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Enantiomeric visualization using proton-decoupled natural abundance deuterium NMR in poly(γ-benzyl-L-glutamate) liquid crystalline solutions

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Abstract

We report the first visualization of chiral molecules oriented in a polypeptide liquid crystalline system (PBLG) using proton-decoupled natural abundance deuterium NMR. The chiral discrimination is observed through measurements of the quadrupolar splitting differences and we demonstrate that the sensitivity of natural abundance deuterium NMR is sufficient to measure the differential ordering effects (DOEs) without the need for isotopic enrichment. The feasibility and the potential of this novel method were investigated using a 5.87 T spectrometer (proton frequency 250 MHz). Several examples of chiral discrimination are presented and particular emphasis is given to demonstrate the potential of this approach.

1. Introduction

An important motivation for research in the field of enantiomeric analysis through NMR is the wish to provide organic chemists with new, efficient and general tools. It has been shown that proton-decoupled deuterium NMR of isotopically labelled enantiomers dissolved in poly(γ-benzyl-L-glutamate) (PBLG) liquid crystalline solutions serve as a very powerful method for the measurement of the enantiomeric excess.[1,2] In this technique, two deuterated enantiomers usually exhibit different quadrupolar splittings, $\Delta\nu_Q^{R \text{ or } S}$ in their proton-decoupled (^{2}H–{^{1}H}) NMR spectra. This enables their discrimination and is shown schematically in Fig. 1. The efficiency of chiral discrimination depends both on the ability of PBLG to interact differently with the two enantiomers, which produces a differential ordering effect (DOE), and the relatively large magnitude of the deuterium quadrupolar coupling constant for a C–D

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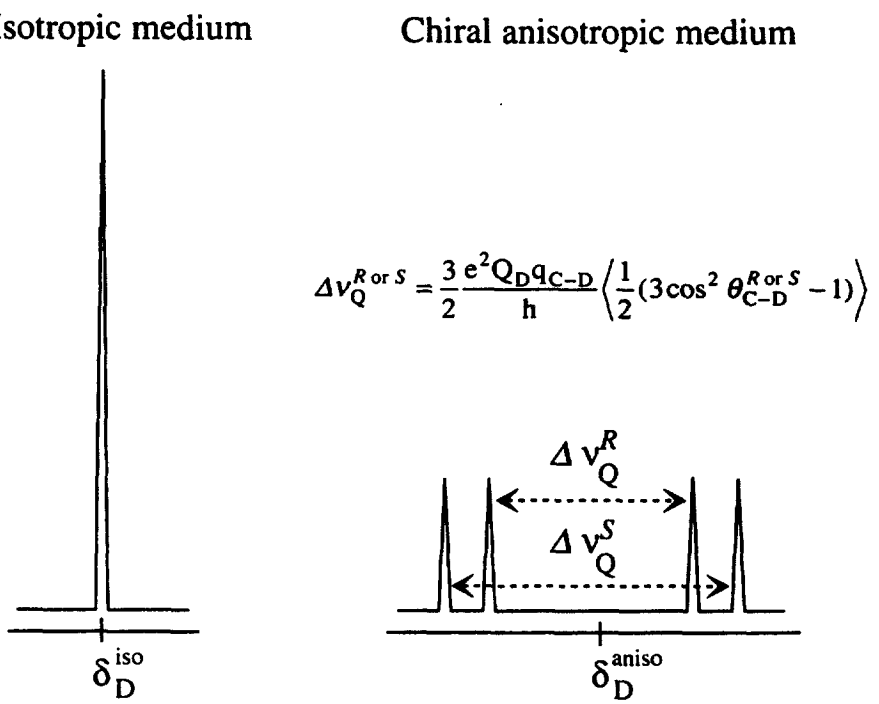

Fig. 1. Schematic $^2H-\{^1H\}$ NMR spectrum of a racemic mixture of a monodeuterated molecule in an isotropic and chiral anisotropic medium. Both spectra are plotted to scale. $\Delta\nu_Q^R$ and $\Delta\nu_Q^S$ are the quadrupolar splittings for each enantiomer (the notations *R* or *S* are arbitrary). Q_D is the deuterium quadrupole moment, q_{C-D} is the electric field gradient (EFG) along the C–D bond and $\theta_{C-D}^{R\text{ or }S}$ is the angle between the EFG and the magnetic field B_0

bond ($e^2Qq/h \approx 170$ KHz). Thus, a small difference in the orientations of the *R* and *S* enantiomers can give a sufficiently large difference in their quadrupolar splitting ($\Delta\nu_Q^R - \Delta\nu_Q^S$), and enables us to visualize each of them.[1,3,4] The method was applied successfully to a large variety of chiral materials, including molecules which are chiral by virtue of isotopic substitution.[2,5] Although this technique has a great potential for enantiomeric analysis, the synthesis of deuterated chiral molecules is a constraining condition, because it may require considerable efforts and time.

In another approach, we have demonstrated that natural abundance carbon-13 (1.1%) NMR could be used to visualize chiral discrimination through the dipolar interactions or the chemical shift anisotropies (CSA).[6,7] Thus, the proton-decoupled natural abundance carbon-13 NMR was sometimes found to be a convenient alternative to the deuterium technique. The advantages of using natural abundance carbon-13 NMR are obvious in spite of the fact that the magnitude of carbon-13 CSA is much lower compared to deuterium quadrupolar interactions.[7] In the first place, no isotopic enrichment or chemical modifications of the chiral material are needed. Secondly, all the rare atoms of the chiral solute can be simultaneously probed, thus increasing the probability of observing a chiral discrimination. Also, the spectral analysis of rare spins in an anisotropic medium is considerably simplified due to the absence of residual dipolar couplings between two rare atoms.[6]

If the low sensitivity of detection of a rare spin is not an insurmountable obstacle to these measurements, then it is pertinent to assess whether the observation of deuterium in natural abundance level (0.015%), in enantiomers dissolved in PBLG solvent, is possible under acceptable experimental conditions. Such an analytical approach is highly attractive and a real challenge, because it would enable us to combine the sensitivity of deuterium quadrupolar interactions to the orientation phenomenon

with the natural abundance NMR advantages. Additionally, we can take advantage of the possibility of choosing the most suitable site in the molecules for the enantiomeric excess measurements.

Although the absolute NMR sensitivity of deuterium is only of the order of 1.45×10^{-6} with respect to the proton, modern high magnetic field NMR instruments permit natural abundance deuterium spectra of neat liquids or highly concentrated samples to be recorded. Deuterium NMR spectroscopy at natural abundance level was developed extensively by Martin et al. for the characterization of molecules and their biological and geographical origin (SNIF NMR), and applied successfully by Pascal et al. for the investigation of kinetic isotope effects (KIEs) in organic and biochemical reactions.[8–11] Recently, natural abundance deuterium solid state NMR experiments have been reported.[12,13] In our case, the situation is more difficult since the chiral molecules under investigation are dissolved in a chiral liquid crystalline phase which diminishes considerably the number of deuterium spins per unit of volume of the sample, and because the quadrupolar splittings reduce the line intensities by a factor of 4 (racemic mixtures) compared to isotropic spectra (cf. Figure 1). Furthermore, the appearance of the solvent signals (PBLG and organic solvent) may interfere with the spectral analysis. However, it can be expected that natural abundance deuterium signals from PBLG itself would not appear in the spectrum, because of their large linewidths compared to those of the chiral solutes.

In this paper we present results showing that the proton-decoupled deuterium NMR (^{2}H–{^{1}H}) at natural abundance level, can provide a novel and simple method for visualizing enantiomers in PBLG liquid crystalline solvent. The purpose of this paper is to show the feasibility of a method using a 5.87 T NMR spectrometer (38.4 MHz for ^{2}H). To illustrate this approach, several examples of chiral discrimination will be presented and discussed. It might be noted that many of the available NMR instruments operate at fields higher than 5.87 T, which implies that our results would certainly be valid and could be achieved more easily using such instruments.

2. Experimental

The samples were prepared using PBLG with a DP=854, MW≈188000 (Sigma). The components of the mixture were weighed directly into a 5 mm o.d. NMR tube which was sealed to avoid evaporation. For efficient mixing and dissolution, the NMR samples were centrifuged back and forth until an optically homogeneous birefringent phase was obtained.

The proton-decoupled deuterium experiments were performed on a Bruker AM 250 high resolution spectrometer equipped with a 5 mm diameter inverse broad-band probe operating at a frequency of 38.4 MHz for deuterium. The NMR tubes were not spun in the field and the temperature was controlled by the Bruker BVT 1000 temperature controller. The sample temperature has to be regulated very carefully in order to keep a good long term thermal stability. Finally, before recording each spectrum, the probe was tuned and matched for optimal sensitivity. The protons were broad-band decoupled using the WALTZ-16 composite pulse sequence (1 W of rf power).[14]

A large number of scans is needed to obtain an adequate signal-to-noise ratio (S/N) which considerably prolongs the NMR measuring time. However, this point is not so critical because deuterium T_1 values of solutes dissolved in PBLG are generally of the order of 1 s, which permits a fast repetition rate of the pulsing (i.e. the acquisition time (AT) plus the relaxation delay (RD)). Compared to the long T_1 values of carbon-13, this situation is more advantageous. With a typical repetition time of the order of 0.5 s, the T_1/(AT+RD) ratio is about 1.5–2, leading to an optimum pulse angle of about 50–60°.[15,16] Under these conditions the deuterium spectra were recorded with a total number of scans between 150,000 and 200,000 with 512 or 1024 data points. The spectral width was about 1500 Hz. The total measurement

time was therefore around 18–24 h, depending on the various NMR parameters. The spectrometer was operated in the unlocked mode. In order to check both the drift of the magnetic field and the long term thermal homogeneity of the sample during the NMR experiment, FIDs were acquired in blocks of 10,000 or 20,000 scans. Each FID's block was added successively to the previous one, prior to the Fourier transformation. Zero filling to 4096 or 8192 data points was used to improve the digital resolution. Specific details for each spectrum are given in the text and in the figure captions. The linewidths in the spectra were typically 3–7 Hz.

3. Results and discussion

3.1. Chiral binary mixtures

Ordinarily, the chiral molecules were added to solutions of PBLG in achiral organic solvents, such as dichloromethane, chloroform, dimethylformamide, dioxane and others, thus forming a 'chiral ternary mixture'. In some particular cases, chiral nematic solutions can be obtained by dissolution of the chiral compounds (or otherwise the molecule under study) in PBLG. We shall denote such solutions as 'chiral binary mixtures'. These cases will be illustrated by the study of PBLG dissolved in phenethyl alcohol and benzyl alcohol. *A priori*, these mixtures offer two advantages: first, the molar concentration and consequently the number of nuclear spins of solute per unit of volume are much larger than in ternary mixtures; and second, the signals of the additional organic solvent are absent from the NMR spectrum.

3.2. Phenethyl alcohol

The NMR sample was prepared from 160 mg of PBLG, and 600 mg of racemic (±)-phenethyl alcohol, corresponding to a weight fraction of PBLG and of each enantiomer of 21.1% w/w and 39.4% w/w, respectively. At room temperature, a very viscous phase (possibly a gel) exists which becomes a liquid crystal above ~310 K.[17]

The $^{2}H-\{^{1}H\}$ spectrum of (±)-phenethyl alcohol is shown in Fig. 2. It was recorded at 320 K using 170,000 scans of 512 data points. The relaxation delay was 0.1 s and the acquisition time was 0.34 s per FID. A Gaussian filtering was applied to improve the spectrum resolution. The signal-to-noise ratio value varies considerably from the resonances of one molecular group to another, reflecting the number of equivalent isotopomers. Thus, the S/N ratio varies between 27 for the methyl group to 4 for the methine group.[18] All deuterons of the chiral molecule were observed in the spectrum whereas no deuterium signals from the PBLG are detected.

As no significant differences exist between the anisotropic proton and deuterium chemical shifts, we were able to determine unambiguously the centre of each doublet in the deuterium spectrum and attribute it to the corresponding isotopomer. Due to the reduction factor of 6.5 of the frequency range, the analysis of the isotropic deuterium spectrum is always more difficult than that of protons. However in our case, the dispersion of the magnitude in the quadrupolar splittings facilitates the reading of spectra, and subsequently their interpretations. The magnitudes of the quadrupolar splittings observed in the spectrum vary from 5.9 Hz to 644.6 Hz, corresponding to deuterons of the methyl group and the para deuteron of the phenyl group, respectively.

In the spectrum presented in Fig. 2, we can distinctly observe 10 out of the expected 12 doublets assuming that all sites are discriminated. A careful analysis of the spectrum clearly shows that chiral discrimination was not visualized for the deuterons of the hydroxyl group for which a single quadrupolar

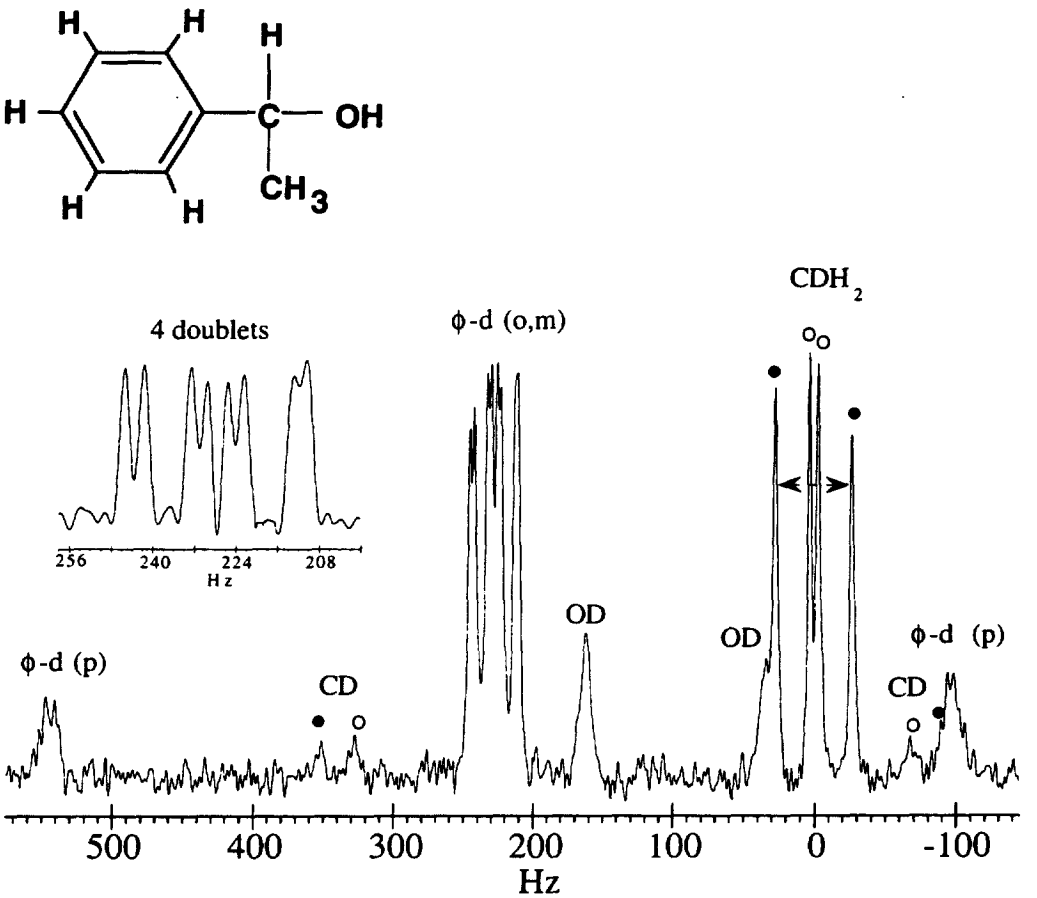

Fig. 2. $^{2}H-\{^{1}H\}$ in natural abundance (±)-phenethyl alcohol dissolved in the PBLG at 320 K. A Gaussian filtering (GB=30%, LB=−3 Hz) and zero filling to 8192 data points were used to improve the spectral appearance and the digital resolution. The peaks arising from the deuterons in the ortho, meta and para positions of the benzyl group are defined by the symbols ϕ-d (o,m) and ϕ-d (p), respectively. In the insert, we show the eight lines associated with the ortho and meta deuterons of the *R* and *S* benzyl groups

splitting of 127.7 Hz is observed. This situation arises because of the fast exchange of the hydroxyl deuterons. The best site to measure an eventual enantiomeric excess in this molecule is the methyl group. The quadrupolar splitting difference of the methyl group between the two enantiomers (47.8 Hz) is sufficiently large, with a good signal-to-noise ratio. The methyl peaks due to each enantiomer are labelled by (○) and (●) in Fig. 2. We are unable to attribute the lines belonging to either the *R*- or *S*-enantiomers since the compound which we measured is racemic. Considering the S/N ratio, the best sites of chiral discrimination would always be that group which possesses the largest number of magnetically equivalent spins that contribute to a given doublet. Therefore, the tert-butyl or trimethylsiloxane groups, (nine magnetically equivalent nuclei) would provide the best probes for natural abundance deuterium NMR measurements.

3.3. Benzyl alcohol

With the same composition as for the phenethyl alcohol, this compound produces a liquid crystalline phase with PBLG from 310 to about 340 K.[17] Although benzyl alcohol is achiral, it possesses a prochiral

methylene group. In the PBLG liquid crystalline phase, the two enantiotopic nuclei in the prochiral methylene group, become magnetically nonequivalent, thus enabling us to distinguish between them through their deuterium NMR spectrum.[4] In fact, the pro-*R* and pro-*S* faces are discriminated through ^{2}H NMR by a difference in the order parameter of each CD bond, S_{CD}.[2,4] This phenomenon was first observed by Samulski et al. and later confirmed by us.[2,19] In natural abundance deuterium NMR of such a molecule, we actually observe the CDH group. In this case, the chiral discrimination is similar to that of molecules which are chiral by virtue of isotopic substitution. Consequently, we would only observe chiral discrimination between the two isotopomers having an *R* and *S* deuteron in the methylene group.

The natural abundance deuterium ^{2}H–{^{1}H} spectrum of benzyl alcohol is shown in Fig. 3. The spectrum was recorded with the same NMR conditions as described above. Again, the S/N ratio changes considerably from one group to another, varying between 59 for the phenyl group to about 13 for the methylene group. As expected, two well-separated quadrupolar doublets are observed for the methylene group, whereas only one doublet is obtained for other isotopomers. The quadrupolar splitting difference for the two chiral isotopomers is 112.2 Hz. It may be noted that the ortho and meta deuteron signals of the benzyl group appear as a single doublet and we believe that this situation is fortuitous for this particular temperature and sample composition. As for the (±)-phenethyl alcohol, the quadrupolar splitting, and subsequently the S_{CD} value, for the deuteron in the para position is much larger (in absolute value) than in the ortho and meta position. This indicates that the ortho and meta C–D bonds lie close to the magic angle. This phenomenon is quite general and has already been reported for other perdeuterated benzylic compounds.[2,20]

3.4. *Chiral ternary mixtures*

The observation and discrimination of enantiomers in binary mixtures is limited to a few cases where a liquid crystalline phase can be formed by dissolution of PBLG in a chiral solvent. Alternatively, ternary mixtures, in which an achiral solvent is included, offer very broad possibilities for the study of enantiomeric analysis. We have therefore investigated the case of chiral ternary mixtures in order to assess the feasibility and the potential of the method through natural abundance deuterium NMR. Samples were prepared by dissolving relatively small amounts of chiral material in organic solutions of PBLG. With selectively deuterated materials, it was shown that optimal results were obtained in solutions containing 12–25% by weight of PBLG and 15 to 30 mg of chiral compounds.[1,2] We must anticipate that to compensate for the very low receptivity of deuterium in natural abundance, larger quantities of non-labelled chiral solute would be needed.

The solvent used for the chiral ternary mixtures was chloroform. Chloroform as a solvent is advantageous, because it contains a single isotopomer and the number of deuterons per unit volume is small. It would also limit the digitization problems associated with the dynamic range of the analogue-to-digital converter of the spectrometer.[21] Solvents such as CCl_4, which do not contain hydrogens, might serve even better but are not good solvents for PBLG.

3.5. *Phenethyl alcohol*

A chiral ternary mixture was made from 100 mg of PBLG, 200 mg of the racemic mixture of (±)-phenethyl alcohol and 300 mg of $CHCl_3$. The natural abundance deuterium spectrum, shown in Fig. 4, was recorded at 301 K using 150000 scans of 800 data points. The relaxation delay was 0.26 s and the acquisition time was 0.34 s per FID, corresponding to a total NMR experiment time of the order of 18 h. No apodisation was applied to enhance the resolution of the spectrum.

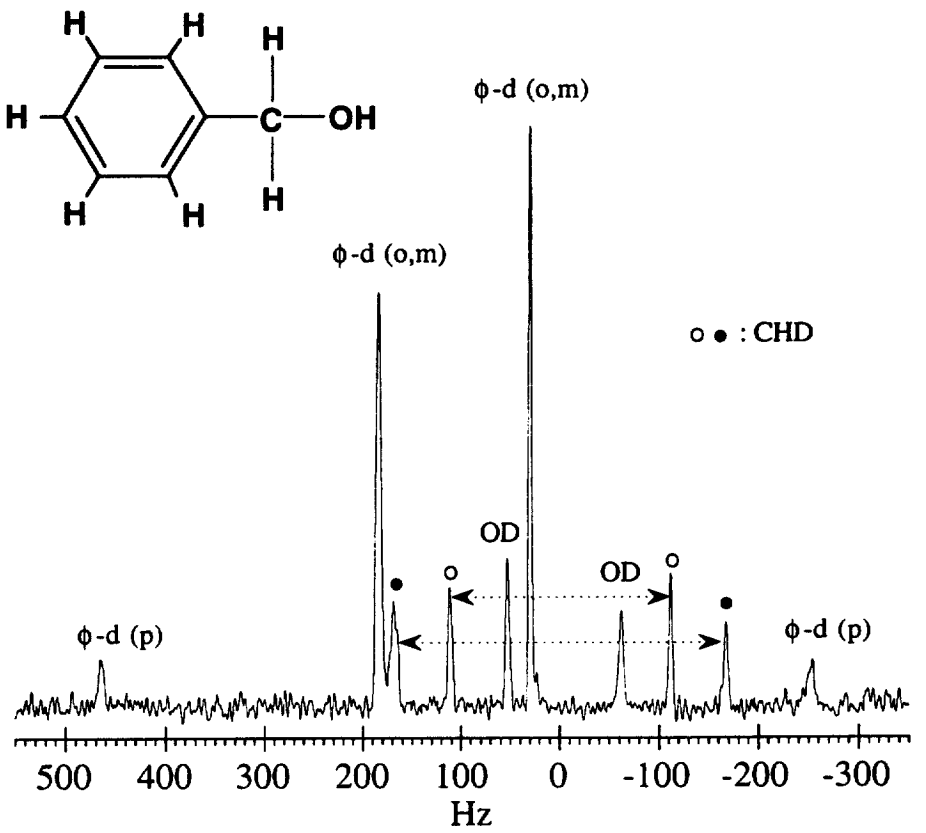

Fig. 3. $^2H-\{^1H\}$ in natural abundance benzyl alcohol dissolved in the PBLG at 320 K. A Gaussian filtering (GB=30%, LB=−3 Hz) and zero filling to 8192 data points were used to improve the spectral appearance and the digital resolution

The quadrupolar splitting of the solvent doublet (natural abundance $CDCl_3$ in $CHCl_3$) is about 350 Hz. This value is typical for such compositions and facilitates its assignment. Moreover, the centre of this doublet can be used as an internal reference (7.3 ppm). In the spectrum, the average linewidth of the solute peaks is 3 Hz, while the linewidths of the solvent peaks are slightly larger, about 5 Hz. This is because the linewidths, in ordered media, increase with the magnitude of the quadrupolar splittings. It should be noted that the hydroxyl group has a large quadrupolar splitting and very broad line, and is hardly observable in the spectrum. Finally, the analysis of the spectrum shows that the methyl group of the molecule exhibits two well-resolved doublets, which enable us to clearly discriminate between the two enantiomers. The S/N ratio measured for these peaks (S/N≈30) enables us to measure the enantiomeric excess with an estimated precision of the order of 15%.

3.6. 3-Butyn-2-ol

In order to explore the lowest possible limit of solute concentration for the enantiomeric analysis with a 5.87 T magnetic field, within reasonable experimental time, we prepared a chiral ternary mixture made from 100 mg of PBLG, 100 mg of the racemic mixture of (±)-3-butyn-2-ol and 350 mg of $CHCl_3$. This corresponds to a concentration of PBLG and enantiomers of 18.2% w/w and 9.1% w/w, respectively. The enantiomeric molar quantity in this case is 7.1×10^{-4} mol. The natural abundance deuterium spectrum was recorded with the same NMR conditions as described above. The spectrum is presented in Fig. 5.

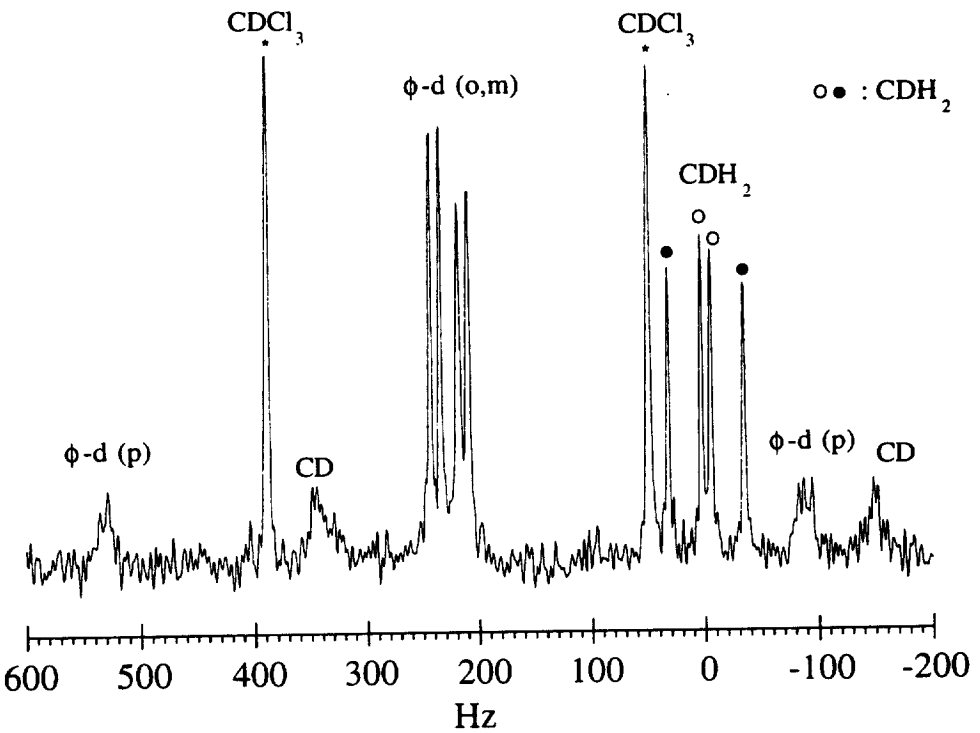

Fig. 4. ^2H–{^1H} in natural abundance of the (±)-phenethyl alcohol dissolved in the PBLG/$CHCl_3$ system at 301 K. No filtering and zero filling to 4096 data points were used

In this example, the percentage of deuterated isotopomers of chloroform is very close to that found for the *R*- and *S*-methyl groups. Consequently, we observe in the spectrum that the intensity of the $CDCl_3$ doublet is close to that of the methyl group for each enantiomer. As in the previous example, the signal of the chloroform does not interfere with other signals of the spectrum. Here again we can observe and discriminate each of the enantiomers for the methyl group signal of the molecule. The quadrupolar splitting difference is very large (151.9 Hz), and the signal-to-noise ratio (S/N≈18) conveniently enables us to evaluate the enantiomeric excess.

3.7. Future potential of the method

In this work, we draw attention to the possibility of observing chiral molecules through their natural abundance deuterium NMR spectra, thus proposing a practical solution to DOE measurements without isotopic enrichment. Our results clearly show the feasibility and the efficiency of this method using simple experiments and standard NMR equipment.

Natural abundance deuterium NMR is characterized by its low sensitivity. An obvious solution is to operate at higher magnetic fields (B_o) as the S/N ratio is proportional to $(B_o)^{3/2}$. On the other hand, for a given S/N ratio, the total experimental time (EXPT) is divided by a factor $(B_o)^3$ when operating at higher fields. Figure 6 shows the evolution of the S/N ratio and EXPT with respect to values needed when a 250 MHz (proton frequency) spectrometer is employed.[18] Thus, when recording the deuterium spectra at 76.8 MHz (500 MHz spectrometer), the S/N ratio would be increased by a factor of 2.8 for the same EXPT. Conversely, the EXPT would be reduced by a factor of 8 to obtain the same S/N ratio that is obtained at 250 MHz. This would also mean that the S/N ratio of 18 measured for the methyl group

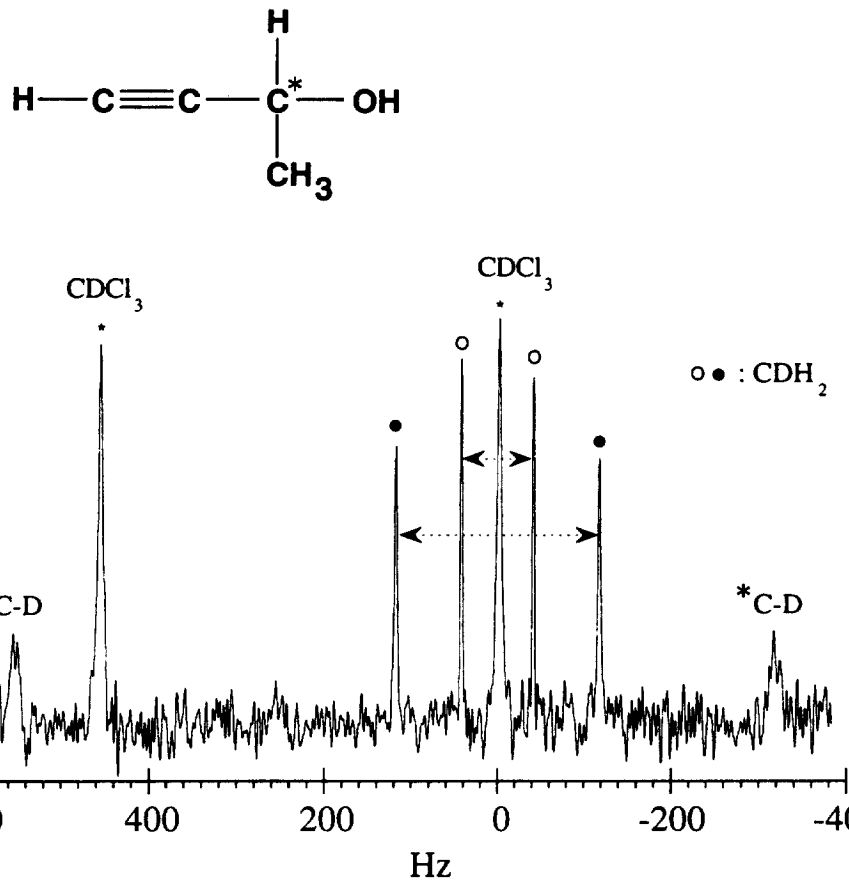

Fig. 5. $^2H-\{^1H\}$ in natural abundance of the (±) 3-butyn-2-ol dissolved in the PBLG/$CHCl_3$ system at 301 K. No filtering and zero filling to 4096 data points were used

in last presented example would become 50 at 500 MHz and approach 100 at 750 MHz with the same experimental conditions. Such an S/N ratio would then result in an acceptable error in the determination of enantiomeric excesses.

Finally, as the NMR sensitivity is also proportional to $(T_2^*)^{1/2}$ (T_2^* being the inverse linewidth), we need to reduce all magnetic field instabilities that contribute to the linewidths, thus degrading the S/N ratio and spectral resolution. For this purpose, the stability of the field must be carefully controlled during the experimental time. The application of a fluorine-19 lock channel should be beneficial for this purpose.

4. Conclusions

We report in this paper that natural abundance deuterium NMR spectroscopy may be employed for the visualization of chiral molecules oriented in organic solutions of poly(γ-benzyl-L-glutamate). We want to draw attention to the feasibility of this method using standard NMR experiments and routine magnetic field strengths. In spite of the low NMR sensitivity of deuterium, these experimental results are very encouraging since with a 5.87 T spectrometer, we were already able to roughly estimate the enantiomeric excesses. It must be emphasized that the use of very high magnetic fields will enable

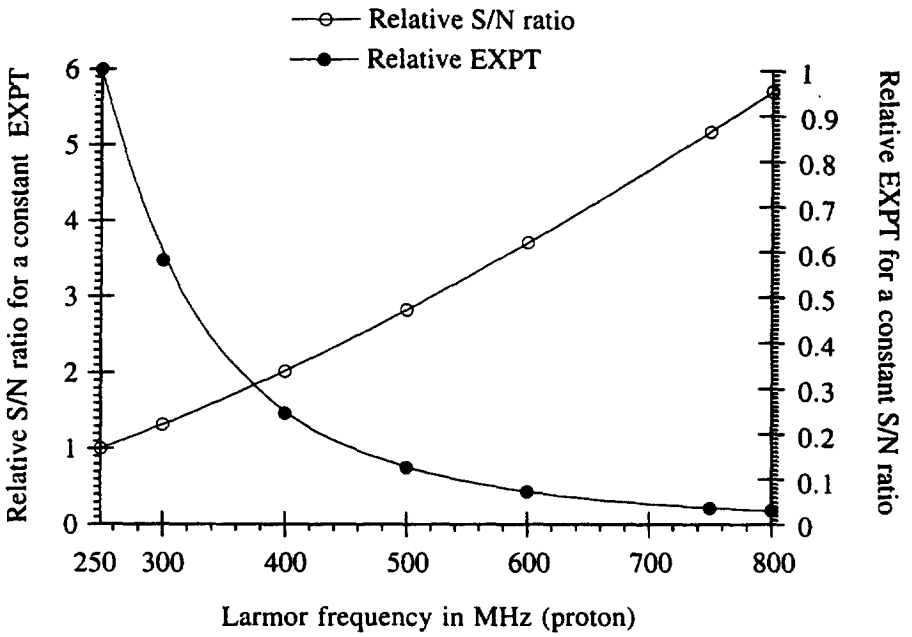

Fig. 6. Evolution of the relative S/N ratio and the relative experimental time (EXPT) as a function of the Larmor frequency with respect to a 250 MHz (proton) spectrometer. The reference value at 250 MHz is 1

the measurement of the enantiomeric excesses with a satisfactory precision. We believe therefore, that natural abundance ^{2}H–{^{1}H} NMR spectroscopy in PBLG will become an efficient NMR technique for the purpose of enantiomeric analysis.

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