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Tunable Alignment for All Polymer Gel/Solvent Combinations for the Measurement of Anisotropic NMR Parameters

Grit Kummerlöwe,[a, e] Elizabeth F. McCord,[b] Steve F. Cheatham,[c] Steve Niss,[d] Russell W. Schnell,[d] and Burkhard Luy*[a, f]

Dedicated to Professor Horst Kessler on the occasion of his 70th birthday

Residual dipolar couplings (RDCs) and other anisotropic NMR parameters provide valuable structural information on the conformation and configuration of biomolecules[1] as well as small organic molecules such as sugars, peptides, and natural or synthetic products.[2] A fundamental requirement for their accurate measurement is the adjustment of a proper degree of partial alignment of the solute molecule.

Mechanically stretched polymer gels allow the scaling of alignment without lower limit in practically all NMR solvents,[3] but the classical way of stretching the gel directly in an NMR tube[2] demands a new sample for each alignment strength. Since the prediction of the orientational order for small organic molecules is still not easily achieved, this frequently leads to unnecessary and time-consuming sample preparations and eventually to a corresponding loss of valuable material. Only with the introduction of a stretching apparatus by the Kuchel group[4,5] the situation changed and it is now possible to rapidly and reversibly adjust the degree of orientation within a single sample using standard liquid state NMR equipment.

While the original stretching apparatus can be used with gelatin[4,6] and other gels containing polar solvents such as D_2O and DMSO,[5] it is incompatible with more apolar organic solvents such as chloroform, dichloromethane, or tetrahydrofuran. The silicone tube as the main part of the device also allows water and humidity to penetrate into the gel sample[5] and leads to an intense, broad signal around 0.1 ppm in corresponding ^{1}H NMR spectra. It is therefore of very limited use for the analysis of most organic compounds. Here we present a novel stretching device based on a tailor-made perfluorinated elastomer tube which overcomes the main existing limitations of the original apparatus and provides the desired stretching capabilities for practically all polymer/solvent combinations.

Following our design of the stretching device equipped with a silicone tube,[5] the flexible perfluorinated elastomer tube is placed inside a cut-open 5 mm NMR tube and fixed at the bottom with a specially designed screw clamp made out of PCTFE (Figure 1). The elastomer tube works as an expandable container for the swollen gel with the solute molecule inside. Upon stretching of the flexible tube the polymer gel is stretched as well, which in turn leads to partial alignment of the solute and measurable anisotropic NMR parameters.

After testing a variety of different materials, Kalrez 8002 perfluoroelastomer,[7] with its superior resistance against most chemicals and its high purity (e.g., no detectable paramagnetic impurities that are present in most other similar

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[a] M. Sc. G. Kummerlöwe, Priv.-Doz. Dr. B. Luy
Department Chemie
Technische Universität München
Lichtenbergstr. 4, 85747 Garching (Germany)
Fax: (+49) 89-289-13210
E-mail: Burkhard.Luy@ch.tum.de

[b] Dr. E. F. McCord
E. I. du Pont de Nemours and Company L.L.C.
Route 141 and Powder Mill Rd., Wilmington, DE 19707 (USA)

[c] Dr. S. F. Cheatham
E. I. du Pont de Nemours and Company L.L.C.
Stine-Haskell Research Center, Newark, DE 19714 (USA)

[d] S. Niss, R. W. Schnell
DuPont Performance Elastomers L.L.C.
300 Bellevue Parkway, Suite 300, Wilmington, DE 19809 (USA)

[e] M. Sc. G. Kummerlöwe
Current address:
Institut für Biologische Grenzflächen
Karlsruher Institut für Technologie
Herrmann-von-Helmholtz-Platz 1
76344 Eggenstein-Leopoldshafen (Germany)

[f] Priv.-Doz. Dr. B. Luy
Current address:
Institut für Organische Chemie
Karlsruher Institut für Technologie
Fritz-Haber-Weg 6, 76131 Karlsruhe (Germany)

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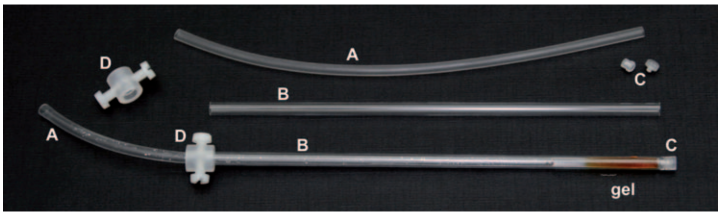

Figure 1. Stretching apparatus for 5 mm standard high resolution NMR probe heads. The flexible Kalrez 8002UP[17] tube (A) is placed inside a cut-open 5 mm NMR tube (B) and fixed with a specially designed PCTFE screw (C) at the bottom. A PCTFE device with nylon screws (D) is used to fix the stretched tube at the top. Inside the assembled apparatus a reddish-brown PAN/DMSO gel is ready to be stretched.

materials), was found to be the ideal material for the given task. A Kalrez 8002UP perfluoroelastomer parts tube with inner/outer diameters of 3.2/4.2 mm was specifically produced at DuPont Performance Elastomers for the current studies.

Although the material is not fully inert towards halogenated hydrocarbons, the tubing can be used with all common NMR solvents, including chloroform and dichloromethane. As an example, we monitored the stepwise stretching of a PDMS/$CDCl_3$ gel[8] containing hydroquinidine as a test molecule by acquiring a number of CLIP-HSQC spectra[9] at different extensions (Figure 2). As reported previously,[4] the alignment strength and therefore anisotropic NMR parameters are approximately proportional to the so-called extension factor Ξ and the plot of ${}^1T_{CH}$ couplings against Ξ or the quadrupolar splitting $\Delta\nu_Q$ reveals highly precise RDCs as the slopes of corresponding linear fits (see Supporting Information).[5]

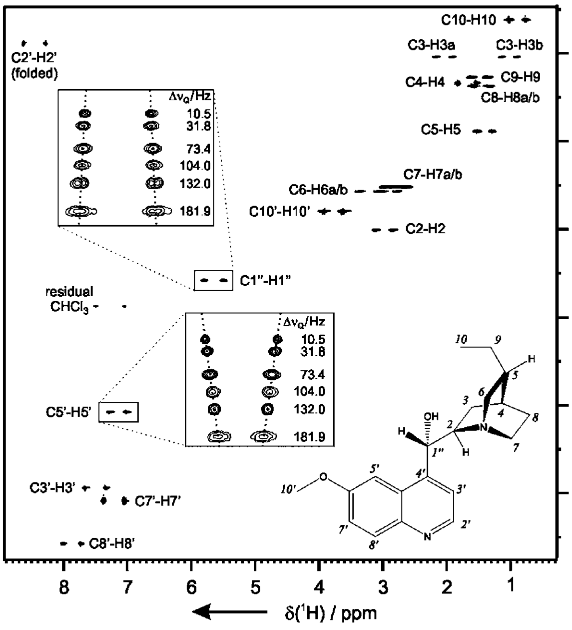

Figure 2. Kalrez 8002UP tubing allows the arbitrary scaling of RDCs even in gels with halogenated solvents: CLIP-HSQC spectrum[9] of hydroquinidine in a PDMS/$CDCl_3$ gel.[8] Insets show two signals (C1″–H1″ and C5′–H5′) at various stages of stretching with peaks separated in the vertical direction according to the corresponding quadrupolar splitting $\Delta\nu_Q$ of the solvent $CDCl_3$. The linear dependence between observed ${}^1T_{CH}$ couplings and quadrupolar splittings is evident.

In addition to the advantage of a wide range of compatible solvents, the quality of ${}^1H$ NMR spectra is substantially improved using the Kalrez 8002UP tubing compared with identical spectra recorded with the silicone tubing, as the perfluorinated elastomer does not contain protons. In Figure 3a and b the 1D ${}^1H$ spectra of two identical gel samples with alanine in gelatin/D_2O stretched within a silicone tube and within a Kalrez 8002UP tube are compared. While the silicone tubing shows an intense, broad signal around 0.1 ppm, there is no additional signal from the Kalrez 8002UP tubing, which not only leads to cleaner spectra, but also to an improved sensitivity as the receiver gain of the spectrometer can be increased.

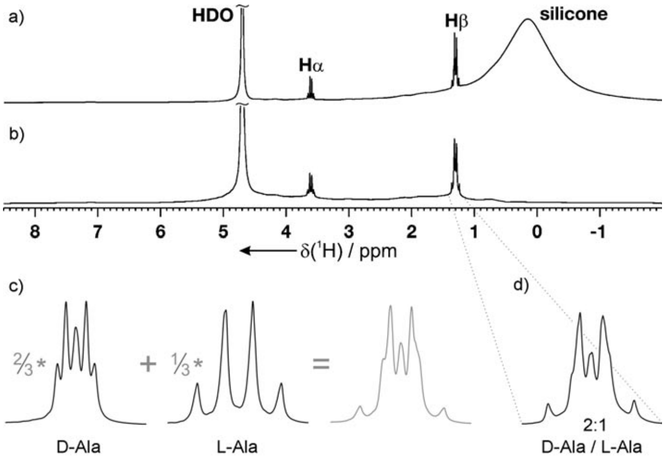

Figure 3. Comparison of ${}^1H$ 1D spectra acquired in a stretching device equipped with silicone tubing (a) and Kalrez 8002UP tubing (b) containing identical samples of 1 M alanine (L-Ala/D-Ala in 2:1 ratio) in 50% gelatin in D_2O. In the case of the silicone tube a broad signal around 0.1 ppm dominates, while the Kalrez 8002UP tube does not contribute to the spectrum; additional low-intensity background contributions originate from the alignment medium gelatin. c) Corresponding samples of 1 M D-Ala and L-Ala in 50 % gelatin/D_2O have been measured with approximately identical stretching. The weighted sum of the spectra (c, dark gray) results in a spectrum similar to the spectrum measured for the 2:1 mixture (d). For clarity only the Hβ region is shown in c) and d); for full spectra and the corresponding Hα region see the Supporting Information.

As has been shown previously, stretched gelatin provides the possibility of distinguishing enantiomers.[4,6,10] The distinction is even more facile with the Kalrez 8002UP tubing, as we demonstrate by repeating a set of 1D ${}^1H$ NMR spectra of different samples of L- and D-alanine for the determination of enantiomeric excess. When adding up the signals of enantiomerically pure amino acids inside equally stretched (chiral) gelatin in the correct mass ratio (Figure 3c) the resulting spectrum is equivalent to the spectrum acquired for the mixture of the two enantiomers (Figure 3d; see also Supporting Information).

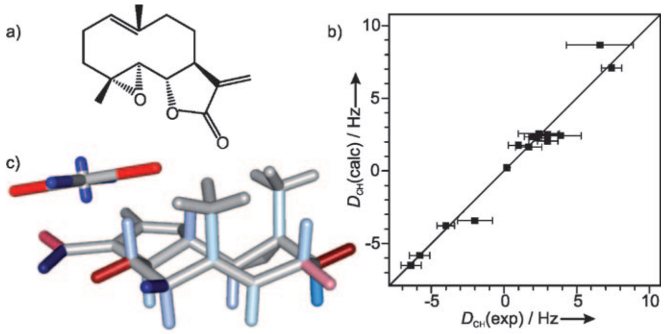

Figure 4. Conformational analysis of the natural product parthenolide (a) by RDCs measured in a PAN/DMSO gel[12] stretched with the apparatus depicted in Figure 1. b) Back-calculated vs measured RDCs for the chair-like conformation confirm the structural model in solution. c) The structural model is shown with color-coded bonds (red: negative; blue: positive RDCs) and the axes of the corresponding alignment tensor next to it.

As another test molecule we investigated the natural product parthenolide, a sesquiterpenic lactone isolated from *Chrysanthemum parthenium*.[11] A set of 16 one-bond RDCs could be obtained for the compound in a PAN/DMSO gel[12] stretched within the stretching apparatus equipped with a Kalrez 8002UP tube. RDCs fitted with the program PALES[13] confirm the presence of a chair-like conformation of the compound, as previously measured by X-ray crystallography[14] and as proposed by MM-calculations,[15] also in DMSO solution (see Figure 4 and Supporting Information).

In contrast to corresponding gels in the silicone-based device, where penetrating humidity leads to shrinking gels within a couple of weeks,[5] the used PAN/DMSO gel is stable for several months as water can not diffuse through the gas-tight walls of the perfluorinated tube.

In summary, the stretching apparatus equipped with the perfluorinated Kalrez 8002UP tube is an ideal tool to partially align molecules for measuring anisotropic NMR parameters, with easily adjustable alignment strength in practically all polymer gel matrices, including those with apolar solvents. In contrast to previously reported stretching devices based on silicone tubes, the perfluorinated elastomer tube does not contribute to ^{1}H NMR-spectra and is generally gas-tight, which, for example, prevents the diffusion of humidity into the sample. Especially in combination with perdeuterated polymer gels,[16] the presented stretching apparatus is of high interest for samples at low concentration as it provides proton spectra void of signals originating from the alignment medium.

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Keywords: alignment · gels · NMR spectroscopy · residual dipolar couplings · structure elucidation

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[1] a) A. Bax, *Protein Sci.* **2003**, *12*, 1–16; b) J. H. Prestegard, C. M. Bougault, A. I. Kishore, *Chem. Rev.* **2004**, *104*, 3519–3540.
[2] a) C. M. Thiele, *Eur. J. Org. Chem.* **2008**, 5673–5685; b) G. Kummerlöwe, B. Luy, *Trends Anal. Chem.* **2009**, *28*, 483-.493.
[3] a) B. Luy, K. Kobzar, H. Kessler, *Angew. Chem.* **2004**, *116*, 1112–1115; *Angew. Chem. Int. Ed.* **2004**, *43*, 1092–1094; b) B. Deloche, E. T. Samulski, *Macromolecules* **1981**, *14*, 575–581; c) R. Tycko, F. J. Blanco, Y. Ishii, *J. Am. Chem. Soc.* **2000**, *122*, 9340–9341; d) J. Sass, G. Musco, S. J. Stahl, P. T. Wingfield, S. Grzesiek, *J. Biomol. NMR* **2000**, *18*, 303–309; e) J. C. Freudenberger, S. Knör, K. Kobzar, D. Heckmann, T. Paululat, H. Kessler, B. Luy, *Angew. Chem.* **2005**, *117*, 427–430; *Angew. Chem. Int. Ed.* **2005**, *44*, 423–426; f) P. Haberz, J. Farjon, C. Griesinger, *Angew. Chem.* **2005**, *117*, 431–433; *Angew. Chem. Int. Ed.* **2005**, *44*, 427–429; g) R. R. Gil, C. Gayathri, N. V. Tsarevsky, K. Matyjaszewski, *J. Org. Chem.* **2008**, *73*, 840–848.
[4] P. W. Kuchel, B. E. Chapman, N. Müller, W. A. Bubb, D. J. Philp, A. M. Torres, *J. Magn. Reson.* **2006**, *180*, 256–265.
[5] G. Kummerlöwe, F. Halbach, B. Laufer, B. Luy, *Open Spectrosc. J.* **2008**, *2*, 29–33.
[6] a) K. Kobzar, H. Kessler, B. Luy, *Angew. Chem.* **2005**, *117*, 3205–3207; *Angew. Chem. Int. Ed.* **2005**, *44*, 3145–3147; b) C. Naumann, W. A. Bubb, B. E. Chapman, P. W. Kuchel, *J. Am. Chem. Soc.* **2007**, *129*, 5340-5341; c) C. Naumann, P. W. Kuchel, *J. Phys. Chem. A* **2008**, *112*, 8659–8664.
[7] http://www.dupontelastomers.com/products/kalrez/indsemi.asp
[8] J. C. Freudenberger, P. Spiteller, R. Bauer, H. Kessler, B. Luy, *J. Am. Chem. Soc.* **2004**, *126*, 14690–14691.
[9] A. Enthart, J. C. Freudenberger, J. Furrer, H. Kessler, B. Luy, *J. Magn. Reson.* **2008**, *192*, 314–322.
[10] a) U. Eliav, G. Navon, *J. Am. Chem. Soc.* **2006**, *128*, 15956–15957; b) G. Kummerlöwe, M. Udaya Kiran, B. Luy, *Chem. Eur. J.* **2009**, *15*, 12192–12195.
[11] A. S. Bawdekar, G. R. Kelkar, S. C. Bhattach, *Tetrahedron Lett.* **1966**, 1225–1226.
[12] G. Kummerlöwe, J. Auernheimer, A. Lendlein, B. Luy, *J. Am. Chem. Soc.* **2007**, *129*, 6080–6081.
[13] a) M. Zweckstetter, A. Bax, *J. Am. Chem. Soc.* **2000**, *122*, 3791–3792; b) M. Zweckstetter, *Nat. Protoc.* **2008**, *3*, 679–690.
[14] A. Quick, D. Rogers, *J. Chem. Soc. Perkin Trans. 2* **1976**, 465–469.
[15] H. Neukirch, N. C. Kaneider, C. J. Wiedermann, A. Guerriero, M. D'Ambrosio, *Bioorg. Med. Chem.* **2003**, *11*, 1503–1510.
[16] a) G. Kummerlöwe, S. Knör, A. O. Frank, T. Paululat, H. Kessler, B. Luy, *Chem. Commun.* **2008**, 5722–5724; b) G. Kummerlöwe, S. Kelch, M. Behl, A. Lendlein, B. Luy, unpublished results.
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