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An Alignment Medium for Measuring Residual Dipolar Couplings in Pure DMSO: Liquid Crystals from Graphene Oxide Grafted with Polymer Brushes

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Abstract: *Residual dipolar couplings (RDCs) have attracted attention in light of their great impact on the structural elucidation of organic molecules. However, the effectiveness of RDC measurements is limited by the shortage of alignment media compatible with widely used organic solvents, such as DMSO. Herein, we present the first liquid crystal (LC) based alignment medium that is compatible with pure DMSO, thus enabling RDC measurements of polar and intermediate polarity molecules. The liquid crystals were obtained by grafting polymer brushes onto graphene oxide (GO) using free radical polymerization. The resulting new medium offers several advantages, such as absence of background signals, narrow line shapes, and tunable alignment. Importantly, this medium is compatible with π-conjugated molecules. Moreover, sonication-induced fragmentation can reduce the size of GO sheets. The resulting anisotropic medium has moderate alignment strength, which is a prerequisite for an accurate RDC measurement.*

Incorporation of RDCs into NMR analytical techniques has emerged as an important tool for stereochemical elucidation in synthetic and natural products chemistry.[1] The anisotropic NMR parameter, which provides exceptionally valuable spatial structural information, has played a key role in determining the relative configurations of remote stereocenters and conformation of flexible molecules.[2] Furthermore, RDC-enhanced structure analysis has also been used to clarify the stereochemistry and conformation of reaction intermediates or products from synthetic chemistry.[3]

In respect to RDC measurements in organic solvents, two main types of alignment media have been developed in the last two decades. These media include 1) stretched polymer gels such as PDMS,[4] PAN,[5] PS,[6] PVAc,[7] PH,[8] PMMA,[9] PEO,[10] PBLG gel,[11] and poly-HEMA;[12] and 2) liquid-crystalline phases such as PBLG,[1d] polyguanidines,[13] polyisocyanates,[14] polyacetylenes,[15] ACHC-rich β-peptides,[16] and disodium cromoglycate.[17] These media are applicable to apolar as well as polar organic solutions. The orienting properties, including advantages and drawbacks of different alignment media, have been extensively discussed in several reviews.[1d,18] In general, lyotropic liquid-crystalline phases are a good choice for measuring RDCs because they allow simultaneous alignment of molecules. However, to date there are no LC phases that are compatible with pure polar and intermediate polarity NMR solvents such as dimethyl sulfoxide (DMSO), which is a widely used solvent in organic chemistry. In our previous work we presented GO-based LCs as a new alignment medium that is compatible with a broad range of solvents (except pure DMSO), and demonstrated several merits, such as high-quality NMR spectra without background signals as a result of the rigidity and high molecular weight of GO molecules.[19] Encouraged by these results, we decided to further optimize the GO-based LC phases to get an alignment medium that would be soluble in pure DMSO solvent. In 2011, Gao and colleagues showed that grafting polymer brushes to GO nanosheets could significantly enhance dispersibility in polar solvents.[20] The resulting polymer brushes, comprising abundant alkyl side chains, exhibited good solubility in organic solvents and reduced viscosity, even at high concentrations. The latter property is a prerequisite for obtaining narrow line shapes in NMR spectra.

Extensive optimization revealed that trifluoroethyl methacrylate (TFEMA) was the optimal monomer for grafting onto GO mono-sheets. The reaction involved a free radical polymerization with initiator 2,2′-azobis-(2-methyl-propionitrile) (AIBN), which is shown in Figure 1 A. To monitor the reaction, FT IR spectroscopy was used to identify the chemical structure of GO-g-TFEMA (Supporting Information, Figure S1). Thermogravimetric analyses (TGA) (Supporting Information, Figure S2) were in agreement with the FT IR spectrum, which confirmed successful synthesis of 2D GO sheets functionalized with TFEMA polymers.

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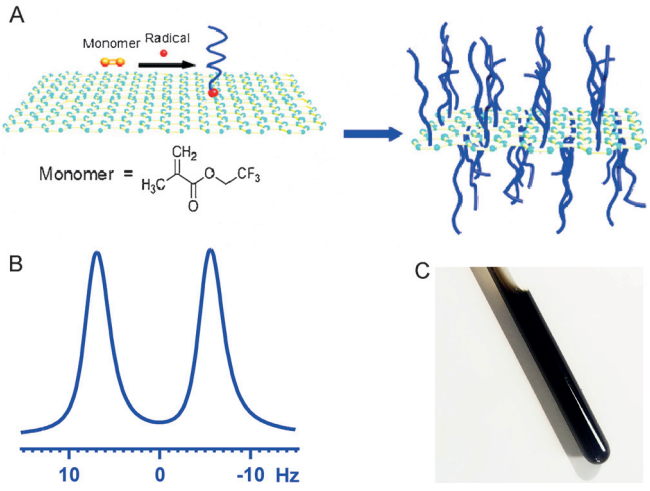

Figure 1. A) Representation of the synthetic procedure for obtaining GO-g-TFEMA. B) 1D ^{2}H spectrum (25 °C, eight scans) of a sample containing 54.3 mg mL^{-1} of GO-g-TFEMA in [D_6]-DMSO with a deuterium splitting of 12.5 Hz (2.5 Hz linewidth). C) Photograph of GO-g-TFEMA in a [D_6]-DMSO dispersion inside an NMR tube.

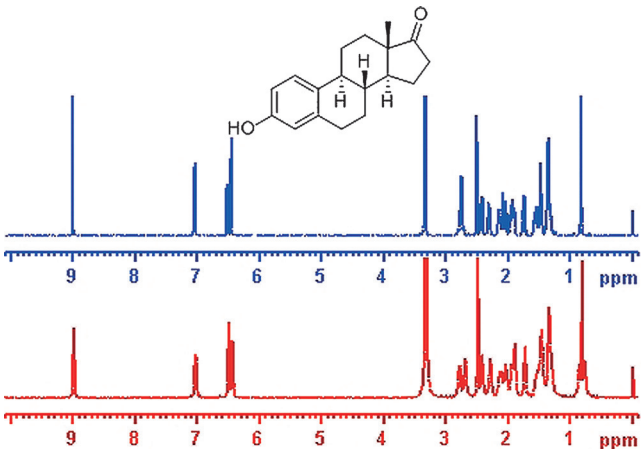

Figure 2. ^{1}H NMR spectra of estrone in isotropic [D_6]-DMSO (blue), and in 9.0 mg mL^{-1} anisotropic GO-g-TFEMA LCs (red).

To study the anisotropic properties of GO-g-TFEMA in DMSO, ^{2}H NMR spectra were recorded using medium concentrations ranging from 1.0–54.3 mg mL^{-1}. Splitting of the deuterium solvent signal occurred at a concentration of 5.4 mg mL^{-1}, and was augmented by increasing concentrations of GO-g-TFEMA (Supporting Information, Figure S5), indicating that the strength of the alignment induced by LCs can be scaled by adjusting their concentration. The NMR signals of the ^{2}H quadrupolar splitting were highly symmetric and a linewidth of 2.5 Hz at a high GO concentration of 54.3 mg mL^{-1} was observed (Figure 1 B). This result suggested that GO-g-TFEMA shows excellent solubility and dispersibility, and exhibits very low viscosity in DMSO. These physical properties of GO-g-TFEMA reveal a significant advantage compared to that of unmodified GO phases previously reported.[19] Thus, GO-g-TFEMA show great potential for application as a standard alignment medium, and could avoid frequently occurring line broadening problems that arise because of the observed high viscosity in unmodified GO LC phases.

Another drawback of our previously reported unmodified GO LCs was the strong π–π interactions that occurred between GO sheets and analytes, which excluded the possibility of RDC measurements on aromatic organic molecules. In the new GO-g-TFEMA phase, potential π–π interactions are expected to be blocked by steric hindrance arising from the alkyl chain brushes grafted onto graphene oxide. To investigate this hypothesis, estrone was employed as a probe. Estrone (10 mg) was added to GO-g-TFEMA LCs (9.0 mg mL^{-1}) with a quadrupolar DMSO splitting of 2.2 Hz. A high quality proton NMR spectrum was obtained under anisotropic conditions (Figure 2), suggesting that aggregation of the GO layer and analytes could indeed be successfully prevented by grafting of GO with polymer chains. In accordance with the unmodified GO LCs, there were no background signals in the NMR spectrum (Figure 2).

Additionally, we used Boc-L-tryptophan to demonstrate the compatibility of aromatic systems with the GO-g-TFEMA LCs medium. Figure 3 A shows a section of the anisotropic CLIP-HSQC spectrum of Boc-L-tryptophan containing a number of carbon proton signals in the aromatic region. The quality of the spectrum in the presence of GO-g-TFEMA LCs was even comparable with the isotropic example, as shown in Figure 3 A. The size of the RDCs of Boc-L-tryptophan and estrone ranged from −25.4–2.2 Hz, and −16.6–28.9 Hz, respectively (Supporting Information, Tables S1 and S2).

Furthermore, we investigated the feasibility of GO-g-TFEMA LCs for structure elucidation of organic molecules by using two test compounds: 1) dihydroartemisinin malaria treatment drug, the discovery of which was awarded the Nobel Prize in 2015; 2) estrone, a well-established sample compound employed in previous RDC and RCSA studies.[21] In a mixture of GO-g-TFEMA LCs (18.9 mg mL^{-1}) and dihydroartemisinin (10 mg mL^{-1}) RDCs range from −11.7 to 27.6 Hz. Experimental RDCs were used to calculate the alignment tensor: 1) from the reference X-ray structure in the case of dihydroartemisinin;[22] and 2) with a density functional theory (DFT) refined structure of estrone,[21] using the singular value decomposition method (SVD)[23] and the program package MSpin.[24] Q-factors of 0.15 and 0.19 were obtained for dihydroartemisinin and estrone, respectively, suggesting that GO-g-TFEMA is a suitable alignment medium for accurate measurement of RDC data (Figure 3 B).

A moderate and tunable degree of alignment is, in principal, of great importance to an LC-based anisotropic medium because it guarantees the precision and accuracy of RDC measurements. As we reported previously, the aligning degree of unmodified GO LCs can be scaled by varying the concentrations of GO monomer, leading to a corresponding quadrupolar splitting of 3.8 to 30.2 Hz in the deuterated DMSO signal. However, after alkyl brushes were grafted onto GO, the maximum quadrupolar splitting dramatically decreased to 6.1 Hz from 30.2 Hz, with the same average size (4 μm) of unmodified and polymer-grafted GO monomers (Figure 4 B). This significant difference can be explained

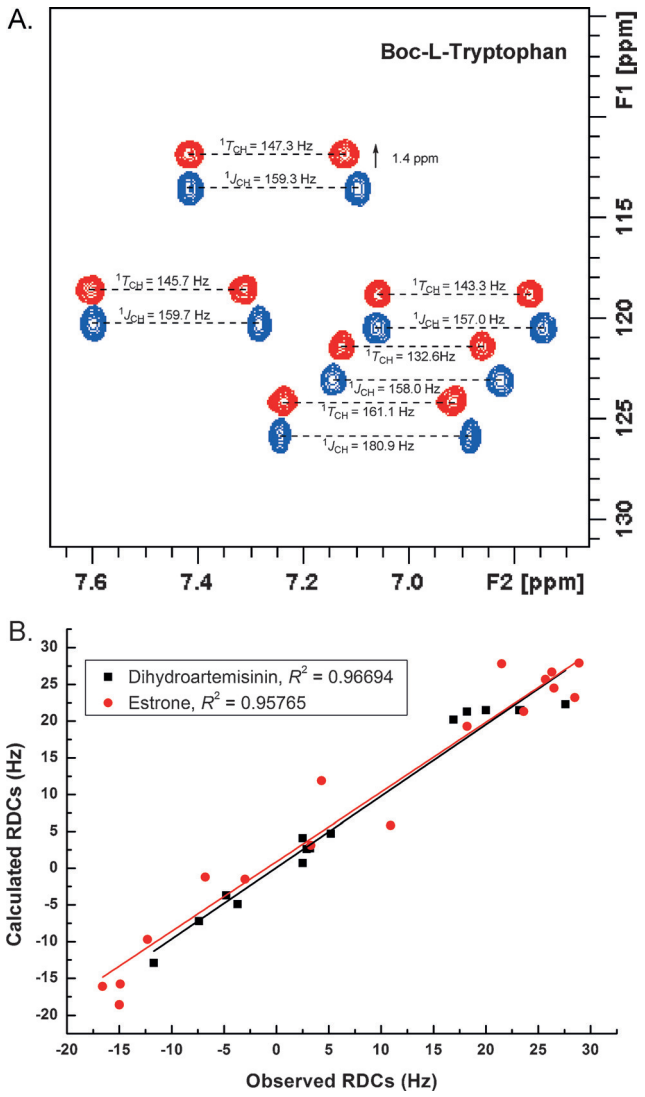

Figure 3. A) Overlay of sections of the 500 MHz [^{1}H,^{13}C]-CLIP-HSQC spectra of Boc-L-tryptophan in the isotropic [D_6]-DMSO phase (blue contours) and in anisotropic 47.80 mg mL^{-1} GO-g-TFEMA LCs (red contours, up-shifted 1.40 ppm in the ^{13}C dimension). The isotropic $^1J_{CH}$ couplings and $^1J_{CH}+{}^1D_{CH}$ couplings under the aligned conditions are shown in the spectrum. B) Correlations between the experimental and calculated $^1D_{CH}$ values of dihydroartemisinin and estrone. RDC fitting was performed using the SVD method. *Q* factors are 0.15 and 0.19 for dihydroartemisinin and estrone, respectively.

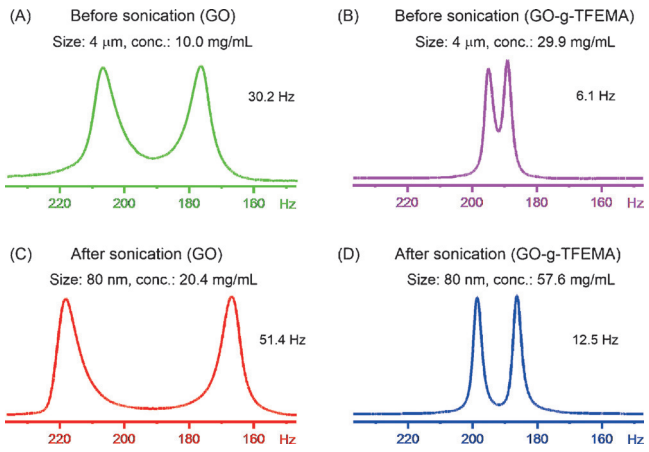

Figure 4. 1D ^{2}H spectrum of: A) 10.0 mg mL^{-1} GO (medium size, averaging 4 μm) before sonication; B) 29.9 mg mL^{-1} GO-g-TFEMA (medium size) before sonication; C) 20.4 mg mL^{-1} GO (small size, ca. 80 nm) after sonication; D) 57.6 mg mL^{-1} (small size) GO-g-TFEMA after sonication.

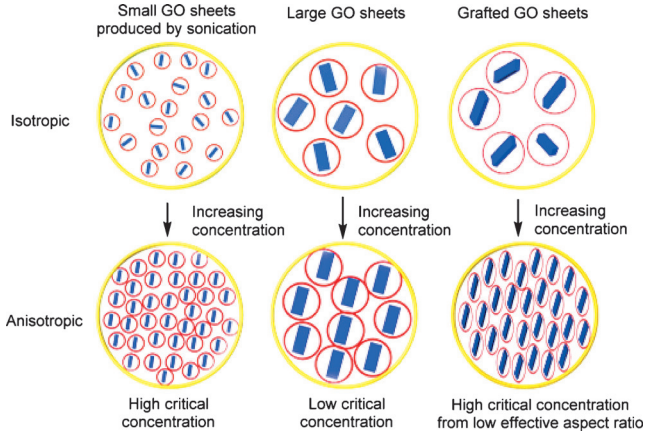

Figure 5. Proposed model for the formation of LC phases of unmodified GO and polymer-grafted GO dispersions by increasing LC concentration with various sheets sizes: the critical concentration for LC formation is higher for smaller GO sheets than for larger GO sheets. However, the critical concentration of the grafted GO sheets with the same average size increases because of a lower effective aspect ratio.

by Onsager's theory,[25] which predicts that the thickness of GO layers increases upon grafting with alkyl brushes, resulting in a lower effective aspect ratio. As illustrated in Figure 5, the critical concentration of liquid-crystalline phase formation of grafted GOs is much higher than that of the unmodified GOs, suggesting that the strength of induced alignment is lower for grafted GOs than for GOs lacking attached polymers at the same concentration.

The limiting factor for inducing stronger alignment by increasing concentration is the high viscosity of LCs, which brings about distortions and line broadenings of NMR signals. A typical asymmetric line shape was readily observed for the GO-g-TFEMA LCs, with a quadrupolar splitting of 6.1 Hz. A recent study by Pasquali and co-workers showed an isotropic-nematic phase transition of graphene dispersed in chlorosulfonic acid at high 20–30 mg mL^{-1} concentrations, indicating that graphene sheets with reduced size could enhance dispersion ability.[26] Inspired by this result, we fragmented the unmodified GOs by sonication. The maximum quadrupolar splitting of the resulting LCs changed from 30.2 to 51.4 Hz, as shown in Figure 4C. After sonication, the average size of GOs was reduced from 4 μm to 80 nm, with a maximum concentration of 20.4 mg mL^{-1} compared to 10.0 mg mL^{-1} for the unfragmented GOs. Subsequently, a quadrupolar splitting of 12.5 Hz was observed for the polymer-grafted GOs (the average size of precursor GOs was 80 nm) with a maximum dispersion concentration of 57.6 mg mL^{-1} in DMSO, as shown in Figure 4D. Using the method described above, the alignment strength of polymer-grafted GOs could be increased two-fold compared to the 4 μm GO LCs at their maximum

dispersion concentrations. Further investigations concerning the extent of grafting are described in the Supporting Information, Figures S13 and S14.

In summary, we presented GO-g-TFEMA LCs as a new tunable alignment medium by grafting polymer brushes onto GO sheets. This work is the first example of an LC-based aligning medium that is compatible with pure DMSO. The excellent solubility and intrinsic low viscosity of the medium allowed us to acquire high-quality NMR spectra with narrow lines. Similar to the previously reported unmodified GO, no background signals were observed because the residual impurity of monomers and polymerization initiators was easily eliminated by efficient washing. Importantly, GO-g-TFEMA is compatible with aromatic compounds because of steric hindrance provided by polymer brushes, which block π–π interactions between GO sheets and analytes. We proposed a sonication-based fragmentation method which enabled us to produce liquid crystals with moderate alignment for accurate RDC measurements. We believe that GO-g-TFEMA holds great promise for RDC measurement applications and structural elucidations of polar organic molecules, while retaining complementarity with existing alignment media.

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Keywords: alignment medium · functionalized graphene oxide · liquid crystals · NMR spectroscopy · residual dipolar coupling

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