NMR Spectroscopy

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Crosslinked Poly(ethylene oxide) as a Versatile Alignment Medium for the Measurement of Residual Anisotropic NMR Parameters**

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In memory of Alexander Dehner

Residual anisotropic NMR parameters like residual dipolar couplings (RDCs), residual chemical shift anisotropies (RCSAs), or residual quadrupolar couplings (RQCs) have proven to provide exceptionally valuable structural information for biomacromolecules[1] as well as small organic molecules with respect to conformation, configuration, constitution, and the distinction of enantiomers when conventional methods are not sufficient for the corresponding structural characterization.[2] Their measurement, however, requires the appropriate weak partial alignment in so-called alignment media for induction of the necessary anisotropy.

Two types of alignment media, liquid-crystalline phases[3] and stretched polymer gels,[4] provide uniaxially anisotropic matrices and are commonly used for aligning samples, both having severe restrictions with respect to compatibility with the solvent and the solute of interest. Today's weak alignment media are either compatible with water,[5] polar organic solvents like dimethylformamide (DMF) and dimethylsulfoxide (DMSO),[6] or with nonpolar solvents like chloroform ($CHCl_3$).[7] Few media are known to be compatible with mixtures like water/DMSO.[8] Only cross-linked poly(vinyl acetate) has been reported as an alignment medium for less frequently used intermediate NMR solvents like acetonitrile (MeCN) and methanol (MeOH),[6b] which, however, has compatibility issues with, for example, peptides as an important class of molecules. For particular solvents like trifluoroethanol (TFE), which is typically used to preserve the secondary structure in α-helical transmembrane peptides in solution,[9] only an alignment medium with strong residual polymer signals is known.[10] For other solvents or solvent mixtures like MeOH/$CHCl_3$ or H_2O/MeOH/$CHCl_3$, commonly applied to study amphiphilic molecules, no compatible alignment media have been reported so far. Our aim was therefore to develop an alignment medium with the largest range of solvent compatibility possible, which should also result in a broad applicability of the corresponding solute molecules. With cross-linked poly(ethylene oxide) (PEO), which is also known as poly(oxyethylene) or poly(ethylene glycol), we found an alignment medium with exceptional compatibility and excellent NMR-spectroscopic properties.

Linear PEO is well-known for its solubility in a wide range of solvents including, for example, water, MeOH, $CHCl_3$, and dichloromethane (DCM). Cross-linked PEO, on the other hand, is typically used as a hydrogel in biological applications.[11] It can be produced from linear PEO either by irradiation or by chemical modification at the terminal OH groups or a combination of both. Because of our previous experience,[6d,e,7d,8b] we initially tried cross-linking by irradiation with accelerated electrons and by γ-irradiation. However, the intense formation of gas bubbles during the irradiation process required a very special protocol to obtain uniform cylindrically shaped polymer sticks useable in NMR applications, which had to allow for substantial degassing during γ-irradiation. We therefore also looked into chemical procedures for cross-linking like the addition of di- and triisocyanates like methylene diphenyl isocyanates for chain extension and cross-linking to a resulting PEO-MDI polymer stick or the use of chemically modified PEO-methylmethacrylate (PEOMMA) for radical-based cross-linking.

Detailed descriptions of the corresponding cross-linking procedures are given in the Supporting Information. As the formation of stable PEO-MDI polymer sticks in our hands was only successful after additional irradiation with accelerated electrons, we focus in the following on the detailed

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examination of PEO cross-linked with γ-irradiation, referred to as γ-PEO, and chemically cross-linked PEOMMA.

The solvent compatibility of both γ-PEO and PEOMMA is outstanding, as can be shown by their swelling behavior: polymer sticks of identical length were equilibrated in various solvents and solvent mixtures and their degrees of volume swelling obtained (Figure 1). With the exception of hexane, considerable swelling was observed with all solvents tested, including nonpolar solvents like benzene, toluene, and dioxane, as well as very polar solvents like MeOH, DMSO, and water.

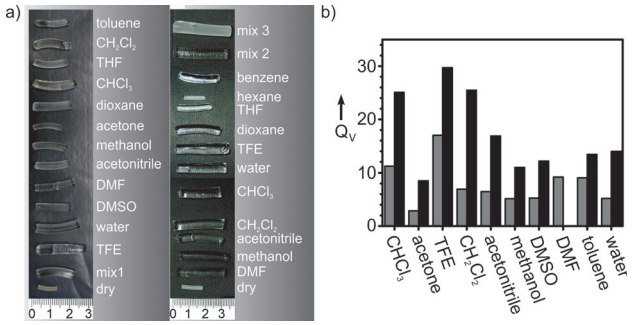

Figure 1. a) PEOMMA (left panel) and γ-PEO (right panel) gels swollen in different solvents or solvent mixtures over 48 h at room temperature; b) corresponding degree of volume swelling Q_V of the swollen PEOMMA (gray) and γ-PEO (black) gels.[12] Mix 1 corresponds to a mixture of TFE/H_2O, mix 2 to MeOH/$CHCl_3$, and mix 3 to H_2O/MeOH/$CHCl_3$ (for details of γ-PEO and PEOMMA preparation and swelling behavior see the Supporting Information).

The range of solvents also includes TFE, for which the observed degree of volume swelling, defined as the ratio of volumes before and after swelling $Q_V = V/V_0$ as an indirect measure for the amount of crosslinking and mesh-size,[13] reaches 30. Tested solvent mixtures also show the desired swelling behavior. Corresponding swelling factors for TFE/H_2O (mix 1), MeOH/$CHCl_3$ (mix 2), and H_2O/MeOH/$CHCl_3$ (mix 3) are shown in Figure 1 and several additional mixtures were successfully tested in NMR tubes.

We generally observed that individual components of the solvent mixture diffuse differently into the PEO matrix but after a certain equilibration time their relative ratios are restored also inside the gel. For D_2O/MeOD, for example, the equilibrium was restored after several days, while for DCM/MeOD it took up to three weeks at room temperature to restore the equilibrium, as monitored by the acquisition of NMR spectra and integration of the corresponding signal intensities.

The NMR spectroscopic properties of γ-PEO are again exceptional: in a 1D ^{1}H experiment a single broad signal at approximately 3.7 ppm is observed without any further impurities (Figure 2b). In the corresponding 2D CLIP-HSQC experiment[14] a single cross-peak appears at a carbon frequency of 70.0 ppm (Figure 2c) with a very small ^{13}C linewidth of approximately 15 Hz. Overlap with signals from any solute molecule of interest are therefore highly unlikely and can almost always be avoided if spectra

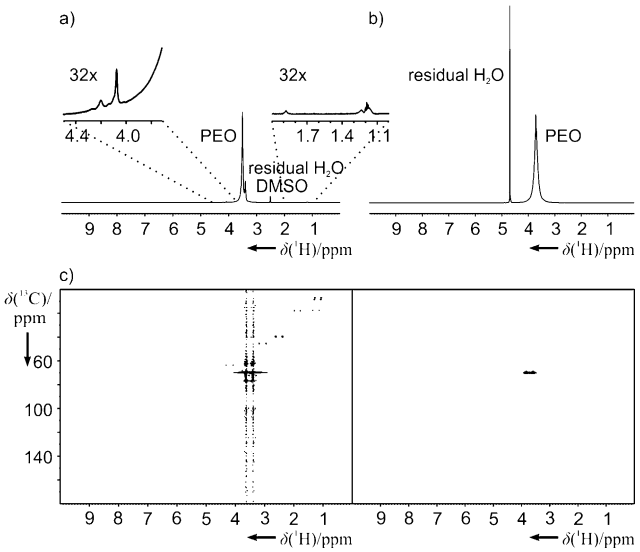

Figure 2. a) 1D ^{1}H spectrum of PEOMMA in [D_6]DMSO; b) 1D ^{1}H spectrum of γ-PEO in D_2O and c) corresponding 2D CLIP-HSQC spectra of PEOMMA (left) and γ-PEO (right). The intensities of the MMA signals of PEOMMA are low and the corresponding spectra have been adjusted to make them visible.

are recorded with sufficient resolution in the carbon dimension. For PEOMMA, in the spectra of the 1D and 2D experiments (Figure 2a,c) additional signals of low intensity in the chemical shift ranges of 1.1 to 4.2 ppm for ^{1}H and of 8 to 70 ppm for ^{13}C appear because of the MMA groups. These additional signals eventually overlap with solute signals. However, signals can be reduced, for example, by corresponding relaxation[15] or diffusion-filtered experiments[10] as MMA is covalently bounded to the polymer network with no undesired residual small molecules present.

Next to the solvent compatibility and good NMR properties, it is also necessary for an alignment medium to have a broad range of applicable solutes. We therefore acquired sets of one-bond RDCs for a number of solutes belonging to different molecular classes.

Corresponding spectra for selected organic solute molecules are shown in Figure 3: norcamphor as an established test molecule for RDCs in PEOMMA/TFE, hydroquinidine as a relatively nonpolar alkaloid in γ-PEO/$CDCl_3$, the steroid sodium cholate in PEOMMA/MeOD/D_2O/$CDCl_3$, a paracyclophane in γ-PEO/MeCN, the cyclic peptide cyclo(Arg-Nal-Ala-Gly-(D-Tyr)-Arg) in γ-PEO/DMSO, and even the uniformly ^{15}N-labeled protein ubiquitin in γ-PEO/90% H_2O/10% D_2O all show beautiful partially aligned CLIP-HSQC spectra from which corresponding $^1D_{CH}$ and $^1D_{NH}$ RDCs can be derived (for extracted couplings and more examples see the Supporting Information). A comparison of the resulting alignment tensors norcamphor in γ-PEO and PEOMMA in various solvents with previously reported alignment tensors of norcamphor using polystyrene and poly(vinyl acetate) as alignment media shows significantly differing alignment tensors for the three polymer types, being of potential importance for the study of dynamics of especially less polar molecules (see the Supporting Information for details).

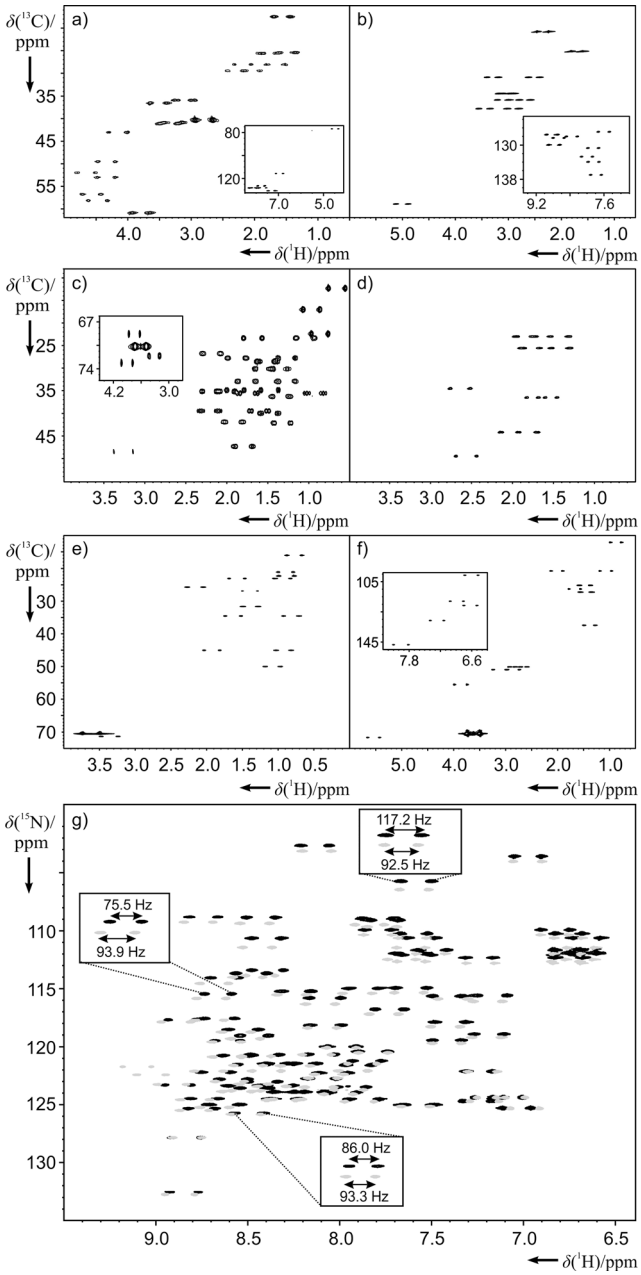

Figure 3. a) NMR spectra of cyclic peptide cyclo(Arg-Nal-Ala-Gly-(D-Tyr)-Arg) in γ-PEO/DMSO; b) paracyclophane in γ-PEO/MeCN; c) sodium cholate in PEOMMA/MeOD/D_2O/$CDCl_3$; d) norcamphor in PEOMMA/TFE; e) menthol in PEOMMA/$CDCl_3$; f) hydroquinidine in γ-PEO/$CDCl_3$; g) spectra of ^{15}N-labeled ubiquitin in stretched γ-PEO/90% H_2O/10% D_2O (black) and as isotropic sample (gray).

PEO gels can also be applied within a stretching apparatus for the convenient tuning of alignment strength.[16] Using perfluorinated elastomer tubing,[17] the stretching device equally covers the broad solvent range accessible by PEO. In Figure 4, the scaling of alignment in a single sample is demonstrated by measuring $^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$ couplings for sucrose in PEO/D_2O, and by measuring ^{15}N-RCSAs[18] for ubiquitin, demonstrating that all anisotropic NMR parameters are indeed accessible with the alignment medium.

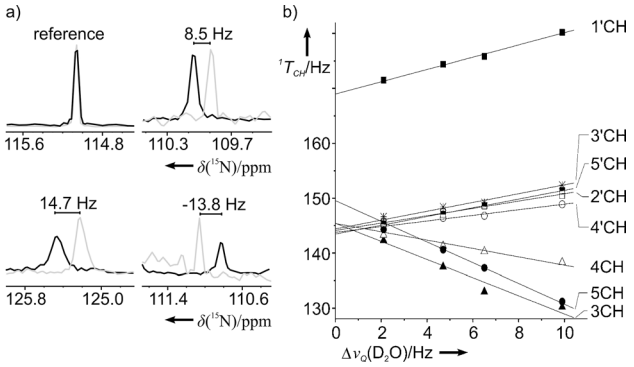

Figure 4. Application of PEO inside a stretching apparatus. a) Residual ^{15}N chemical shift anisotropies are measured at 60.774 MHz resonance frequency from 1D traces of ^{15}N-labeled ubiquitin in stretched (black) and isotropic (gray) γ-PEO/90% H_2O/10% D_2O. The Gly76 signal was arbitrarily chosen as chemical shift reference for ΔΔRCSA measurements according to Ref. [17a]. b) In a similar way, residual dipolar couplings of sucrose in γ-PEO/D_2O at different degrees of stretching can be determined with high accuracy.

We conclude that PEO seems to be the desired all-in-one alignment medium. The strength of alignment of PEO thereby follows the previously observed rules of an increase with enhanced cross-linking and a larger diameter of the dry polymer stick relative to the NMR tube.[15] As fine tuning of the alignment is easily possible with the mentioned stretching device and as a very large number of conditions would have to be tested, we did not attempt to systematically measure the alignment strength for the various solvents, stick diameters, and degrees of cross-linking accessible; we rather tried to show a number of useful examples covering most solvents, solutes and polymer sticks that can be used to extrapolate the conditions for a desired application.

The wide compatibility of PEO comes with only few restrictions. It should be noted that the polymer is known to be degraded by hydrochloric acid and temperatures above 50 °C should be avoided since polymer sticks will lose their shapes because of softening. As mentioned, PEO is only incompatible with very nonpolar substances like alkanes, for which we refer to other alignment media like poly(dimethyl siloxane).[7d] Furthermore, nonchiral PEO alone obviously cannot be used to distinguish enantiomers. The addition of adequate chiral adducts like cyclodextrines, however, certainly will also enable the determination of enantiomeric excess.[19] Besides these rare limitations, the polymer will be applicable to the structural investigation of almost all solutes and solvents by anisotropic NMR parameters. In addition, the broadly compatible alignment medium will also open new avenues like, for example, detailed structural studies under varying solvent conditions, the identification of preferred binding sites by solvent-dependent dynamics, or the measurement of anisotropic NMR parameters in titration-type studies of otherwise incompatible components. Together with its excellent NMR spectroscopic properties, we therefore foresee an important role of cross-linked PEO for the general study of structure and dynamics for almost any class of solvents and solute molecules.

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