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Stretched Poly(acrylonitrile) as a Scalable Alignment Medium for DMSO

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Anisotropic NMR parameters in partially aligned samples, such as residual dipolar couplings (RDCs), provide valuable structural information for obtaining conformation and configuration of biomolecules[1] as well as organic molecules, such as natural products, sugars, or peptides (refs 2 and 3 and references cited therein). For weak anisotropic effects to be investigated by NMR, alignment media need to be of sufficiently low alignment strength to enable RDCs and other measurements to be made while still maintaining the high resolution of liquid-state NMR spectra. While a multitude of adequate alignment media exists for aqueous solutions[1] and apolar organic solvents (refs 4 and 5 and references cited therein), only few orienting media with limited applicability are reported for the standard solvent in pharmaceutical NMR, DMSO.

Mixtures of pentaethylene glycol monododecyl ether ($C_{12}E_5$) with D_2O and DMSO as a lyotropic nematic phase have a narrow range of alignment.[6] Stretched gels based on cross-linked poly(vinyl acetate) are freely scalable in their alignment for a large number of small molecules, but cyclic peptides as an important class of molecules do not diffuse into it (ref 7 and unpublished results). Finally, poly(acrylamide)-based PH-PAA gels worked well with a number of peptides, but they exclude many molecules due to their negative charge.[8] Therefore, the aim of our research was the development of a freely scalable, uncharged alignment medium applicable to a wide range of molecules.

We started to look systematically for polymers with a minimum amount of NMR signals and with desired solubility properties. Polyglycolide, polylactide, and the halogenated polymers poly(vinyl fluoride), poly(vinylidene fluoride), and poly(vinylidene chloride) all showed insufficient solubility in DMSO. However, poly(vinyl chloride) (PVC) as well as poly(vinyl alcohol) (PVAlc) and poly(acrylonitrile) (PAN) are highly soluble in the polar organic solvent and were further examined. Following the successful example of poly(dimethylsiloxane) cross-linked by accelerated electrons,[5] we tried to cross-link these polymers by irradiation in order to avoid undesired NMR signals originating from chemical cross-linking. Unfortunately, PVC without stabilizing additives degrades slowly after treatment with fast electrons. PVAlc and PAN, instead, remain stable after cross-linking with accelerated electrons. Since DMSO is also used because of its aprotic properties and as PVAlc provides hydroxyl groups, we concentrated on PAN.

In a first step, sticks were obtained by precipitating PAN out of DMSO solution (see Supporting Information for details). The polymer sticks were then cross-linked by irradiation with accelerated electrons.[4] Depending on the amount of cross-linking, irradiated polymer sticks swelled to approximately 15 times their original volume in DMSO and 20 times their volume in DMF. Swelling in water or less polar organic solvents, such as methanol or chloroform, was not observed (Figure 1A). By letting the polymer stick swell inside an NMR tube, stretching of the gel is achieved (Figure 1B) and anisotropic NMR interactions such as the quadrupolar splitting of DMSO-d_6 in the deuterium 1D can be measured.

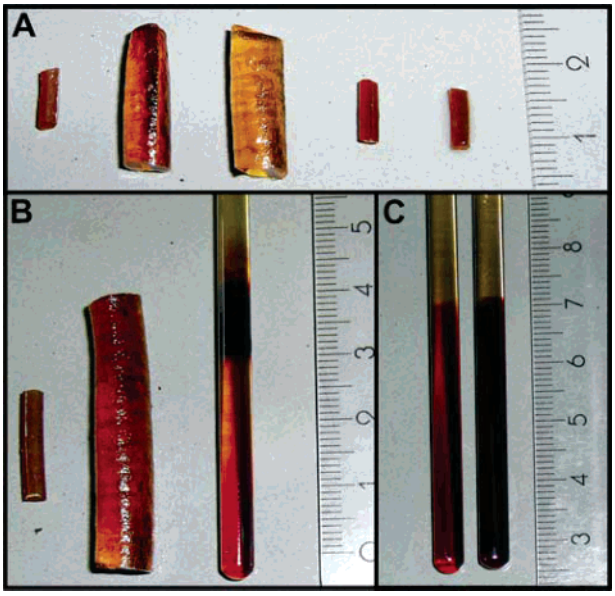

Figure 1. Swelling of reddish-brown PAN/DMSO gels in various solvents and readily prepared samples for RDC measurements. (A) From left to right: dry PAN polymer stick of 2.4 mm diameter and identical sticks after 5 days in DMSO, DMF, methanol, and chloroform. (B) From left to right: Polymer stick of 3.2 mm diameter unswollen, freely swollen in DMSO, and swollen and stretched inside an NMR tube. (C) Samples prepared from highly concentrated PAN/DMSO solution irradiated inside the NMR tubes with 280 (left) and 480 kGy (right) accelerated electrons and subsequent addition of DMSO on top of the gel. NMR tubes are darkened by the accelerated electrons.

A second, more elegant and robust way of obtaining stretched polymer gels is the irradiation of a highly concentrated PAN/DMSO solution by accelerated electrons directly in the NMR tube. If DMSO is added on top of the in-tube cross-linked polymer, the gel swells further and is automatically stretched (Figure 1C).

The ^{1}H NMR spectrum of a PAN/DMSO gel prepared directly in the NMR tube is shown in Figure 2A. The cross-linked PAN results in two broad signals at 2.0 (CH_2 groups) and 3.2 ppm (CHCN groups). In addition, a triplet from $^{14}NH_4^+$ (7.0 ppm) and signals of unknown impurities (3.8, 6.0, 6.3 ppm) are observed. These impurities are already visible in non-cross-linked PAN, but their intensities seem to increase slightly upon irradiation.

Figure 2B,C shows the ^{1}H amide region and a coupled ^{1}H,^{13}C HSQC of 8 mg cyclo(-D-Ala-Ala-Ala-(*N*Me)Ala-Ala-)[9] diffused into a PAN/DMSO gel with a final concentration of the two observed

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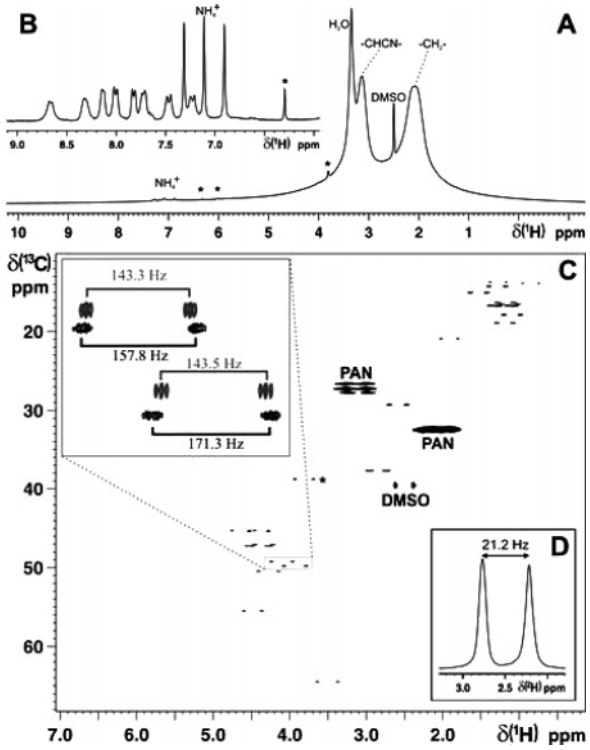

Figure 2. Various spectra of a PAN/DMSO gel used for measuring RDCs on cyclo(-D-Ala-Ala-Ala-(*N*Me)Ala-Ala-). (A) ^{1}H 1D of the gel without peptide added. Asterisks indicate small impurities, and other signals are annotated correspondingly. (B) Amide region of the peptide diffused into the gel. The two conformations of about equal intensities are visible. (C) The coupled ^{1}H,^{13}C HSQC for the extraction of one-bond coupling constants. The inset in the upper left corner shows two example cross-peaks, with the unaligned spectrum in gray for comparison. (D) The deuterium spectrum of the sample yields a quadrupolar splitting of DMSO-d_6 of 21.2 Hz, which can be used to characterize the relative alignment strength of PAN/DMSO gels.

Table 1. ^{1}H,^{13}C RDCs Obtained from Spectra Shown in Figure 2C for the Two Conformers of Cyclo(-D-Ala-Ala-Ala-(*N*Me)Ala-Ala-)

	Conformer 1		Conformer 2	
	Cα–Hα	Cβ–Hβ	Cα–Hα	Cβ–Hβ
D-Ala1	5.7 Hz	0.4 Hz	22.8 Hz	−5.5 Hz
Ala2	14.5 Hz	−0.8 Hz	27.8 Hz	7.3 Hz
Ala3	12.4 Hz	2.0 Hz	36.4 Hz	−0.1 Hz
*N*Me-Ala4	7.4 Hz	1.8 Hz	19.5 Hz	−5.4 Hz
Ala5	6.0 Hz	−4.5 Hz	17.0 Hz	1.6 Hz

conformers of ∼16 mM. As the intensive polymer signals do not overlap with the signals of the peptide, the full set of one-bond D_{CH} RDCs could be obtained (see Table 1).

Since other solute molecules might well have overlapping cross-peaks, we investigated several possibilities of reducing the backbone polymer signals. Presaturation of one or both of the backbone signals leads to a significant decrease of peak intensities but might also infer efficient saturation of the solute molecule. We obtained best results by applying a CPMG-based relaxation filter[10] or a less power consuming *z*-relaxation filter[2] (see Supporting Information for spectra).

As with all gel-based alignment media, the degree of alignment in PAN/DMSO gels can easily be scaled. Monitoring the quadrupolar splitting of the deuterium signal of DMSO-d_6 reveals that the strength of alignment depends on the amount of cross-linking (i.e., the irradiation dose), the chain length of the non-cross-linked polymer, and the concentration of the PAN/DMSO solution irradiated inside the NMR tube (see Supporting Information). We obtained gels with quadrupolar splittings between 3 and 40 Hz.

A drawback for the presented technique is the slow equilibration of the stretched gels due to the viscosity of the solvent, which typically takes several weeks at room temperature. Higher temperatures and silylated NMR tubes help to speed up this process.

To explore the range of solutes compatible with PAN/DMSO gels, we measured ^{1}H,^{13}C one-bond RDCs for eight compounds: norcamphor as a small organic compound, the standard sugars glucose and sucrose, the peptides cyclo(-*a*AA(*N*Me)AA-), cyclo(-R(Pbf)GD(O*t*Bu)fK-), cyclo(-RNalAGyR-), and hymenistatin,[11] and the natural product cylindramide[12] (see Supporting Information for selected compounds). All compounds tested so far readily diffused within a couple of days to a week into the pre-equilibrated PAN/DMSO gels and resulted in high-quality spectra. Structural work for two of the peptides and the natural product is in progress and will be published elsewhere.

In summary, we have shown that stretched poly(acrylonitrile)/DMSO gels are very useful alignment media for the measurement of anisotropic NMR parameters. The few broad NMR signals resulting from the polymer backbone can be reduced by relaxation filters, and small detected impurities can be neglected. As the polymer is freely scalable, uncharged, and compatible with peptides and other molecular classes, it is widely applicable and even complementary to existing alignment media.

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Supporting Information Available: The effect of irradiation dose, polymer chain length and concentration on the alignment strength of PAN/DMSO gels, data concerning their equilibration process, spectra demonstrating the effectiveness of relaxation filters for polymer signal suppression, experimental data for norcamphor, sucrose, and the cyclic pentaalanine, and a description of gel preparations are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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