NMR Spectroscopic Methods (1)

Stretched Poly(vinyl acetate) Gels as NMR Alignment Media for the Measurement of Residual Dipolar Couplings in Polar Organic Solvents**

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Residual dipolar couplings (RDCs) have revolutionized the NMR-based structure determination of large biomolecules[1–4] and lately demonstrated their potential in the field of small molecules.[5–12] The measurement of RDCs relies on the partial orientation of the molecule of interest, for which a so-called alignment medium is necessary. In aqueous solution a variety of alignment media are known, for example,

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[**] B.L. and H.K. thank the Fond der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (Emmy Noether fellowship LU 835/1-2; Ke 147/37-2) for financial support.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200461241

bicelles,[13] filamentous phage,[14] liquid-crystalline phases,[15] or stretched polyacrylamide-based gels.[16,17] For relatively apolar organic solvents, liquid crystals such as poly-γ-benzyl-L-glutamate (PBLG)[5–7,18] or deuterated 4-*n*-pentyl-4′-cyanobiphenyl (PCBP)[19] as well as stretched polystyrene (PS)[11] and polydimethylsiloxane (PDMS)[12] gels are used to obtain structural information from RDCs. For polar organic solvents such as methanol or DMSO, however, no alignment medium is available yet. Herein we propose the use of stretched poly(vinyl acetate) (PVAC) gels for partial orientation of molecules dissolved in polar organic solvents.

Crosslinked PVAC-polymer sticks were produced in a similar way to that described previously for PS.[11] Glass tubes with inner diameters of 2.4 mm, 3.4 mm, and 4.0 mm were sealed by melting one end and dried carefully. They were then treated with a 1:1 mixture of chlorotrimethylsilane and dichloromethylsilane for 18 h to ensure hydrophobic glass surfaces. After being washed with dichloromethane, the tubes were dried at 50 °C. Vinyl acetate (Fluka) and adipic acid divinyl ester (ABCR) were filtered with basic aluminum oxide (pH 10) and distilled under reduced pressure. The monomers then were degassed for 15 min under vacuum in an ultrasonic bath and ventilated with argon. Immediately afterwards, vinyl acetate, adipic acid divinyl ester, and azoisobutyronitrile were mixed in the desired concentrations and transferred to the glass tubes. The open end was sealed by melting, and polymerization was carried out for 5 days at 45 °C and for another 2 days at 60 °C.

As a first test, we put crosslinked PVAC sticks in various organic solvents and watched the swelling behavior. The sticks exhibited swelling in essentially all tested solvents, such as chloroform, THF, dioxane, benzene, ethyl acetate, acetone, acetonitrile, methanol, and dimethyl sulfoxide (DMSO). We repeated the swelling inside NMR tubes and added $CDCl_3$ (5% *v*/*v*) to the non-deuterated solvents to allow the monitoring of any induced anisotropy by the deuterium quadrupolar splitting ν_Q. In all cases, significant splittings with relatively sharp lines could be observed (Table 1) after allowing the gels to equilibrate for 13 days. Furthermore, we recorded the dependence of the quadrupolar splitting on the amount of crosslinker used for polymerization for four solvents (DMSO, MeOH, THF, and dioxane; Figure 1). The resulting curves are similar to that observed for PS gels,[11] corroborating the general trend that higher crosslinking concentrations lead to stronger induced anisotropies.[12]

In a next step we tested the alignment properties of norcamphor in PVAC gels swollen in the four deuterated solvents available in our laboratory. Norcamphor (Table 2) was chosen

Table 1: Quadrupolar deuterium splittings [Hz] of $CDCl_3$ (5% *v*/*v*) added to PVAC sticks (0.1% *v*/*v*) cross linker, 3.4 mm and 4.0 mm inner diameter) swollen in various non-deuterated solvents.

	MeCN	Acetone	EtOAc	Benzene	$CDCl_3$
3.4 mm	36.6	33.8	49.4	83.1	72.8
4.0 mm	47.5	54.7	67.7	–[a]	106.1
	DMSO	MeOH	THF	Dioxane	
4.0 mm	10.0	35.2	54.6	39.5	

[a] Gel disrupted during swelling.

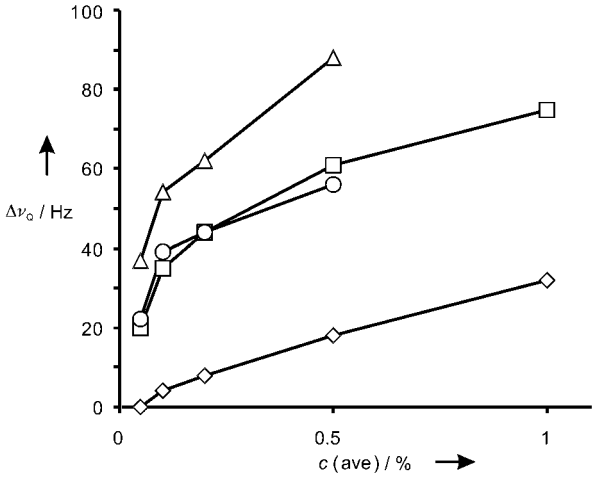
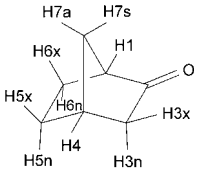


Figure 1. Dependence of the quadrupolar deuterium splitting ν_Q of $CDCl_3$ on the amount of crosslinker adipic acid divinyl ester (AVE). To obtain the splittings, $CDCl_3$ (5% *v*/*v*) was added to the PVAC sticks swollen in the otherwise non-deuterated solvents (◇: DMSO, □: MeOH, △: THF, ○: dioxane).

Table 2: RDCs and alignment-tensor parameters of norcamphor[a] dissolved in PVAC gels that are swollen in different deuterated solvents.



	[D$_6$]DMSO	CD_3OD	CD_3CN	$CDCl_3$
$\Delta\nu Q(CDCl_3)$	18.5 Hz[b]	70.4 Hz[b]	85.2 Hz[c]	21.7 Hz[d]
RDCs				
C_1–H_1	−2.37	−0.86	1.24	0.31
C_3–$H_{3,exo}$	−3.61	−12.61	−1.04	−0.44
C_3–$H_{3,endo}$	−15.51	−7.24	−8.51	−2.07
C_4–H_4	10.31	18.22	6.21	1.29
C_7–$H_{7,anti}$	15.71	−7.79	0.61	−3.70
C_7–$H_{7,syn}$	−10.83	8.22	−6.18	−3.97
A_{xx}	-1.6180×10^{-4}	4.1254×10^{-5}	-3.1161×10^{-5}	-8.9383×10^{-6}
A_{yy}	-3.6670×10^{-4}	9.2611×10^{-4}	-2.1580×10^{-4}	$-7.5429\times^{-5}$
A_{zz}	5.2850×10^{-4}	-9.6736×10^{-4}	$2.4696\times^{-4}$	$8.4368\times^{-5}$
D_a	2.6425×10^{-4}	-4.8368×10^{-4}	1.2348×10^{-4}	4.2184×10^{-5}
D_r	6.8302×10^{-5}	-2.9495×10^{-4}	6.1546×10^{-5}	2.2164×10^{-5}
R^2	0.992	0.990	1.000	0.854

[a] Norcamphor: ≈130 mg/sample. [b] PVAC: 0.5% crosslinker, stick diameter: 4.0 mm. [c] PVAC: 0.2% crosslinker, stick diameter: 4.0 mm. [d] PVAC: 0.1% crosslinker, stick diameter: 2.4 mm.

as a sample for two reasons: it is highly soluble in almost every organic solvent and its protons all point in different directions. In [D_6]DMSO, CD_3OD, CD_3CN, and $CDCl_3$ we were able to measure at least six D_{CH} RDCs and derive the alignment tensors by using the program PALES with the *bestFit* option[20] (Table 2). Interestingly, the four alignment tensors differ significantly: D_a/D_r ratios differ for the four solvents and the axial component for methanol even has the opposite sign compared to the others. We can only speculate about this different behavior, but most probably specific interactions of the solvents with the polymer and/or the solute lead to this phenomenon. However, the most important result for the use of stretched PVAC gels as alignment media is that RDCs in the desired range of ± 20 Hz can be measured in all cases.

RDCs should be especially important for the structure determination of natural products. We therefore applied the method also to the well-characterized antibiotic sphaeropsidin A as a more realistic test sample.[21–23] After diffusion of ≈ 6 mg of sphaeropsidin A into an already swollen PVAC/DMSO gel with a quadrupolar $CDCl_3$ splitting of 10.1 Hz, two coupled HSQC spectra, optimized for aliphatic and olefinic nuclei, were acquired on a Bruker DMX900 spectrometer (Figure 2). Compared to sphaeropsidin A cross peaks, relatively strong PVAC and DMSO signals are present in both spectra, but D_{CH} RDCs can still be measured in most cases since sphaeropsidin A and PVAC signals do not overlap. Only the methylene protons attached to C_{11} show significant overlap and second-order effects so that a reliable RDC measurement was not possible. All couplings were carefully measured by extracting slices out of the 2D spectra and applying the phase correction approach described in detail elsewhere.[9] As a starting point we looked at the eight C–H vectors containing the atoms C1, C2, C3, C5, and C14. The diastereotopic protons of the methylene groups of C2 and C3 were easily derived by using the simple method to distinguish between axial and equatorial C–H vectors.[9] The initial alignment tensor calculated from six unambiguous D_{CH} couplings (also including C–H vectors from C5 and C14) then allowed the prochiral assignment of the methylene groups containing C1 and C12 (for methodology used, see references [6,7]). This is remarkable, because in both cases the difference in distance of axial/equatorial or *syn*/*anti* protons, respectively, to unambiguously identified neighboring protons such as 5-H or the methyl group containing C17 is only ≈ 0.1 Å, too small to be identified reliably by NOE studies. By deriving D_{CC} RDCs from the measured D_{CH} couplings with the formula given in reference [7], finally also the methyl groups at C4 could be assigned. RDCs of spin pairs containing C15, C16, and C17 could not be fitted to any reasonable structural model. This most probably is indicative for intrinsic dynamics that lead to averaged RDCs at this part of the molecule. Taking into account the significant changes in chemical shifts of C14–C16, and 14-H–16-H, it might even be concluded that the gel introduces a difference in population of the exchanging conformations in this region. However, this effect is not yet understood. The 12 remaining assigned RDCs were used to refine the alignment tensor that is shown together with the correspondence of experimentally determined and back-calculated RDCs in Figure 3b–d. The correlation factor R^2 of 0.966 in this case is a clear indication that the structural model is consistent with the experimental data, which also can be seen easily in Figure 3e.

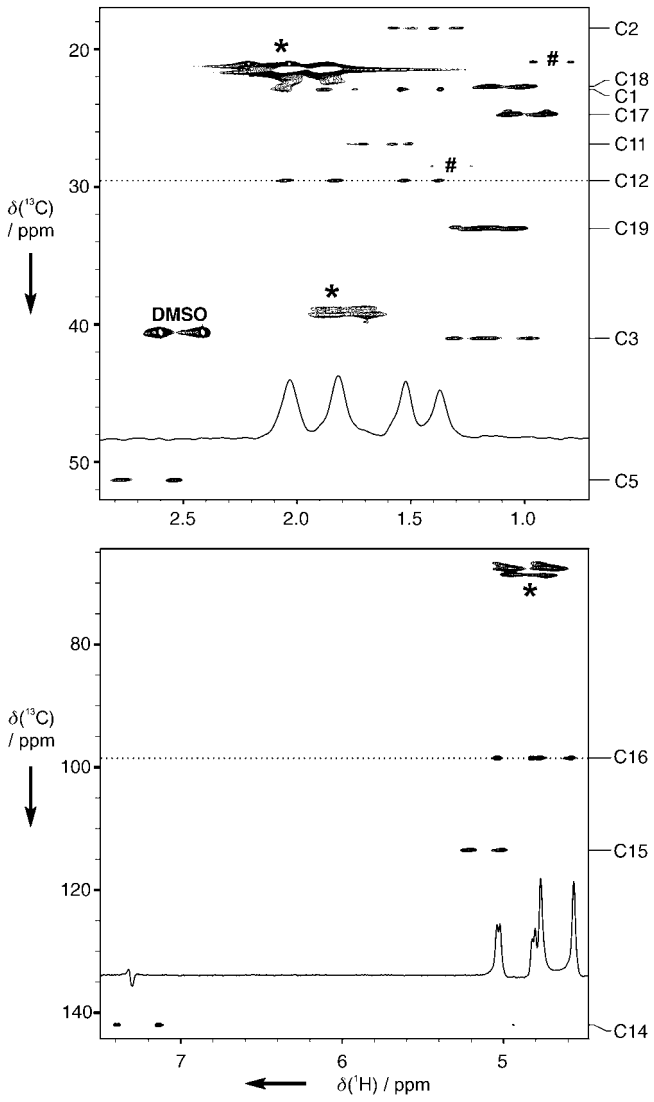


Figure 2. Aliphatic (top) and olefinic (bottom) coupled $^{13}C,^{1}H$-HSQC spectra acquired on ≈ 6 mg of sphaeropsidin A dissolved in a PVAC/DMSO gel. DMSO signals (DMSO), PVAC signals (*), and signals originating from unpolymerized monomers (#) are indicated in the spectra. Slices at the dotted lines are shown to give an impression on the overall quality of the cross peaks.

In summary, stretched PVAC gels can be used as scalable alignment media for polar organic solvents. With this possibility the gap between relatively apolar solvents such as dichloromethane or chloroform and aqueous solutions is closed and RDCs can now be measured in practically all common NMR solvents. The applicability of the new alignment method was demonstrated on norcamphor and the antibiotic sphaeropsidin A. Although PVAC signals did not pose a serious problem in heteronuclear correlation experiments, more sophisticated NMR spectroscopy methods such

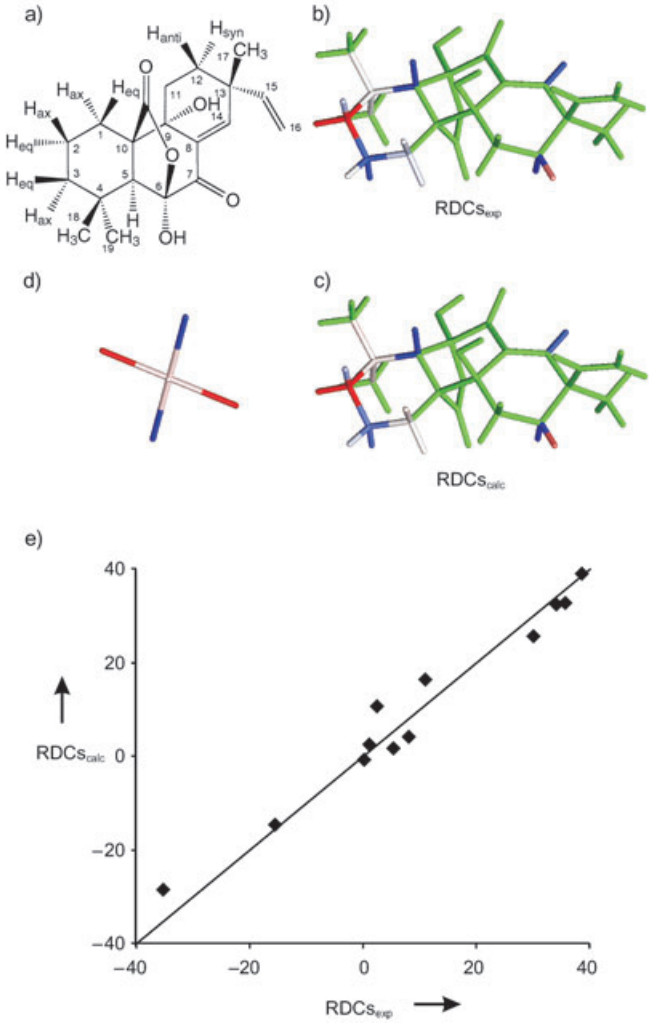


Figure 3. Correspondence between experimental and back-calculated RDCs of sphaeropsidin A dissolved in a PVAC/DMSO gel. a) Structure and numbering of sphaeropsidin A. b) and c) color representation of the experimental and back-calculated RDCs, respectively, onto sphaeropsidin A. Red corresponds to negative, blue to positive, white to intermediate, and green to no RDCs measured. d) Representation of the alignment tensor in the coordinate frame of sphaeropsidin A as shown in b, c) with red being negative and blue positive tensor axes. e) The plot of experimental versus back-calculated RDCs shows a good correlation.

as relaxation filters should be included in experiments to obtain spectra of higher quality.[24]

Received: July 8, 2004
Revised: August 9, 2004

Keywords: gels · NMR spectroscopy · partial alignment · residual dipolar coupling

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