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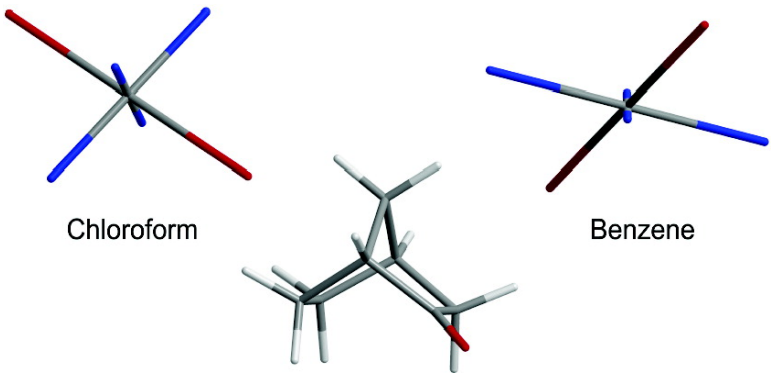

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Orientational Properties of Stretched Polystyrene Gels in Organic Solvents and the Suppression of Their Residual ^{1}H NMR Signals

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Abstract: While residual dipolar couplings (RDCs) are an established method in high-resolution biomolecular NMR, their use for structure determination of small molecules in organic solvents is limited by the alignment media available. Only recently stretched polystyrene (PS) gels were introduced for the measurement of RDCs on small compounds that allowed urgently needed free scalability of the induced anisotropy. Here, the properties of such stretched PS gels in different organic solvents as well as for different magnetic field strengths and temperatures are studied and practical NMR-spectroscopic aspects are discussed.

Introduction

NMR spectroscopy in partially oriented media was first discovered in 1963,[1] and in a very fundamental paper Saupe was also able to present the essential theory to describe and understand the observable phenomena only 1 year later.[2] After this initiation a flood of NMR spectra in various liquid crystalline phases was reported (see e.g. refs 3−7) using mainly nematic mesophases but also smectic,[8−10] destroyed cholesteric,[4,11] and lyotropic nematic mesophases.[12,13] In today's high-resolution NMR of biomacromolecules the measurement of residual dipolar couplings (RDCs) can be viewed as a standard method for obtaining structural information, and a large abundance of aqueous alignment media such as lipid bicelles,[14,15] stretched polyacrylamide gels,[16,17] filamentous phages,[18] and other liquid crystalline phases[19,20] is used. In the field of small-molecule NMR, however, only recently the potential of NMR spectroscopy in partially oriented samples with RDCs as a powerful structural parameter was rediscovered and few, nevertheless impressive, demonstrations were published.[21−35]

A limiting step for the application of RDCs to small molecules is still the availability of alignment media for organic solvents. Liquid crystalline phases such as poly(γ-benzyl-L-glutamate) (PBLG)[11,25,27,36] are known to align organic molecules in $CDCl_3$ and similar apolar organic solvents. Liquid crystals, however, have the disadvantage that for the phase transition a minimum concentration is needed and therefore a minimum anisotropy is induced in the sample. The development of specially designed crystalline phases with lower minimum alignment, as shown in

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the case of 4-*n*-pentyl-4′-cyanobiphenyl (PCBP)[37] and poly-(γ-ethyl-L-glutamate) (PELG),[26] will improve the applicability of such systems.

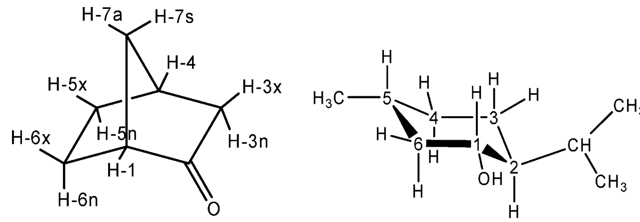
A second technique for partial alignment which is not limited by a minimum anisotropy is strain-induced alignment in a gel (SAG), based on the pioneering work of Deloche and Samulski.[38] The technique was extensively used to study the properties of polymer gels by means of high-resolution deuterium NMR,[39] but only lately gel alignment was used to induce RDCs in molecules dissolved into the gel.[16,17] SAG allows the unrestricted scaling of alignment over a wide range and can be used for aqueous as well as organic solvents, depending on the polymer used.[32−35,40,41] As a first example in organic solvents, RDC measurements in stretched polystyrene (PS) gels swollen in $CDCl_3$ were reported as a promising alignment method.[33] After the proof of principle we want to give a more detailed characterization of the alignment and NMR properties of the PS gel in this article: the influence of physical parameters, i.e., the temperature and the static magnetic field, as well as the effect of some solvents suitable for swelling the gel on the induced anisotropy was studied. Also some characteristics of NMR spectra measured in PS gels are analyzed like potential line width limitations due to gel heterogeneity, chemical shift changes, and the appearance of undesired PS signals. Finally, partial PS-signal suppression using so-called relaxation filtering methods is shown, in which context a *z*-filter-based relaxation filter is introduced.

Materials and Methods

Cross-linked polystyrene sticks were prepared in glass tubes with inner diameters of 3.4 and 4.0 mm. The glass tubes were carefully dried, sealed on one end by melting, and treated with a 1:1 mixture of chlorotrimethylsilane and dichlorodimethylsilane for 18 h to ensure apolar surfaces. After being washed with dichloromethane (5 times), the tubes were dried again at 50 °C. Styrene (99%, Fluka) and divinylbenzene (80%, Fluka) were filtered (basic aluminum oxids, pH 10, Fluka) and distilled under reduced pressure. Immediately before polymerization the monomers were degassed for 15 min by ultrasound in vacuo and ventilated in an argon atmosphere. After careful mixing of styrene, divinylbenzene, and 2,2′-azobis(2-methylpropionitrile) (AIBN) to desired concentrations, the mixture was filled into the prepared glass tubes and their tops were sealed. Polymerization was performed for 5 days at 45 °C and 2 days at 60 °C. After the glass tubes were broken, bubble-free parts of the polymer sticks were cut into pieces of 1.0−1.5 cm length. All samples used for measurements were prepared by putting polystyrene sticks of defined diameters directly into NMR tubes and letting them swell in the chosen solvent for 1 to 14 days. The swelling processes were monitored by acquiring ^{2}H NMR spectra of the deuterated solvents: with progressing swelling and stretching of a polymer the initially sharp single signal broadens irregularly, then turns into two broad lines, and finally ends up with two relatively sharp lines for well-equilibrated samples. Swelling times necessary to obtain equilibrated samples differ significantly for different solvents used and different diameters of the polymer stick taken. Shortest swelling times were observed for samples swollen in dichloromethane; in this case, most of the polystyrene sticks of 3.4 mm diameter reached equilibrium state within 24 h. Similar sticks swollen in chloroform were ready to use in about 2−3 days, while slowest swelling was observed for dioxane and benzene in which PS sticks required up to 2 weeks to reach equilibrium. Within limits, the speed of the swelling process could be increased by increasing the sample temperature. In the end, all PS sticks swollen in one of the solvents discussed in the text resulted in nicely equilibrated samples with uniform splittings in the ^{2}H NMR spectra.

In contrast to results published in ref 33 where polymerization took place at 80−120 °C using dibenzoyl peroxide as radical starter, we could not find a significant dependence of the induced anisotropy on the radical starter in the procedure described above. This is probably due to overall longer polymer chains produced at lower polymerization temperatures.

Chart 1. Structures of Norcamphor (**1**) and Menthol (**2**)



1 **2**

The derivation of alignment tensors of norcamphor (Chart 1) in different solvents involved several steps. First, norcamphor was added on top of already prepared PS-gel samples, which were kept at room temperature for 2−3 days to allow for diffusion of the small molecule into the gel. The samples were then used to acquire standard coupled ^{1}H−^{13}C HSQC spectra with sensitivity enhancement and phase sensitive echo/antiecho gradient selection.[42−44] All 2D spectra were measured on Bruker DMX600 spectrometers with 3.5 ppm spectral width in the ^{1}H-dimension (4096 complex points) and 70 ppm (256 increments) in the indirect ^{13}C-dimension. With 2 transients acquired/increment the total experiment time for each HSQC spectrum was about 16 min. Spectra were processed with exponential multiplication (with an additional line broadening of 1 Hz) in the directly recorded dimension and a 90°-shifted squared sine-bell window function in the indirect dimension. Coupling constants were measured with the aid of the program SPARKY[45,46] using automated peak picking and by individually phasing the doublet components at slices along the directly detected dimension in cases of slight phase twists (cf. procedure described in ref 30). It turned out that the latter procedure gave more reliable results with deviations relative to the automated peak picking of up to 2 Hz. The estimated errors of the coupling constants determined by the more elaborate procedure were generally below $\pm$0.5 Hz, which was used as a conservative estimate for subsequent calculations. The alignment tensors were finally derived using only RDCs from signals without second-order effects with the program PALES[47] using the bestFit option. Relative orientations in 5D space and errors estimations of the alignment tensor calculations were calculated with the corresponding options in the PALES program. Samples for various solvents containing menthol were prepared and measured in the same way as norcamphor samples, but no attempt to derive alignment tensors was pursued since only four C,H-vectors point in different directions.

Temperature dependence was measured on a Bruker DMX750 spectrometer with a calibrated variable-temperature unit, using nitrogen as flowing gas for room temperature and above and liquid nitrogen for

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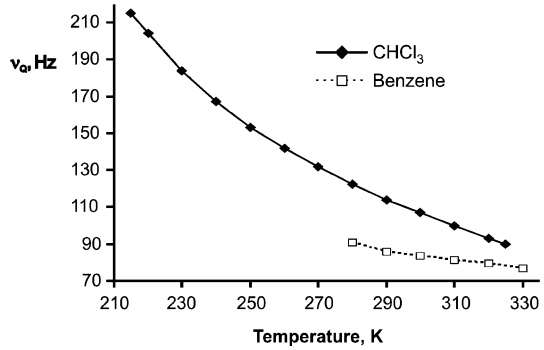

Figure 1. Temperature dependence of the quadrupolar deuterium splitting ν_Q of a stretched PS-gel sample swollen in $CDCl_3$ (◆, solid line) and C_6D_6 (□, dashed line).

cooling. ^{2}H-1D spectra were measured in the range of 215−325 K in steps of 5 or 10 deg, allowing 10−15 min for calibration after each spectrum. As a control for temperature equilibration spectra were acquired twice: once after cooling and once after heating to the desired temperature. Since no hysteresis could be observed in the temperature plot of the RDCs, samples were considered to be equilibrated.

Field dependence of the quadrupolar splitting of $CDCl_3$ in ^{2}H-1D spectra was measured on Bruker DMX spectrometers with proton frequencies of 250, 600, 750, and 900 MHz. After a defined temperature (293 K) on the first spectrometer was set, the chemical shift difference of the hydroxyl and methyl signals of a standard methanol sample was determined carefully. Afterward the same methanol sample was used on all other spectrometers to adjust the temperature to the identical chemical shift difference, before measuring the quadrupolar splitting in the PS-gel sample. This way identical temperatures for the measurements could be accomplished.

To test relaxation filter methods, spectra using the pulse sequences shown in Figure 6 were acquired on a Bruker DMX600 spectrometer equipped with a ^{1}H, ^{13}C, ^{15}N, ^{19}F-quadruple resonance *x*,*y*,*z*-gradient probehead. The spectral widths for all HSQC-based spectra were 6009 Hz (2048 complex points) and 24 154 Hz (128 increments) in the ^{1}H and ^{13}C dimensions, respectively. A total of 4 transients/FID were acquired with a recycle delay of 1 s, leading to an overall time of 9 min/experiment. The spinlock used for suppression of the polystyrene signal as described in Figure 6A was calibrated to 3 kHz. Zero filling up to 256 points was applied in the ^{13}C dimension, and 90°-shifted squared sine-bell apodization functions were multiplied in both dimensions prior to Fourier transformation.

Results and Discussion

Alignment Properties of PS Gels. In ref 33 we could show that cross-linked polystyrene swollen in chloroform under the boundary conditions of an NMR tube can be effectively used as an alignment medium to measure residual dipolar couplings. However, the method certainly has its limitations and the aim of the investigations presented here is to find out the range of conditions under which stretched PS gels still show alignment properties that allow RDC measurement. A physical parameter of central importance is the sample temperature. Most liquid crystals show a phase transition with a specific transition temperature below or above which no partial alignment can be achieved.[7,14] We therefore studied the anisotropy change of stretched PS gels, monitored by the quadrupolar deuterium splitting, over the full temperature range of liquid $CDCl_3$ and C_6D_6. The result is shown in Figure 1: no abrupt changes could be observed, only a steady increase of the observed splitting of 1.1 and 0.35% on average per degree toward lower temperatures. Therefore no general limitation of the method can be seen in the temperature range of $CDCl_3$ and C_6D_6 as solvents, but for practical applications it should be noticed that a defined temperature must be chosen to guarantee identical alignment conditions if RDCs measured in different experiments shall be compared.

Table 1. Magnetic Field Strength Dependence of Quadrupolar Splitting of $CDCl_3$

	B_0 field, MHz			
	250	600	750	900
splitting, Hz	58.8	59.0	59.0	58.9

An important parameter for the mechanism of alignment is the static magnetic field dependence of the induced anisotropy. While gel alignment due to mechanical stretching should be independent of the magnetic field, autoalignment of polymer chains as previously observed for macromolecules[48,49] and liquid crystalline phases[25] should be field dependent. After careful calibration of the temperature, a series of quadrupolar splittings of a PS/$CDCl_3$ gel sample was measured for four different magnetic field strengths with the results shown in Table 1. The measured splittings are all within the error due to temperature variations and spectral noise, which we conservatively estimate to be about ±0.2 Hz. From this result we can deduce that the anisotropy of the gel originates from mechanical stretching only and no autoalignment of the polymer chains occurs, which is consistent with the finding that non-cross-linked PS dissolved in $CDCl_3$ does not cause any measurable quadrupolar deuterium splitting (data not shown). This also implies that no lower anisotropy limit is imposed by the PS-gel alignment method since the mechanical stretching of the polymer can be varied continuously.

Of course, the alignment properties of a stretched PS gel also depend on the consistency of the gel itself. As was shown previously,[33] the anisotropy induced by the gel is dependent on the amount of cross-linking agent used for polymerization. We repeated the study at lower polymerization temperature (45 °C) with 2,2′-azobis(2-methylpropionitrile) (AIBN) as a more reliable radical starter and different amounts of divinylbenzene (DVB) as cross-linker ranging from 0.05% to 5% (v/v). Five samples/concentration were swollen in $CDCl_3$, and quadrupolar deuterium splittings were recorded. The resulting graph is shown in Figure 2A: anisotropies corresponding to quadrupolar deuterium splittings in the range of 0−540 Hz for PS sticks with initial diameter of 4.0 mm could be achieved, which should basically cover all needs for partial alignment.

A point of major interest in the applicability of PS gels is the range of solvents in which molecules can be aligned. We therefore did a series of experiments where we tried to swell PS sticks in a number of organic solvents. In line with the very low solubility of non-cross-linked PS in these solvents (see e.g. ref 50), no swelling could be observed for very apolar solvents such as octane and relatively polar solvents such as acetone or acetonitrile. However, dichloromethane (DCM), tetrahydrofuran (THF), benzene, and dioxane showed significant swelling and were used for further experiments. The DVB dependence of

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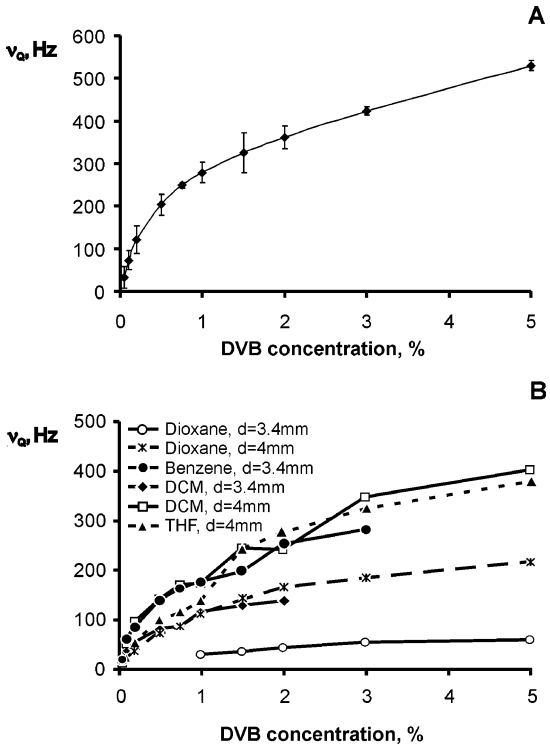

Figure 2. Deuterium quadrupolar splitting ν_Q of $CDCl_3$ as a measure of induced anisotropy with respect to amount of cross-linking agent DVB used for PS-stick polymerization. (A) ν_Q values of $CDCl_3$ are averaged over 4−5 different samples for each data point with standard deviations as error bars. Samples were prepared with PS sticks of 4 mm diameters. (B) ν_Q values are shown for 5−10% $CDCl_3$ added to the solvents dichloromethane, tetrahydrofurane, dioxane, and benzene. Samples were prepared with PS sticks of 3.4 and 4 mm diameter for the four solvents as indicated in the graph. Only one sample/data point was prepared. Deviations from a smooth curve as in (A) are probably due to variations in inner diameters of NMR tubes and glass tubes used for polymerization and slight distortions from a perfect cylindrical shape of some PS sticks used.

the induced anisotropy was measured by adding 5−10% $CDCl_3$ to the otherwise undeuterated solvents (Figure 2B). Quadrupolar deuterium splittings in the range of 0−400 Hz could be obtained in a few days for DCM and THF as solvents, showing overall similar behavior to chloroform. Benzene and dioxane both showed a relatively slow swelling with a different behavior concerning quadrupolar splittings. Samples of 3.4 mm initial polymer diameter swollen in benzene result in similar quadrupolar splittings as chloroform samples with 4 mm initial PS-stick diameter. Samples swollen in dioxane, on the other hand, show smaller quadrupolar splittings than observed for the other solvents.

To further test the alignment properties of the PS gel in the different solvents in more detail, we chose norcamphor (Chart 1) as a test sample which is soluble in all solvents of interest and allows an accurate determination of alignment tensors with only few measured heteronuclear RDCs. A total of 4 out of 10 signals in the HSQC experiments showed strong coupling artifacts and were not used for the alignment tensor determination. The remaining six dipolar D_{CH} couplings are summarized in Table 2 together with the parameters of the alignment tensors as derived by the program PALES.[47] A first inspection of RDCs obtained already reveals that alignment for the five different solvents is very similar but not identical: all derived alignment tensors have a strong negative A_{zz} component, but their orientation and the rhombic components differ slightly. The

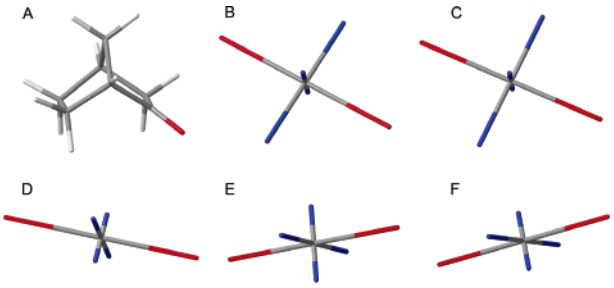

Figure 3. Illustration of the alignment tensors of norcamphor in stretched PS gels prepared in different organic solvents: (A) orientation of norcamphor as reference frame for the alignment tensors (axes A_{xx}, A_{yy}, and A_{zz} drawn with their length proportional to the magnitude of the Eigenvalues and with the orientation according to the Eigenvectors of the alignment tensors) in (B)−(F). Alignment tensors were derived with the program PALES for samples swollen in chloroform (B), dichloromethane (C), dioxane (D), tetrahydrofuran (E), and benzene (F) (cf. Table 2). Positive components of the alignment tensors are shown in red, and negative tensor components, in blue. The view angle relative to the molecule was chosen to pronounce the differences in alignment tensors.

relative angles of the resulting alignment tensors relative to each other are summarized in Table 3, varying from 10 to 21° for the different solvent combinations. The alignment tensors for DCM and chloroform are very similar, but for THF, dioxane, and benzene as solvents the three eigenvector components A_{xx}, A_{yy}, and A_{zz} are tilted with respect to each other. In Figure 3 the tensors for the five different solvents are shown in an orientation that pronounces the differences in alignment.

We also measured sets of ^1H,^{13}C-RDCs for menthol in PS gels swollen in the five different solvents. ^1H,^{13}C-RDCs are summarized in Table 4. Although the limited number of differently oriented C−H vectors in menthol does not allow the reliable determination of alignment tensors, the comparison of RDCs leads again to the conclusion that alignment in PS gels is solvent dependent. So, for example, many RDCs measured in THF show sign inversion compared to RDCs from other solvents, which is unambiguous evidence that the alignment tensors differ in this case.

The difference in alignment of norcamphor and menthol in the five different solvents can be explained in many ways: most likely there are specific interactions of the solvent with either the organic molecule of interest and/or the PS polymer. Also, the structure of the polymer or the solute itself might change in the different solvents. Maybe the different alignments can also be explained by variations in the dielectric constants for the solvents used (on this subject see e.g. ref 51). Solvent dependent alignment was also observed for poly(vinyl acetate) as a polymer,[35] and it will be very interesting to study these effects with additional solutes and polymer gels. However, the interpretation of the presented data in terms of a more or less sophisticated model cannot be the topic of this paper.

NMR Properties of PS Gels. Spectra of partially aligned samples are different from conventional isotropic liquid samples. In general, more complex multiplet patterns because of additional dipolar couplings through space must be expected that mostly lead to what looks like a single broad line (cf. Figure 5). On the other hand, narrowed signals can be observed in few cases when RDC's of opposite sign reduce the splitting due to already existing *J*-couplings (e.g. left signal 23 in Figure 5B,C). The appearance of a spectrum depends on many parameters such

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Table 2. Measured $^1H,^{13}C$-RDCs of Directly Bound Atoms at ^{13}C Natural Abundance and Resulting Alignment Tensor Parameters (Axial and Rhombic Components (D_a, D_r), Correlation Factor R, and Principal Axes A_{xx}, A_{yy}, and A_{zz} with Their Corresponding Eigenvectors) for Norcamphor in Organic Solvents As Calculated with the Program PALES

assgnt	$CDCl_3$	DCM	dioxane	THF	benzene
D_{C1-H1} (Hz)	5.9	3.5	3.3	12.9	2.5
D_{C3-H3n} (Hz)	1.1	0.2	−2.9	−3.1	−2.5
D_{C3-H3x} (Hz)	14.0	7.9	8.2	20.1	6.1
D_{C4-H4} (Hz)	−3.9	−1.0	0.2	−1.0	1.0
D_{C7-H7a} (Hz)	−13.3	−10.3	−11.2	−23.9	−8.8
D_{C7-H7s} (Hz)	15.7	8.4	7.6	19.1	5.2
D_a(av/std dev)	$-2.37 \times 10^{-4}/3.89 \times 10^{-6}$	$-1.40 \times 10^{-4}/5.00 \times 10^{-6}$	$-1.48 \times 10^{-4}/1.19 \times 10^{-5}$	$-3.75 \times 10^{-4}/4.98 \times 10^{-6}$	$-1.01 \times 10^{-4}/7.52 \times 10^{-6}$
D_r(av/std dev)	$-7.68 \times 10^{-5}/4.37 \times 10^{-6}$	$-4.72 \times 10^{-5}/6.91 \times 10^{-6}$	$-5.45 \times 10^{-5}/1.57 \times 10^{-5}$	$-6.18 \times 10^{-5}/6.18 \times 10^{-6}$	$-3.12 \times 10^{-5}/9.62 \times 10^{-6}$
R	1.000	0.999	0.992	0.998	0.995
A_{xx}	1.22×10^{-4}	7.07×10^{-5}	7.21×10^{-5}	2.83×10^{-4}	5.76×10^{-5}
A_{yy}	3.51×10^{-4}	2.08×10^{-4}	2.20×10^{-4}	4.66×10^{-4}	1.42×10^{-4}
A_{zz}	-4.74×10^{-4}	-2.79×10^{-4}	-2.92×10^{-4}	-7.49×10^{-4}	-2.00×10^{-4}
EV A_{xx}	−0.27, −0.51, 0.82	−0.24, −0.41, 0.88	−0.21, −0.28, 0.94	0.10, −0.67, 0.74	0.21, −0.64, 0.74
EV A_{yy}	−0.74, 0.65, 0.16	−0.70, 0.70, 0.13	0.77, −0.64, −0.23	0.76, −0.43, −0.49	0.75, −0.38, −0.54
EV A_{zz}	0.61, 0.57, 0.55	0.67, 0.59, 0.45	0.61, 0.71, 0.35	0.64, 0.61, 0.47	0.63, 0.67, 0.40

Table 3. Relative Angles (deg) of the Alignment Tensors of Norcamphor in Solvents (See Table 2) in Five-Dimensional (5D) Space As Calculated with the Program PALES

	5D-angles				
	$CDCl_3$	DCM	dioxane	THF	benzene
$CDCl_3$		10.5	21.2	14.6	20.9
DCM	10.5		16.9	13.4	18.2
dioxane	21.2	16.9		20.1	17.6
THF	14.6	13.4	20.1		10.4
benzene	20.9	18.2	17.6	10.4	

Table 4. Measured $^1H,^{13}C$-RDCs for Menthol in Stretched PS Gels Swollen in Organic Solvents

coupling	$CDCl_3$	DCM	dioxane	THF	benzene
D_{C1-H1} (Hz)	−3.6	−8.7	−7.2	2.7	−3.0
D_{C2-H2} (Hz)	−3.3	−9.7	−2.4	6.3	−2.0
D_{C3-H3a} (Hz)	−4.9	−12.7	−17.7	16.5	1.2
D_{C3-H3e} (Hz)	−0.9	−2.9	−13.6	−3.1	4.2
D_{C4-H4a} (Hz)	−4.4	−20.2	−7.1	−0.9	−5.6
D_{C4-H4e} (Hz)	6.2	−5.0	−10.3	−14.0	6.2
D_{C5-H5} (Hz)	−4.4	−10.7	−5.9	−0.4	−5.0
D_{C6-H6a} (Hz)	−4.8	−16.9	−11.4	1.2	−2.8
D_{C6-H6e} (Hz)	0.1	−3.1	−8.4	−5.3	−0.2

as the density of NMR-active nuclei, the strength and orientation of alignment, and the *J*-coupling network.

But what is the line width that can be principally achieved in a stretched PS gel? The consistency of the polymer can be considered quite heterogeneous with a wide distribution of shorter and longer polymer chains and varying concentrations of cross-links between chains which might affect the line shape of a sample. We therefore tried to get a good shim for a swollen PS gel with an intermediate induced anisotropy ($\nu_Q(CDCl_3) \sim$ 110 Hz): a line width below 1 Hz for the $CHCl_3$ proton signal was easily obtained as shown in Figure 4A. Line shape distortions due to gel microheterogeneity therefore must be of minor importance and can be neglected.

Besides the multiplet pattern also resonance frequencies are affected by the stretched PS gel (Figure 5B,C). Two effects are expected: first, PS works as a cosolvent that shifts all resonances upfield compared to the conventional liquid $CDCl_3$ sample; second, a change in chemical shifts is directly induced by the anisotropy of the stretched gel, leading to so-called residual chemical shift anisotropy (RCSA).[52−56] Chemical shift changes in aliphatic regions as shown in Figure 5B,C are mainly due to the cosolvent effect since CSA is relatively small in this case

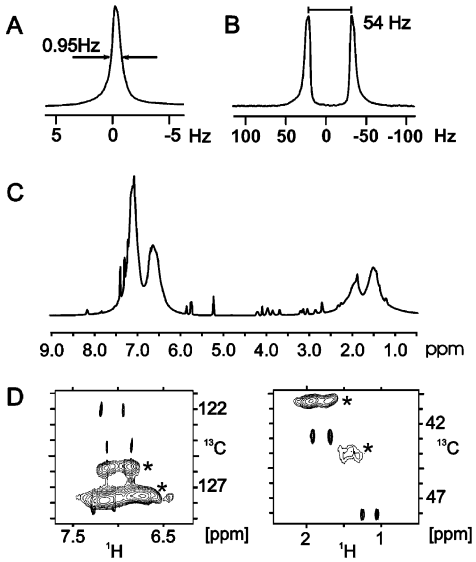

Figure 4. Achievable line width and polymer signals in a stretched PS gel. (A) Signal of residual $CDCl_3$ in the gel: Although most signals are broadened due to residual dipolar couplings, the experimentally achievable line width in a PS gel is not significantly larger than in conventional liquid samples. (B) Typical quadrupolar splitting of $CDCl_3$ as observed in equilibrated PS-gel samples. (C) A 1D-spectrum of a 50 mg strychnine sample in a stretched PSgel is shown. The strong broad signals in the aliphatic and aromatic regions originate from PS. (D) The polymer signals do not interfere with the signals of strychnine in heteronuclear 2D-experiments. In the case of strychnine the full set of $^1H-^{13}C$ heteronuclear RDCs can be measured. 2D-contours resulting from PS-gel signals are marked with asterisks.

(for sizes of measured RCSA, see e.g. ref 7). Aromatic ^{13}C-chemical shifts, however, might be significantly shifted due to relatively strong RCSA. The main disadvantage resulting from the chemical shift changes is that in certain cases it might be necessary to repeat parts of the assignment process.

Finally, the main drawbacks of PS gels as alignment media are the undesired NMR signals originating from the polymer itself. In Figure 4C the proton spectrum of a strychnine PS-gel sample is shown with broad PS signals in the aromatic and aliphatic region. The difference in line width of strychnine and

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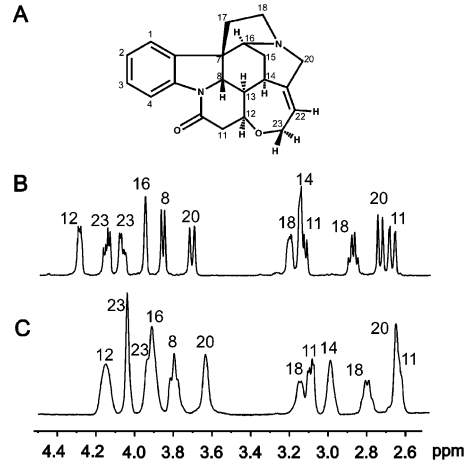

Figure 5. Chemical shift changes of strychnine (A) due to PS gel and section of 1D-spectra of strychnine acquired in $CDCl_3$ (B) and in a stretched PS gel swollen in $CDCl_3$ (C). PS as cosolvent causes upfield changes in chemical shifts, and small additional changes due to RCSA might be present. Chemical shifts in both spectra were referenced to internal TMS.

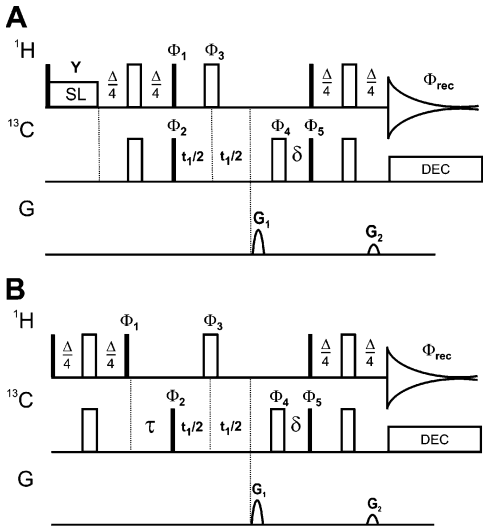

Figure 6. HSQC-pulse sequences with building blocks used to suppress signals originating from PS: (A) standard HSQC experiment with additional spin lock period (SL) as a T_2 relaxation filter; (B) standard HSQC experiment with extended *z*-filter delay τ which uses the difference of $^1H-^1H$ NOE relaxation rates of the polymer network relative to the small molecule observed. Phase cycles are as follows: $\Phi_1 = y$; $\Phi_2 = x, -x$; $\Phi_3 = x, x, -x, -x$; $\Phi_4 = \Phi_5 = x, x, x, x, -x, -x, -x, -x$; $\Phi_{rec} = x, -x, x, -x, -x, x, -x, x$. Filled and open bars correspond to 90 and 180° pulses, respectively, with *x*-phase unless indicated otherwise. $\Delta = 1/^1J_{CH}$, and δ compensates for G1 gradient duration. Gradients are of equal length (1 ms) with ratio G1:G2 = 80:20.1 for $^1H,^{13}C$-correlation. Phase sensitive detection in the indirect dimension is achieved by cycling G1, Φ_2, and Φ_{rec} according to the echo−antiecho mode.

PS signals allows their clear distinction, and the measurement of coupling constants out of one-dimensional experiments is possible but difficult. In two-dimensional spectra the situation is strongly improved, since the probability of signal overlap is reduced and data analysis can be accomplished in a conventional way. Strychnine e.g. allows the measurement of a complete set of $^1H-^{13}C$ dipolar coupling constants from an uncoupled HSQC spectrum because only one aromatic signal partially overlaps with PS, for which the reliable measurement of RDCs is still possible (Figure 4D[33]). However, suppression of PS signals would increase the overall quality of the spectra and allow the measurement of less concentrated samples. We therefore tested

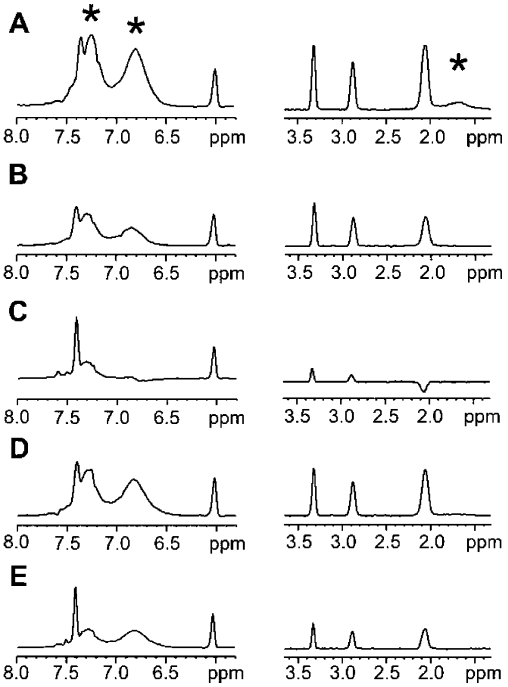

Figure 7. (A−E) Traces of aromatic and aliphatic regions from HSQC spectra with relaxation filters implemented as described in Figure 6. No filtering (A), spin lock filtering with 100 ms (B) and 400 ms (C) spin lock times, and *z*-filtering with 100 ms (D) and 400 ms (E) *z*-filter delays were applied in the experiments with otherwise identical parameters. Broad polymer signals are indicated with asterisks; all other signals originate from strychnine dissolved into the gel. While aliphatic signals can easily be suppressed by both methods (their intensities are already strongly reduced in the HSQC experiments without relaxation filter (A)), suppression of the more flexible aromatic PS signals can only be achieved with significant loss of desired strychnine signals. Spin locking (with 3000 Hz rf-amplitude used) can lead to inversion or even suppression of strychnine signals (C), an effect not observed for the *z*-filtering method (E).

two relaxation filtering approaches for PS-signal reductions: The first filter uses the difference in T_2 relaxation rates of the small molecule of interest compared to the large polymer and is accomplished by a spin lock period in which the magnetization is kept in the *xy*-plane (Figure 6A). A second method uses a *z*-filter period to suppress spin pairs with efficient relaxation pathways for the operator $2I_zS_z$. The relaxation in this case is mainly determined by $^1H-^1H$ NOE which is quite efficient for the PS-polymer network (Figure 6B). The separation in this case is therefore best if the molecule of interest has a correlation time with vanishing nuclear Overhauser enhancement. The quality of suppression for both approaches can be seen in Figure 7 for various filter periods: both methods work well for the backbone aliphatic PS signals which are already strongly reduced in the conventional HSQC (Figure 7A) and can be suppressed completely with relaxation filter delays of 100 ms (Figure 7B,D). The aromatic PS signals instead appear to have relatively short correlation times, probably due to the increased flexibility in the side chain. Long relaxation filter periods are necessary for partial suppression of these signals, and even after 400 ms relaxation filter periods, aromatic PS signals remain visible (Figure 7C,E).

In comparison of the two methods introduced in Figure 6, spin locking provides the slightly better PS-signal suppression for identical filter periods. However, the approach can lead to offset dependent suppression and even inversion of desired signals (Figure 7C) if the spin lock field used cannot cover the

bandwidth of the spectrum. In addition, irradiation of medium to high power rf for longer periods will lead to significant sample heating. Here, an extended *z*-filtering delay appears to be a very attractive alternative.

Conclusion

A detailed characterization of stretched polystyrene gels as alignment medium for organic molecules in high-resolution NMR has been given. Important results are that the induced anisotropy is completely independent of the static magnetic field strength but depends strongly on temperature. Cross-linked PS sticks cannot be used in very apolar solvents such as octane and more polar solvents such as acetone or acetonitrile, but good alignment properties are obtained e.g. in chloroform, DCM, dioxane, THF, and benzene. We find a variation of alignment for norcamphor and menthol in different solvents applied.

NMR spectra in well-equilibrated PS gels can in principle be shimmed to line widths below 1 Hz, but signals show, of course, significantly changed multiplet patterns due to many short- and long-range RDCs. Upfield chemical shift changes are caused by PS working as a cosolvent, and additional chemical shift changes are expected to result from RCSA. Serious problems for practical applications are residual PS NMR signals: while aliphatic PS signals can be readily suppressed using either spin lock or newly derived *z*-filter-based relaxation filters, signals of the more flexible aromatic side chains survive even relaxation filter periods of 400 ms. In addition, chemically synthesized polymers very likely contain traces of polymerization initiators and monomers that will lead to additional signals with narrow lines that cannot be suppressed by any relaxation filter. It therefore must be concluded that especially samples at low concentrations (less than ~50 mM) with resonances in the aromatic region cannot be used effectively with PS gels as alignment media. In this case only deuterated PS gels or other alignment media like poly(dimethylsiloxane)-based polymer gels[34] provide a viable alternative. Still, for organic molecules chemically inert stretched PS gels seem to be among the most promising alignment media known so far.

The *z*-filter-based relaxation filter proposed in this article appears to be a considerable alternative to spin-lock methods since no additional sample heating is induced and offset-dependent artifacts are avoided. It can be applied in any heteronuclear correlation experiment in which the operator $2I_zS_z$ is produced as an intermediate, as is for example the case in all INEPT-based transfer steps.

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