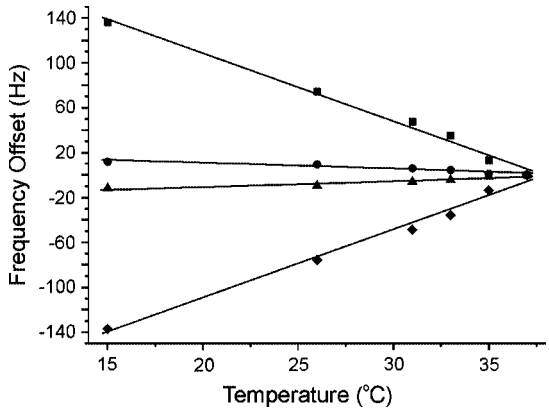

Figure 5. Temperature dependence of ^{2}H NMR quadrupolar splitting for DMSO-d_6 in a stretched high-melting-temperature glycerinated gelatin gel (extension factor 1.0, 100% gelatin, w/v; 56% glycerol, 29% D_2O, 15% DMSO-d_6, all v/v). The gel needed to be heated to >80 °C to become sufficiently liquid to enable drawing into the silicone tubing. There was a linear decrease of the quadrupolar splittings with rising temperature until at 37 °C isotropic properties were achieved. Squares and tilted squares represent the larger quadrupolar doublet, circles and triangles the smaller one.

reversibly adjusting the amount of stretching, we obtained quadrupolar splittings that ranged from 0 to more than 1000 Hz for the same deuterium spin system. This allowed us to differentiate chiral and prochiral centers in small molecules.

Gelatin gel samples can be cycled from a stretched to a compressed state and vice versa. We demonstrated, in detail (Figure 2), how a typical sample was moved from a compressed to a stretched state in 11 intermediate stages. The inclusion of ^{13}C-labeled glycine into the gel demonstrated the change of the sign of the residual dipolar coupling on going from a compressed to a stretched state. We often observed splittings for the compressed gels of more than 50% of those obtained with the corresponding stretched gel before it was melted. In the aforementioned case of a gel that was stretched from a

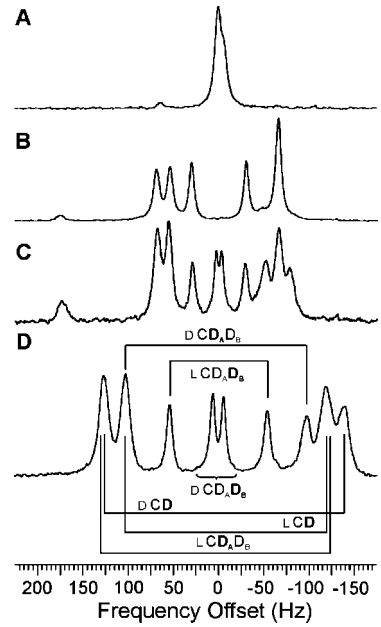

Figure 6. Resolution in ^{2}H NMR (61.4 MHz) spectra of deuterated DL- and L-serine-*1*,*2*,*2*-d_3 enantiomers (3%, w/v) embedded in gelatin (30%, w/v) by using the different quadrupolar splitting of each deuteron. By doing this, compounds that had overlapping peaks were successfully analyzed. (A) L-Serine, unstretched gel. The two resonances for the CD and CD_2 deuterons almost coincided. DL-Serine yielded an identical spectrum. (B) L-Serine stretched 0.9-fold. The prochiral methylene group showed separate quadrupolar splittings for each deuteron. The small signals at high frequency belong to D_2O. (C) DL-Serine stretched 0.9-fold. (D) DL-Serine stretched 1.4-fold. Measuring the frequencies of the ^{2}H NMR quadrupolar signals for DL- and L-serine, and taking into account that the splits were symmetrically arranged around the original chemical shifts, led to the assignments shown in (D). A double-quantum-filtered ^{2}H NMR COSY spectrum (or a ^{2}H NMR EXSY spectrum with a short mixing time) correlated each quadrupolar pair (see the Supporting Information).

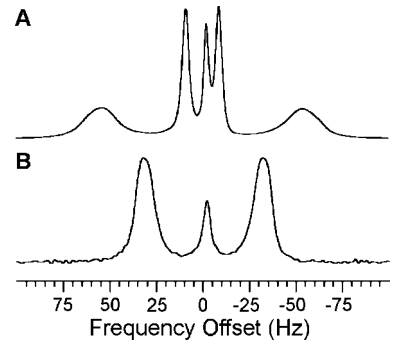

Figure 7. Incorporation of human erythrocytes into gelatin: upon stretching there was ^{2}H NMR (15 °C, 61.4 MHz) spectral resolution between the anisotropic gel and the isotropic inside of the erythrocytes. Key: (A) 3.6% (v/v) DMSO-d_6 in 30% (w/v) gelatin containing a final hematocrit of the suspension of ~25%, extent of stretching 0.9. (B) 3.9% (v/v) dimethyl sulfone-d_6 in 30% (w/v) gelatin containing a final hematocrit of ~25%, extent of stretching 1.1.

compressed to a stretched state, the difference in splitting for equidistant compressed and stretched states was typically more than 70%; see the small difference of the slopes of the resulting graphs (splitting versus stretching; see Figure 2 and the Supporting Information). However, recall that the observed quadrupolar, or dipolar, splitting of a resonance is likely to be the weighted average of a small amount of this resonance being in an anisotropic environment and in fast exchange with the isotropic bulk.[10] It is possible that a small increase in the lifetime

of association between the collagen helices and the guest occurs on switching from a stretched to a compressed gel. This would increase the apparent quadrupolar splitting for the compressed state relative to the stretched one.

The gel can be melted at 37 °C and reused. The whole process appeared to be reversible at any stage. The gels were stable to changes in pH and to moderate inclusion of monovalent halide salts. Gelatin was dissolved in polar solvents such as water, and water−polar solvent mixtures; thus, it was possible to dissolve moderately lipophilic compounds in DMSO and add sufficient water to prepare the gel without precipitating the guest. There is a temperature limitation to using gelatin gels. We have not yet been able to obtain an anisotropic gelatin-based gel at 37 °C, but stretched and compressed gels that were kept below 25 °C were stable for weeks.

The inverse linear relationship of the quadrupolar splitting with temperature seen in Figure 5 was typical of other studies of the temperature dependence of this spectral parameter. The decrease in splitting with increasing temperatures reflects the likely decrease in order that is brought about by thermal motion. So far we have not seen the effects that are described for liquid crystal solutions of poly(γ-benzyl-L-glutamate) (PBLG) in organic solvent where the quadrupolar splitting increases in some cases with higher temperatures.[4c] However, gelatin gels seem to be much more temperature sensitive than PBLG: a quadrupolar splitting of 1000 Hz can collapse to 0 Hz within a 20 °C range.

Future applications could include natural abundance ^{2}H NMR measurements, particularly when commercial (Bruker) ^{2}H NMR-specific CryoProbes become more generally available.[12] Gelatin as a peptidic material is relatively inert, but it may interact with guest peptides and cause unfolding to give non-natural structures. In addition, we found that incorporation of large (on a molecular scale) objects such as Sephadex beads or red blood cells did not destroy the anisotropic phase of the gel; this is a property that has already been used to advantage in biophysical studies on red blood cells.[10]Another future aim will be the incorporation of peptides into the gel to obtain a range of residual dipolar couplings. The shortening of the ^{13}C relaxation times of guest peptides caused by the gel may be offset by deuteration. For sodium acetate solutions we found that the ^{13}C T_1 value for the CH_3 group in solution was close to that measured for the CD_3 group of deuterated sodium acetate in 60% (w/v) gelatin gel.

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Supporting Information Available: Additional data for the gel described in Figure 2 and two additional gel samples and double-quantum-filtered ^{2}H NMR COSY spectra for stretched gelatin gels containing L- and DL-serine-*1*,*2*,*2*-d_3. This material is available free of charge via the Internet at http://pubs.acs.org.

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