HRMAS ^{1}H NMR as a tool for the study of supramolecular gels

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HRMAS ^{1}H NMR is reported for the first time as a useful technique to gain insight into the dynamic properties of aggregates present in supramolecular gels. The study of several low molecular weight gelators with this technique in toluene and acetonitrile is described.

The structural characterization of soft matter provides solid ground for developments associated to a rational design of new materials with desired properties. For example, scattering, microscopy and spectroscopy related techniques are used commonly to characterize soft matter.[1] However, the detailed structural characterization of soft materials at molecular level (conformation and intermolecular interactions) represents generally a challenge. For example, in many cases X-ray diffraction is not very informative due to the reduced or even non-existent crystallinity of these materials. Additionally, a very powerful technique for structural determination in solution such as NMR, presents serious limitations when studying condensed phases due to the reduced mobility of the molecules leading to line broadening and loss of spectral resolution. Despite these difficulties, interesting data have been recently gained using solid-state NMR in the study of soft materials such as, for example, amyloid aggregates.[2] Of special interest in the study of soft materials is the use of high resolution magic angle spinning (HRMAS) NMR.[3] HRMAS NMR is appropriate for investigating interfaces between a translationally mobile (liquid) and an immobile or less mobile (solid support, gel, microparticle), more particularly for detecting NMR resonances from a conformationally mobile chemical moiety, grafted to or interacting with the immobile phase. Thus, if the mobile moiety displays sufficient isotropic rotational mobility (correlation time of *ca.* 10^{-11} s or less) because it is dipped in the non-viscous liquid phase, the dipolar interactions for its particular NMR signals are naturally eliminated, leading to vanishing of the dipolar broadening exactly as in classical homogeneous liquid NMR; this in turn results in reasonably sharp resonances, even though they usually remain somewhat broader (a few Hz line-width in ^{1}H NMR) than in actual homogeneous solutions. By contrast, for moieties lacking such a conformational mobility, the magic angle spinning rate used in HRMAS NMR—around 4 kHz—is not sufficiently high to average dipolar broadening, but the resulting resonances are then so broad (like in solid-state NMR) that they just vanish into the spectrum noise. In this way, valuable information is obtained only about interface moieties that are grafted to the immobile support but dipped in the liquid phase. Additional discrimination between such grafted but conformationally mobile moieties, and translationally free species in the liquid phase is achieved by so-called diffusion filtered HRMAS NMR.[4] In short, this strategy exploits gradient pulsed techniques that encode translationally free diffusing species, while the grafted ones do not. Thus, in this way, such gradient pulse techniques enable one to suppress signals from species with translational motion freedom while those without remain unaltered. Using a diffusion filter takes advantage of the very low diffusion rate of networked molecules as compared to free ones. Applications of HRMAS NMR to the study of polymers, whole cells and tissues have been reported following the pioneering work of Shapiro and Keifer in this area.[5]

Supramolecular gels have received increasing attention in recent years. These materials are formed in most cases by extended supramolecular aggregates that are built through the anisotropic 1-D assembly of low molecular weight molecules. The self-assembled fibrilar networks formed in this way percolate the solvent and induce gelation.[6] The interest for this type of materials has grown enormously in the last years due to their potential applications resulting from their reversible, stimuli-responsive formation and other intriguing properties.[7] It has been shown that the study with conventional ^{1}H NMR of supramolecular gels results in NMR signals which should be ascribed to the gelator molecules which move freely in solution and are in equilibrium with the NMR-silent solid-like gel network.[8] Here we report for the first time on the use of HRMAS ^{1}H NMR for the characterization of supramolecular gels with the aim of gaining insight into the structure and properties of the aggregates in the fibrillar network. A previous attempt to obtain signals from the fibrillar network of a supramolecular gel with HRMAS ^{1}H NMR was unsuccessful.[9]

For this study, molecules, the gelation properties of which have been studied previously in our group, were used (see Scheme 1).[10] To test the influence of solvent polarity in the gels on their spectroscopic visibility using HRMAS NMR, the studies were performed in acetonitrile, a polar solvent, and in toluene, a rather non-polar solvent. The minimum gelation concentration for the studied molecules is listed in Table 1.

The presence of free gelator molecules in supramolecular gels may represent a serious drawback for the study of the gel network with HRMAS ^{1}H NMR because the signals of free and networked molecules overlap. As mentioned above, using a diffusion filter in pulsed field gradient spin echoes (PFGSE) experiments, enables one to eliminate signals from free moving molecules, as demonstrated previously.[11] For example, the HRMAS ^{1}H NMR spectrum in CD_3CN of the gel formed by compound **2**, a gelator with catalytic L-proline units which has been described recently,[10c] is quite similar to that obtained for a solution of this compound (Fig. 1A). However, it can be noticed that upon application of the diffusion filter[12,13] the

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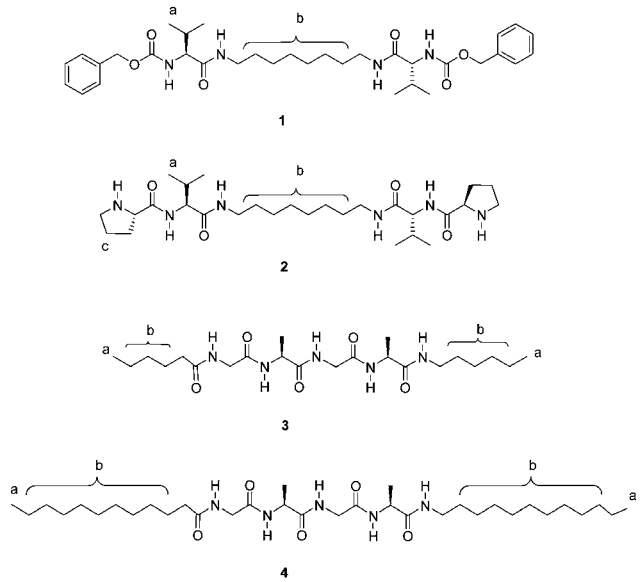

Scheme 1

Table 1 Minimum concentration for gel formation (mM) of the studied molecules at 25 °C in toluene and acetonitrile[10]

Compound	Toluene	Acetonitrile
1	7	13
2	11	14
3	9	4
4	8	8

intensity of the signals decrease considerably (Fig. 1B) indicating that the observed resonances in Fig. 1A correspond mainly to free, non-aggregated gelator molecules.

The diffusion filtered HRMAS ^{1}H NMR spectra of the gels formed by compound **1** in CD_3CN and toluene-d_8 showed interesting differences (Fig. 2). In acetonitrile the resonances of the valine methyl groups and those of the aliphatic central chain were observed after applying the diffusion filter. These results indicate that only those parts of the molecule are rotationally mobile enough in the aggregates to give rise to HRMAS signals. It has been shown that hydrogen bonding is a main driving force for aggregation of this molecule and, therefore, it is reasonable to assume that those parts close to the hydrogen bonding units are rather stiff in the aggregates, leaving only the aliphatic bridging and side-chains to be sufficiently

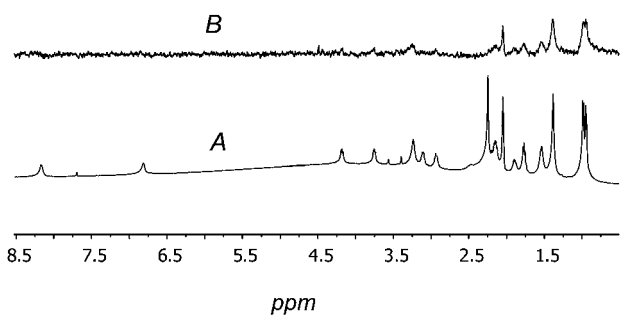

Fig. 1 HRMAS ^{1}H NMR spectra of a gel formed by compound **2** in CD_3CN: (A) no diffusion filter; (B) 50% diffusion filter.

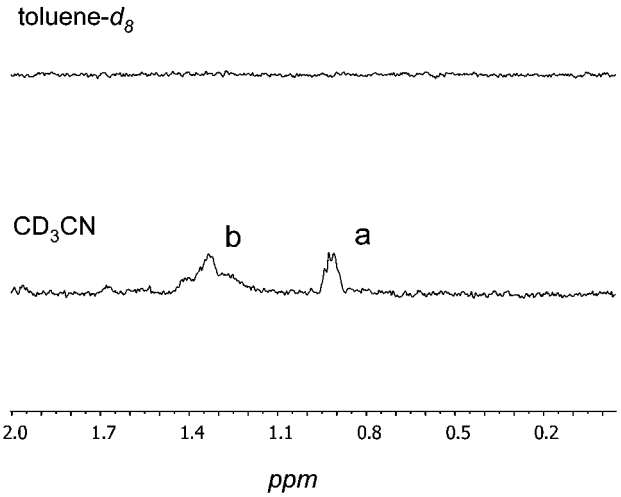

Fig. 2 Diffusion filtered (70%) HRMAS ^{1}H NMR spectra of gels formed by compound **1** in CD_3CN and toluene-d_8. See Scheme 1 for signal labeling.

conformationally mobile to be HRMAS NMR visible. Interestingly, the signals of the terminal benzyl units were not observed, indicating that this moiety is also rigidified in the aggregates, possibly through π-stacking.

On the other hand, no NMR signals from compound **1** could be detected after applying the diffusion filter in toluene. This behavior can be ascribed to the stronger hydrogen bonding interactions among gelator molecules in this solvent which would result in quite rigid aggregates. This fact correlates well with the lower minimum gelation concentrations of **1** in toluene (see Table 1) which can also be rationalized taking into account the stronger intermolecular interactions in this solvent.

The diffusion filtered ^{1}H HRMAS NMR spectrum of the gel formed by compound **2** in acetonitrile (Fig. 3) revealed signals corresponding to the valine methyl groups and the central aliphatic chain in analogy with the results found for compound **1**. Interestingly, in toluene, a new signal raising from the L-proline ring, is the most intense one and, additionally, the relative intensity of the signal of the

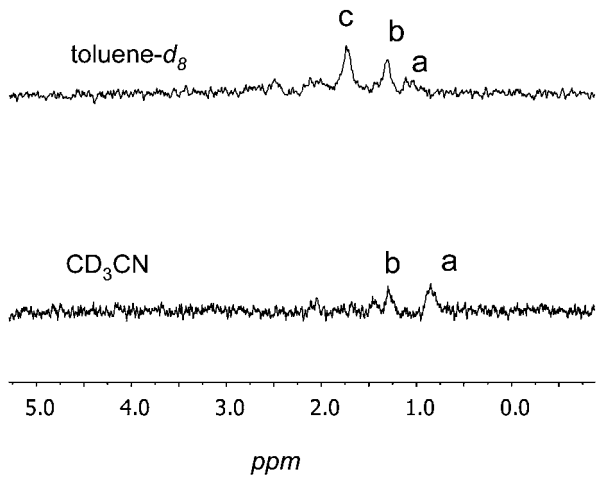

Fig. 3 Diffusion filtered (70%) HRMAS ^{1}H NMR spectra of gels formed by compound **2** in CD_3CN and toluene-d_8. See Scheme 1 for signal labeling.

central aliphatic chain is reduced when compared to that of the methyl groups.

The enhanced mobility detected for the proline units in toluene as compared to acetonitrile seems counterintuitive because of the stronger intermolecular hydrogen-bonding interactions expected in toluene. This result suggests a different structural arrangement in the aggregates in both solvents which would result in enhanced mobility of the proline unit in toluene.

Compounds **3** and **4** are organogelators which contain a tetrapeptidic core attached to terminal aliphatic end chain which have been shown to influence the gelation properties.[10b] The aggregation of these compounds to produce gels is based both on hydrogen bonding interactions through the peptidic moiety and van der Waals and solvophobic interactions through the aliphatic chains. Interesting results were obtained upon comparison of the ^{1}H NMR HRMAS spectra obtained for these compounds in toluene and acetonitrile. As shown in Fig. 4, in acetonitrile, compound **3** gave ^{1}H NMR HRMAS signals which correspond to the aliphatic side chains, the tetrapeptidic moiety being NMR silent. This result is reasonable considering that the main driving force for gelation is hydrogen bonding and that the central part of the molecule is rigidified in the gel network. The overall poor signal-to-noise ratio of the spectra of **3** and **4** indicates poor swellability and hence poor conformational mobility of the gels in CD_3CN for **3** and none at all for **4**, which is reasonable considering the dominant presence of non-polar moieties in both species, with much longer chains in compound **4** than **3**.

The gel formed by compound **4**, with its longer aliphatic end chains than compound **3**, showed no signal by ^{1}H NMR HRMAS in acetonitrile (Fig. 4). This observation suggests that the polarity of acetonitrile rigidifies the aliphatic moieties in compound **4** through intermolecular van der Waals and solvophobic interactions, this effect being less important in compound **3** due to its shorter chains. This line of reasoning is supported by the results obtained with toluene (Fig. 5). Noticeably, in this apolar solvent, no signals could be detected for compound **3** but rather intense signals were recorded for compound **4** which are assigned to the aliphatic chain (the assignment was achieved by comparison with the NMR spectrum of the compound dissolved in DMSO-d_6). This situation is the opposite of that found in acetonitrile and can be rationalized on the basis of hydrogen bonding strength and affinity of the aliphatic chains for the

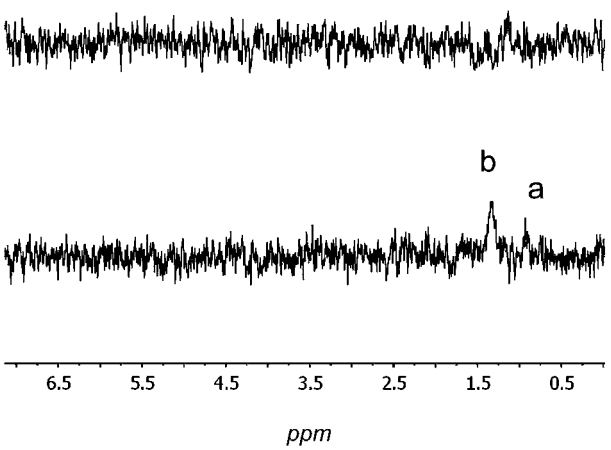

Fig. 4 Diffusion filtered (70%) HRMAS ^{1}H NMR spectra of gels formed by compounds **3** (down) and **4** (up) in CD_3CN. See Scheme 1 for signal labeling.

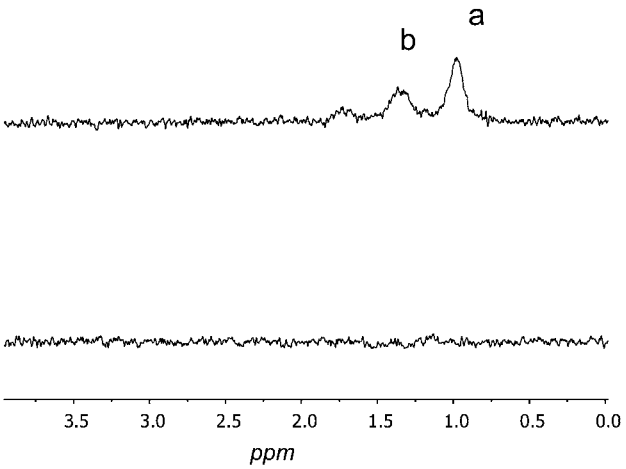

Fig. 5 Diffusion filtered (70%) HRMAS ^{1}H NMR spectra of gels formed by compounds **3** (down) and **4** (up) in toluene-d_8. See Scheme 1 for signal labeling.

solvent. In the case of compound **3**, the lack of HRMAS ^{1}H NMR signals in toluene would reflect the stronger hydrogen bonding interactions in the aggregates formed in this solvent as compared to acetonitrile, resulting in stiff assemblies, including the too short aliphatic chains, which are NMR silent, accordingly being immobilized. By contrast, the much longer aliphatic chains of compound **4** in the aggregated form produce intense HRMAS ^{1}H NMR signals in toluene but are not detected in acetonitrile. It seems that affinity of these long apolar chains for toluene results in enhanced mobility due to extensive solvation of this part of the aggregates.

In conclusion, the results described reveal that diffusion-filtered ^{1}H NMR HRMAS can provide useful information about the conformational behaviour of supramolecular gels as a function of the polarity of the solvent. To the best of our knowledge this is the first report on the successful use of ^{1}H NMR HRMAS in the study of supramolecular gels. The thermodynamically reversible nature of the formation of this type of soft matter requires the use of diffusion filters in order to eliminate the signals from free, non-aggregated molecules which are in equilibrium with the gel network. It is remarkable that in general only some parts of the gelator are detected with this technique. This behaviour can be related to the differentiated mobility of different gelator moieties, the flexible parts only being observable. ^{1}H NMR HRMAS is especially useful to highlight the role played by the solvent in the formation of the aggregates that produce gelation. The use of low polar solvents as toluene increases the strength of intermolecular hydrogen bonding interactions and rigidify aggregates. Interestingly, in the case of gelator **4**, which contains long terminal alkyl chains, the apolar parts of the molecule are rigidified in acetonitrile and would contribute to gelation through solvophobic effects. However, in toluene the affinity of these aliphatic chains for this solvent result in high mobility and therefore would not contribute to the aggregation process significantly.

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12 All high resolution magic angle spinning (HRMAS) NMR spectra were acquired on an Avance2 Bruker 500 spectrometer using a dedicated HRMAS triple channel probe tuned to the resonance frequencies of ^{1}H (500.13 MHz), ^{13}C (125.77 MHz) and ^{119}Sn (186.50 MHz) nuclei, equipped with a *z*-gradient coil reaching a maximum of 53.5 G cm^{-1} of field gradient intensity. Only the proton channel was used in this work. Unless indicated otherwise, the HRMAS spectra presented were obtained in the diffusion-filtered mode, using the longitudinal eddy current delay (LED) diffusion-filtered pulse sequence available in the standard Bruker pulse programme library as "ledgp2s1d", with 70% of the maximum gradient intensity, using SIN.100 gradient pulses. The number of scans was 64, with 16 dummy scans, a relaxation delay $D1 = 3$ s, the diffusion delay was 30 ms, and the gradient recovery delay 3 ms. For standardproton acquisitions, the basic parameters were the same except for the 8 rather than16 dummy scans. The samples were prepared by immersion of the HRMAS rotor in a vial containing a hot solution of the gelator. Upon cooling to room temperature a gel was formed inside the rotor which was then carefully removed from the vial and its outer surface cleaned up. The gels were mechanically stable at the moderate magic angle spinning rate of 4 kHz used in all the HRMAS experiments, and no sample instabilities resulting from centrifugation related phenomena were detected.

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