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New solid state-NMR techniques for investigating structure and dynamics in soft solids and biomaterials

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The potential of high-resolution solid-state NMR to address the problem of establishing quantitative relationships between macroscopic properties of soft solids and biomaterials and their structure and dynamics at the molecular level is investigated. An analysis of advanced proton recoupling pulse sequences to provide unique insights into the structure and dynamics of such materials at molecular level is given. In particular, the excitation of multiple-quantum coherences under rotational resonance conditions is analyzed with respect to quantifying the cross-peak intensities measured from the corresponding 2D ^{1}H-^{1}H correlation spectra in terms of the characteristic order parameter, which provides structural and dynamical information for the investigated materials.

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1. Introduction

Physical characterization of soft-solids of technological and bio-medical interest like elastomers, related mobile polymeric systems, biopolymers, tendons, collagen, etc., represents an important area of research in materials science. For instance, block copolymers in the solid and rubbery states are used as thermoplastic elastomers with applications such as impact modification, compatibilization and pressure-sensitive adhesion [1,2]. More recently a lot of attention was paid to applications in the biomedical field for drug delivery [3,4]. Due to the rapid progress in all these areas block copolymers are nowadays on the verge of a new generation of sofisticated materials applications, in which particular nanostructures will play a crucial role [2].

Biodegradable polymers represents another important class of synthetic biomaterials that are widely used in temporary therapeutic applications such as wound closure, tissue, regeneration and drug delivery. Among the bioabsorbable polymers developed thus far, polyglycolide, poly(lactide) and their copolymers constitute promising materials for the use in the field of surgery and pharmaceutics [5,6]. Unlike non-degradable implants, these materials degrade to harmless products and therefore obviate the need for surgical removal after they have served their purpose. Beside biocompatibility and biodegradability, these type of copolymers has a “memory” that allows them to be deformed into a temporary configuration and then to be restored to the original pattern geometry by applying heat in few seconds [6]. Compared to other shape-memory materials, such as shape memory alloys, the polymers offer much greater deformation capabilities, substantially easier shaping procedure, high shape stability, they are cheaper and in the case of biomedical use biocompatibility and biodegrability.

Collagen is an extracellular-matrix protein that plays an important role in the formation of tissues and organs and is involved in various functional expressions of cells [7]. But, the collagen that is prepared in a matrix form such as a gel for tissue reconstruction is mechanically insufficient. To overcome the disadvantages of collagen while maintaining its biological performance a bioartificial polymeric material was developed by blending or mixing biomolecules and synthetic materials[8]. The chief purpose for developing such a bioartificial polymer material is to overcome the poor biological performance of synthetic polymers and to enhance the mechanical characteristics of biomolecules. To control cell adhesiveness and to increase mechanical strength simultaneously, collagen must be modified by cross-linking or mixing with synthetic polymers [8].

Considering all these applications, the rheological behavior of soft polymeric systems is of central (technological) concern, yet the establishment of quantitative relationships between macroscopic properties and structure and dynamics at the molecular level remains an open challenge. An understanding of microscopic structure and dynamics is necessary in order to manufacture such materials with improved macroscopic properties. Unlike mechanical characterization, the high-resolution solid-state NMR techniques are particularly well suited to address this problem, because they provide chemical site resolution [9].

In our previous paper [10] we have shown the possibility to exctract structural and dynamical informations from organic molecules with 13 C Solid State NMR method.

The investigation of local structure by solid state NMR often requires recoupling of weak dipolar interactions, which otherwise are efficiently averaged out by magic angle spinning (MAS). By rotational resonance (R^2) phenomenon [10] one can selectively reintroduce part of the dipolar interaction between two chemically distinct nuclear sites, *j* and *k*, provided that their isotropic chemical shift difference matches a small integer multiple of the rotor frequency, i.e. $\delta_{jk} = \omega_j - \omega_k = n\omega_R$. A two-dimensional (2D) double-quantum (DQ) experiment under rotational resonance (R^2) conditions has been introduce [12] for evaluating dipolar couplings in rotating solids. An analytical model was developed for isolated spin-pairs and compared with experimental results. In the present work, this simplified model is extended to a 6-spin system made of the protons in the monomeric unit of natural rubber, thus representing a more realistic approximation of spin dynamics in soft solids. The particular conditions under which the DQ-R^2 technique can provide valuable structural and dynamical information in such materials is thoroughly analyzed. Special emphasis is put on quantifying the cross-peak intensities measured from the corresponding 2D ^{1}H-^{1}H correlation spectra in terms of the so-called order parameter, which characterizes the scaling of the residual dipolar interaction under rapid chain motion.

2. Experimental

The experiments were performed on a Bruker DSX 500 NMR spectrometer operating at 500.45 MHz for ^{1}H resonance frequency. The DQ spectra were obtained employing the pulse sequence which is depicted in Fig.1. The excitation of the DQ coherences is obtained using the nonselective two-pulse sequence, where an additional central π-pulse eliminates the frequency-offset dependence of the DQ signal (Fig.1b). This pulse divides the full excitation time into two equal periods of $\tau = m\tau_R$, with m an integer and τ_R the rotor period. For reconversion of the DQ coherences to observable magnetization, a similar pulse sequence is used, but with a π/2 phase shift of its pulses. For recording 2D DQ spectra the TPPI procedure, combined with a four- step phase cycling of the excitation pulses [13] was used.

The investigated system is represented by protons of various functional groups in a cross-linked natural rubber. It is a well-characterized compound and thus can be used to test the performance of the proposed method. The DQ experiments described above was performed at those R^2 conditions for which no R^2-induced line splitting was obtained in conventional SQ spectra. Specifically, this refers to n = 1 rotational resonance condition with respect to the CH-CH$_2$ lines (ν_R = 1.53 kHz) and the CH-CH$_3$ lines (ν_R = 1.74 kHz). To simplify the presentation, the CH, CH$_2$ and CH$_3$ proton resonance frequencies are designed in the following by ω_1, ω_2 and ω_3. Also the notations $\sigma_{jk} = \omega_j + \omega_k$ and $\delta_{jk} = \omega_j - \omega_k$ are used.

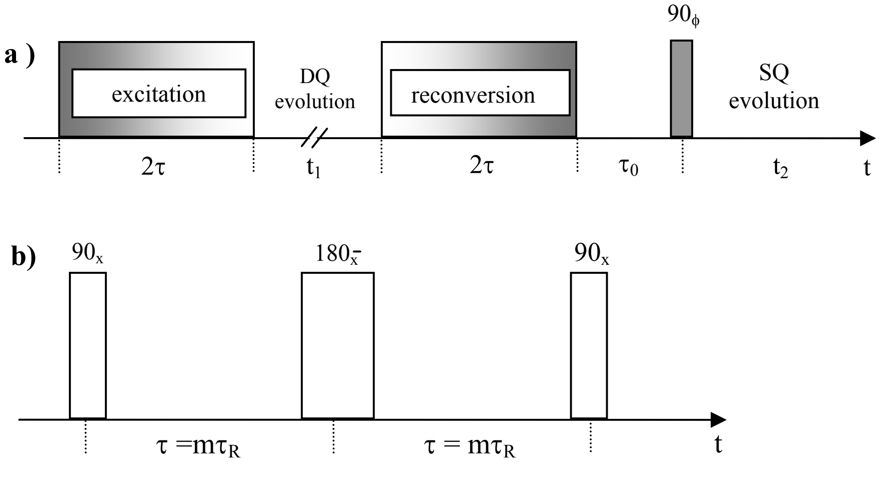

Fig.1. (a) Schematic representation of the 2D DQ MAS experiment. This basic scheme can be used with different excitation/reconversion pulse sequences. The z-filter is represented by a rotor-synchronized purging period τ_0 (inserted delay between reconversion and detection to allow undesired coherences to decay). (b) The pulse sequence used for exciting DQ coherences. For reconversion a similar pulse sequence was used, except that the phases were shifted by π/2.

Figure 2 shows the ^{1}H R^2-DQ spectra recorded with a total excitation time of $2\tau = 16\tau_R$ at the spinning frequencies $\nu_R = \delta_{12}$ (Fig.2a) and $\nu_R = \delta_{13}$ (Fig.2b). This corresponds to the maximum efficiency of the DQ excitation process. In the both cases, a symmetric four-peak pattern determined by the corresponding R^2 recoupled dipolar interaction is obtained, as described in the caption of Fig. 2. The pair of J lines that occurs is correlated with the DQ coherences generated by the J-coupling between the CH-CH$_2$ protons. This can be understood based on the very small values of the other two J coupling constants, compared with $J_{\mathrm{CH-CH2}}$. As an estimation of the relative strengths between the relevant J coupling in natural rubber, one can take for instance, the typical value of the J coupling constants between protons in similar structural arrangements in liquids, i.e., $J_{\mathrm{CH-CH2}} \cong 10$ Hz, $J_{\mathrm{CH-CH3}} < 3$Hz, and $J_{\mathrm{CH2-CH3}} < 1$Hz [14].

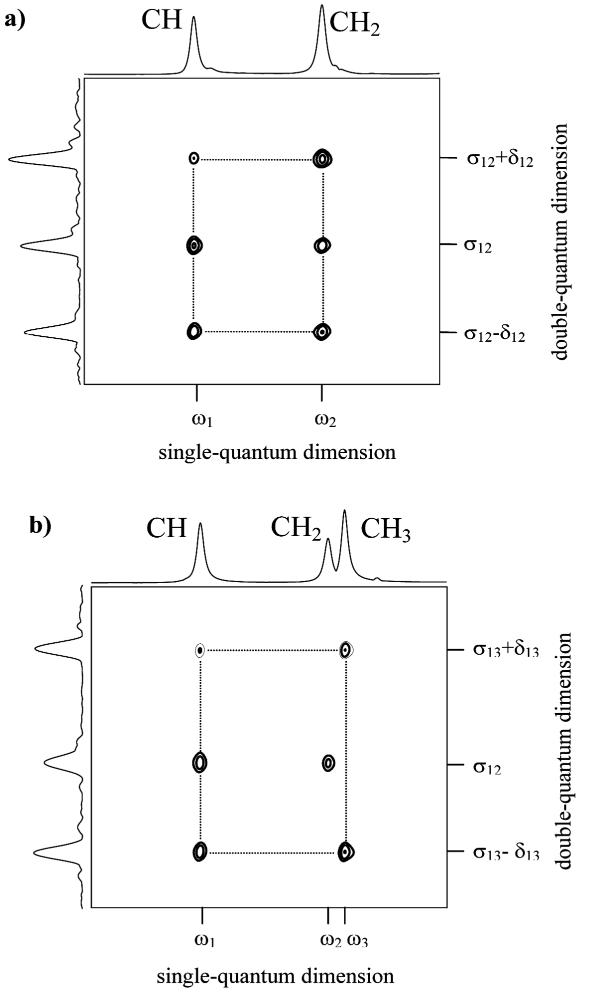

Fig.2. (a) The 2D R^2-DQ spectrum of ^1H in a crosslinked natural rubber (phr = 1), obtained at $\nu_R = \delta_{12} = 1.53$ kHz (n = 1 R^2 condition corressponding to the CH-CH$_2$ lines), using a total excitation time of $\tau = 8\tau_R = 5.23$ ms. The notations ω_1, ω_2 and ω_3 along the SQ dimension represent the ^1H resonance frequencies of the CH, CH$_2$ and CH$_3$ protons. Along the DQ dimension, $\sigma_{jk} = \omega_j + \omega_k$ and $\delta_{jk} = \omega_j - \omega_k$. (b) Similar to case (a), except that the n = 1 R^2 condition corresponding to the CH-CH$_3$ lines is employed now ($\nu_R = \delta_{13} = 1.74$ kHz).

3. Results and discussion

To describe the residual dipolar couplings we consider a model in which the effect of intense molecular motion is assumed to consist only of scaling the rigid-body dipolar couplings by so-called order parameter, $\langle P_2 \rangle$ [15]. This is due to topological constrains induced by crosslinking in elastomers. Specifically, the molecular motion is rendered anisotropic with respect to the axes connecting consecutive cross-link points (either sulfur bridges, or physical entanglements of the polymer chains), considered fixed on the time-scale of the polarization transfer process. Taking a linear chain of N statistical segments, fixed at its extremities, the dipolar coupling constant between any two spins, j and k, is given by $\langle \omega(j,k) \rangle = \omega_0(j,k) \cdot \langle P_2 \rangle$, with $\omega_0(j,k)$ the rigid body coupling constant, and where the exact relationship between the order parameter and N essentially depends on the adopted statistical chain model [16].

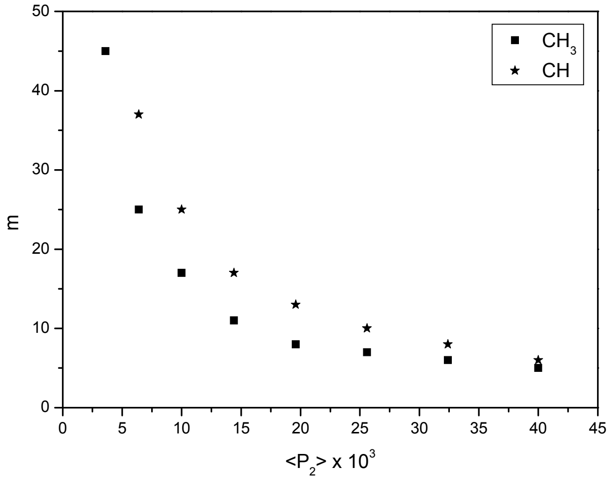

Fig. 3. The order parameter dependence of the maximum in the DQ buildup curves computed for the specified chemical groups in the natural rubber monomer.

Analyzing the spectra from Fig.2 we provide a quantitative estimation of the underlying order parameter. For this purpose, first, we simulate the DQ buildup curves in a system of 6 spins ½, corresponding to protons from CH$_3$, CH$_2$ and CH groups, where the rigid body dipolar couplings are scaled by the order parameter $\langle P_2 \rangle$. These curves are obtained by studying the evolution of the DQ excitation efficiency with increasing the excitation time, $\tau = m\tau_R$, in the pulse sequence depicted in Fig. 1. We consider only the J coupling between CH$_2$ and CH groups, $J_{\mathrm{CH-CH2}} \cong 10$ Hz, the other having negligibly small values, and n = 1 R^2 condition corresponding to the CH-CH$_3$ lines. Such DQ buildup curves have been computed for different values of the order parameter $\langle P_2 \rangle$ ranging between 0.0036 and 0.04. In particular, the maximum amplitudes of the corresponding DQ buildup curves obtained for the CH and CH$_3$ lines are represented as a function of the involved $\langle P_2 \rangle$ values, and they are illustrated in Fig. 3.

Considering the value m = 8 determined experimentally, one can conclude that our investigated natural rubber sample is characterized by an order parameter $< P_2> = 0.02$.

The DQ buildup curves corresponding to CH_3 and CH groups simulated for this particular experimental parameters, and scaled dipolar couplings, are represented in Fig. 4. As can be seen, the CH resonance appears to give rise to larger DQ cross-peaks. However, in this particular case one should take into account that both, the CH_3-CH residual dipolar interaction, recoupled under rotational resonance condition, and the CH_2-CH J coupling, contribute to generating the observed DQ signal. By contrast, in the case of CH_3 line, only the former mechanism is responsible for DQ excitation.

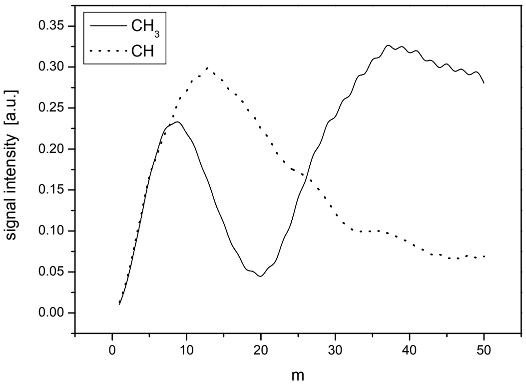

Fig. 4. The DQ buildup curves corresponding to the CH_3 (straight line) and CH (dot line) groups simulated under the conditions of maximum DQ excitation efficiency at $< P_2> = 0.02$, i.e., $\tau = 8\tau_R$.

As demonstrated above, a comparison between the theoretical and experimental DQ buildup curves (obtained under rotational resonance conditions with respect to different resonance lines in the ^{1}H NMR spectrum) is in principle sufficient to provide an accurate evaluation of the order parameter induced by the characteristic molecular motion in the investigated soft solid material. However, for very small values of the order parameter, the maximum in the DQ buildup curve is obtained at relatively large excitation time values. For instance, in highly mobile polymer chains, the inter-group residual dipolar couplings are scaled down values of the order of 10 to 100 Hz, which requires extended hundreds of ms for getting an efficient excitation of the DQ coherences. However, for such long times one can no longer neglect the effect of relaxation, which will interfere with the coherent evolution of the spin system in defining the maximum in the DQ buildup curve. In such extreme situations, the order parameter can be alternatively evaluated from the relative intensities of the DQ peaks generated in the 2D R^2-DQ ^{1}H spectrum by the recoupled dipolar interaction, and J coupling, respectively. This procedure is illustrated in Fig. 5 with the example of the DQ projection of the cross peaks in the 2D R^2-DQ spectrum simulated for the case of the rotational resonance condition with respect to the CH-CH_3 groups. Comparing the computed pattern with the experimental spectrum (Fig. 2b), one can conclude that this procedure can provide a reliable estimation of the underlying order parameter, even in the cases when the decay of the DQ buildup curve is strongly affected by relaxation.

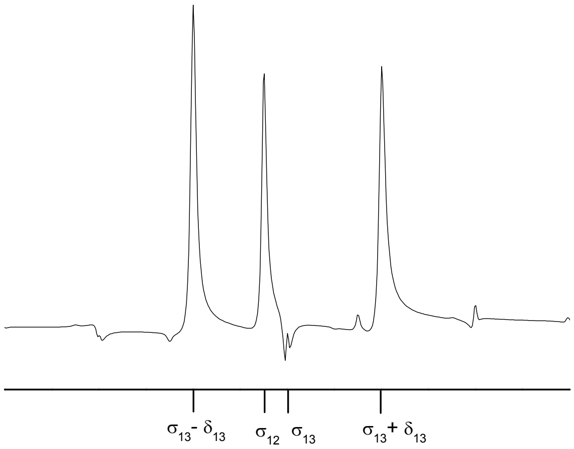

Fig.5. Projection along the DQ dimension of the 2D R^2-DQ spectrum of ^{1}H in a crosslinked natural rubber (phr = 1), obtained at $\nu_R = \delta_{13} = 1.74$ kHz (n = 1 R^2 condition corresponding to the CH-CH_3 lines), using a total excitation time of $\tau = 8\tau_R = 5.23$ ms. As previously, we use the notations, $\sigma_{jk} = \omega_j + \omega_k$ and $\delta_{jk} = \omega_j - \omega_k$, where ω_1, ω_2 and ω_3 represent the ^{1}H resonance frequencies of the CH, CH_2 and CH_3 protons.

3. Conclusions

The potential for practical applications of the R^2-DQ technique to many organic materials that fall in the category of soft solids (elastomers, related mobile polymeric systems, biopolymers, certain biomaterials, tendons, collagen, etc.) is demonstrated both, experimentally and theoretically.

It is shown here, that this solid-state NMR method can provide unique structural insights into such materials with chemical site resolution, unlike other analytical methods which are based on their mechanical characterization.

The theory necessary for the basic understanding of the resulting 2D R^2-DQ spectra was derived in a previous work [11] by confining the analysis to simplified 3-spin model.

Here, one extends this treatment to a more realistic 6-spin model, which is applied to quantify the experimental data recorded on a crosslinked natural rubber.

A good agreement between the theory and the measured data is obtained in this case, which leads to the conclusion that the proposed R^2-DQ experiment represents a powerful technique that enables one a reliable estimation of the ^{1}H-^{1}H inter-group residual dipolar couplings, as well as of the underlying order parameter.

Acknowledgments

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