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Application of z-COSY experiment and its variant for accurate chiral discrimination by ^{1}H NMR

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ABSTRACT

We report the application of z-COSY experiment and a band selected version of it by employing a selective 90° pulse entitled BASE-z-COSY for precise chiral discrimination, quantification of enantiomeric excess and the analyses of the ^{1}H NMR spectra of chiral molecules aligned in the chiral liquid crystalline solvent poly-γ-benzyl-L-glutamate (PBLG). We have demonstrated their applicability for obtaining very high resolution in the ^{1}H NMR spectra of small organic molecules. It is well known that the commonly employed z-COSY experiment disentangles the spectral complexity, provides pure phase spectra with high resolution, aids in the complete spectral analyses, in addition to yielding information on relative signs of the couplings. The BASE-z-COSY experiment possesses all these properties, permits the measure of enantiomeric excess, in addition to large saving of instrument time.

1. Introduction

The discrimination of enantiomers and the quantification of excess of one form over the other are of paramount importance in pharmaceutical industry as well as in asymmetric synthesis. It is well known that enantiomers cannot be distinguished by NMR unless diastereomorphic interactions are imposed. Several chiral aligning media [1–8] have therefore been reported for their visualization, although the extensive studies are carried out using the polypeptide liquid crystal poly-γ-benzyl-L-glutamate (PBLG) [9]. In the PBLG aligning liquid crystalline medium, the difference in the orientational parameters, consequently the order sensitive NMR parameters enable enantiodiscrimination. For nuclei with spin $I > ½$ such as deuterium (^{2}H), the majority of the work using PBLG is focused on ^{2}H NMR in natural abundance exploiting the large strengths of quadrupole couplings (Q_i) compared to other NMR interaction parameters. The use of ^{13}C NMR experiments has also been reported [10,11]. Though ^{1}H detection is largely ignored there are several advantages because of its high sensitivity and the ubiquitous presence of protons in all the chiral molecules. It is also imperative that when dealing with spin ½ ^{1}H nuclei the only available option is to work with chemical shift anisotropies ($\Delta\sigma_H$), the homonuclear (D_{HH}) and heteronuclear (D_{XH}, where X = nucleus other than protons) dipolar couplings.

Though rich in information content, the large number of short and long distance couplings experienced by each inequivalent proton results in broad and featureless spectra. In addition there is also an overlap of spectra from both the enantiomers. For bigger molecules the analyses of ^{1}H NMR spectra and the derivation of any meaningful information is challenging [12] and hence its routine application is severely hindered. This is the reason for extensive application of ^{2}H NMR and a recent review describes the large quantity of work [9]. In spite of several difficulties, there are experimental schemes reported to visualize enantiomers using ^{1}H NMR and a brief discussion is available in a report [13]. Recently there are also reported experimental methodologies for both differentiation and analyses of complex ^{1}H NMR spectra [14–18].

The homonuclear two-dimensional experiments such as Soft-COSY and BASE-β-COSY provided better spectral resolution and aided the determination of spectral parameters [16,17]. The BASE-β-COSY has also been demonstrated to be superior over previously discussed methodologies. The drawback of these correlation experiments is that the overall intensities of the cross peak multiplicities in BASE-β-COSY are less than diagonal peak components. Also the cross peak multiplets have absorptive line shapes while the diagonal peaks are of phase twisted in nature.

For very high resolution ^{1}H spectra the phase twisted line shapes are undesirable and the pure phase spectra would be of significant

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help. In achieving good line shapes and resolution the SERFph [19] and improved SERFph [20] experiments which are the modified versions of original SERF experiment [21] have been reported. The improved SERFph pulse sequences resulted in better line shapes and chiral discrimination but demand several single- or bi-selective excitations for obtaining complete spectral parameters, especially when the coupled spin network is reasonably big, thereby limiting their applications.

In circumventing this problem we apply the well known z-filtered correlation experiment (z-COSY) [22,23] and utilize a modified version of it, entitled BASE-z-COSY. The pulse sequences for these experiments are given in Fig. 1. These experiments are much superior to the previous experiments [16,17] in terms of pure absorption mode line shapes providing cleaner spectrum aiding accurate chiral discrimination and the precise measure of couplings of small magnitudes hidden within the line widths, in a single experiment. The study is demonstrated on a chiral molecule possessing six interacting spins not only for better chiral discrimination but also for the precise analyses of broad and overlapped ^{1}H NMR spectra. The sample preparation and analysis of one-dimensional spectrum being well known, is given as a Supplementary information.

2. Two-dimensional z-COSY experiment

The well known z-COSY pulse sequence (Fig. 1A) [22,23] results in reduced spectral multiplicity, with comparable intensities for both the diagonal and cross peaks and absorption mode line shapes. The z-COSY experiment also provides information on the relative signs of the couplings. Its application has been demonstrated in the automated analyses of the spectra by pattern recognition [24], and by using energy level connectivity information [25–27]. However, in spite of several advantages there are very few examples of the application of z-COSY technique for the analyses of the NMR spectra of bigger biomolecules [28].

Though one might argue that z-COSY experiment is less sensitive compared to previously reported Soft-COSY and BASE-β-COSY experiments [16,17], it is never a hindrance when proton is detected. Instead, one can derive benefits of pure phase mode, and with comparable intensities between cross peaks and diagonal

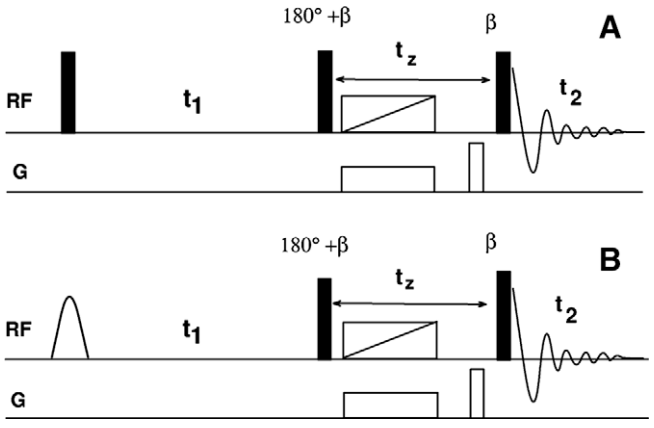

Fig. 1. (A and B) The z-COSY and BASE-z-COSY pulse sequence. The duration of the 90° hard pulse in z-COSY is 11.2 μs. The small flip angle pulses, β, are 15°. The selective excitation soft pulse in BASE-z-COSY is EUBRP-2 of duration 6.25 ms. t_z is the zero quantum suppression period. For suppression of the zero quantum coherences [28,34], the swept 180° pulse used in both the sequences are smoothed CHIRP with bandwidth of 31 kHz and of 31 ms duration. The homo-spoil gradient pulse is of strength 11 G/cm for the duration of 4 ms. The simultaneous gradient pulse of strength 4 G/cm is applied during CHIRP. Two step phase cycling has been used to suppress the axial peaks. The phase of the first pulse and receiver has been cycled according to x, $-x$. Other pulses are of phase x unless and otherwise mentioned.

peaks. However, the inherent problem of z-COSY sequence is the presence of zero quantum coherences between the second and the third small flip angle pulses resulting in unwanted dispersive components of the spectrum. One of the ways of discarding this unwanted coherence is to repeat the experiment with stochastic variation of delay (t_z in Fig. 1) between the pulses. Alternate and efficient way has been reported wherein the z-filter is modified to eliminate the zero quantum coherences in a single experiment by applying a swept frequency 180° pulse between the two small flip angle pulses with the simultaneous application of a gradient pulse [29]. In the present study we have employed Thrippleton–Keeler element for the suppression of zero quantum coherences [29].

The z-COSY spectrum of (*R*/*S*)-propylene carbonate is reported in Fig. 2. The reduced multiplicity pattern and the pure phase spectrum with high resolution are clearly evident from the expanded regions of cross peak multiplet pattern. The distinct and identifiable coupling pattern for each enantiomer enable the precise chiral discrimination. The differentiated peaks for *R* and *S* enantiomers are marked for selected peaks. The further evidence for the pure phase character is reflected in the particular F_2 cross section, represented by an arrow, plotted below the 2D spectrum. There is also a separation of active and passive couplings in the direct and indirect dimensions at the respective chemical shift positions of each proton. The separations providing $({}^nT_{HH})^{R/S}$ (where T_{HH} is the total coupling, $J_{ij} + D_{ij}$ and the superscript n refers to the coupled proton that is n bonds away) are marked. The analysis of cross peak multiplicity pattern of z-COSY spectrum is identical to BASE-β-COSY spectrum [17].

The large size of the z-COSY 2D data matrix required for obtaining the spectrum with acceptable resolution puts heavy demand on the instrument time. In circumventing this constraint we explored the feasibility of aliasing the part of the spectrum in the indirect dimension [30,31]. The methyl peak aliased spectrum is reported Fig. 3. In the inset of the figure is given the full spectrum, with the expansion of the region marked with broken rectangle. This is compared with identical expanded part of the aliased spectrum. The transitions of the methyl group are well isolated and the method resulted in considerable saving of the instrument time. This is a specific example where the aliasing did not result any overlap with other regions of the spectrum. But in realistic situations the technique of aliasing lacks wide generality and versatility. In getting around the problem of time constraint, we have used a band selective version of z-COSY, entitled as BASE-z-COSY (Fig. 1B), which combats both resolution and experimental time constraints in a single experiment.

For demonstrating the application of this methodology, the 2D methyl group selected BASE-z-COSY spectrum of (*R*/*S*)-propylene carbonate was recorded and is reported in Fig. 4. The beneficial effect of the present experiment is evident when compared with BASE-β-COSY spectra recorded both in phase sensitive and magnitude modes under identical experimental conditions. The F_2 cross sections represented by an arrow are plotted below each spectrum. The BASE-z-COSY sequence provides cleaner spectrum with pure phase, lower line width compared to large line widths and distorted phase in BASE-β-COSY spectra. The measure of differences in their line widths provides more clarity. For example, the line widths of the particular cross section plotted below for BASE-z-COSY and magnitude mode BASE-β-COSY are, 1.3 and 1.9 Hz, respectively. It is clear that the peaks corresponding to *S* enantiomer, marked with tilted broken rectangle, is completely masked in phase sensitive version of BASE-β-COSY spectrum and are visible in the magnitude mode spectrum but with relatively larger line width.

In Fig. 5 the 2D BASE-z-COSY spectrum pertaining to protons H6 and H7 and its counter part of BASE-β-COSY recorded in magnitude

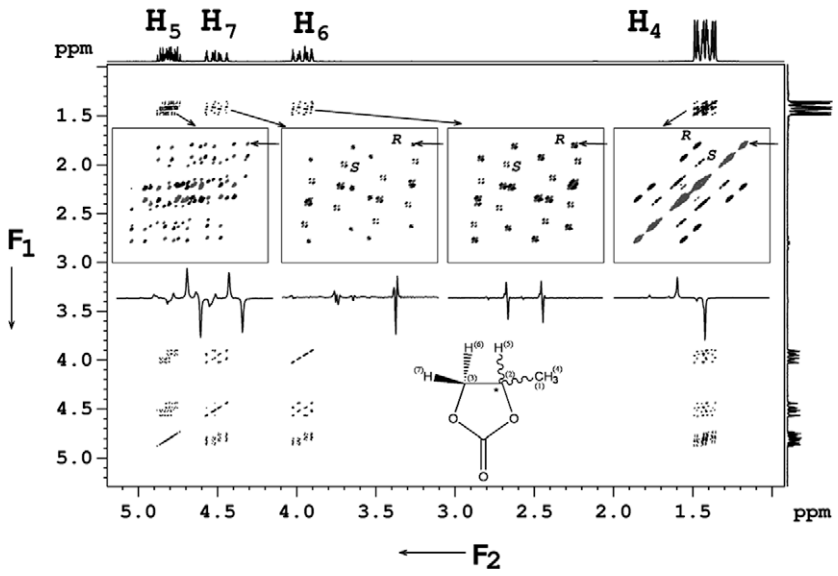

Fig. 2. (A) The 500 MHz 2D z-COSY spectrum of (*R*/*S*)-2-propylene carbonate aligned in the solvent PBLG with suppression of zero quantum coherences, recorded at ambient temperature with corresponding F_1 and F_2 projections. Assignment of resonances to individual protons is also marked. Sample was not spun during acquisition. The size of 2D data matrix is 4096 × 6806. Spectral widths are 2750 and 3094 Hz in F_1 and F_2 dimensions. Number of accumulations for each t_1 increment is 2. The relaxation delay is 2.0 s. The data was zero filled to 4096 and 8192 points and processed with an exponential window function. The digital resolution in F_1 and F_2 dimensions are 0.7 and 0.4 Hz, respectively. The racemic structure of molecule is given inside the 2D matrix. The expansion for each proton is given in solid rectangle and is identified by an arrow. Further below each of these expansions the particular F_2 cross section denoted by an arrow is plotted to depict the pure phase character of the peaks.

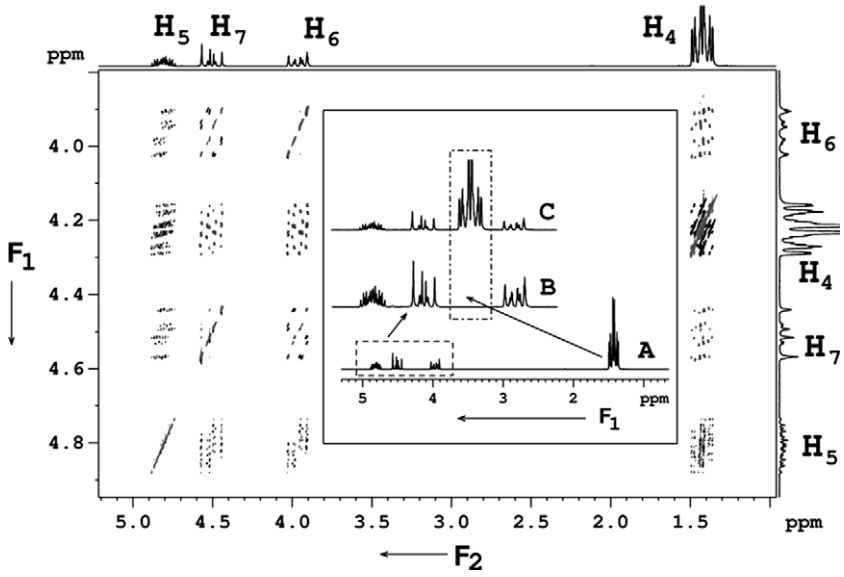

Fig. 3. The 500 MHz 2D z-COSY spectrum of (*R*/*S*)-2-propylene carbonate with suppression of zero quantum coherences, recorded at ambient temperature and permitting the aliasing of methyl protons in the F_1 dimension. The size of 2D data matrix is 1024 × 8192. Spectral widths are 700 and 3754 Hz in F_1 and F_2 dimensions. Number of accumulations for each t_1 increment is 2. The relaxation delay is 10.0 s. The data was zero filled to 2048 and 16384 points and processed without any window function. The digital resolution in F_1 and F_2 dimensions are 0.7 and 0.4 Hz, respectively. Given in the inset of a solid rectangle are the F_1 projections of (A) z-COSY spectrum, (B) expanded portion of A marked with broken rectangle and (C) expanded portion with aliased z-COSY.

mode are compared. The advantage of pure phase and better clarity in the resolution is clearly visible from the expanded regions of a particular cross peak marked with broken rectangle. The peak separations of 2.4 and 1.0 Hz could be easily measured from the BASE-z-COSY spectrum, whereas in the BASE-β-COSY spectrum, the multiplet pattern is poorly resolved. This becomes clearer from the F_2 cross sections, plotted below each of these expansions.

Another problem to be combated in phase sensitive COSY experiment is the presence of artifacts [32,33]. There are additional unacceptable artifacts in BASE-β-COSY spectrum, which are represented by a tilted broken line (This figure is given as a Supplementary material). The zero quantum filter retains only longitudinal magnetization and suppresses all unwanted coherences in BASE-z-COSY spectrum.

The major benefit is derived in large saving of the experimental time. As a matter of comparison, experimental time requirement for the conventional z-COSY is 6–8 h, it is nearly 2 h for the aliased spectrum, and is 1½ h and 45 min, respectively, for the phase sensitive version of BASE-β-COSY and BASE-z-COSY experiments with same number of t_1 increments required for the measurement of all the couplings. As far as deriving the spectral information is considered the analyses of the spectrum at individual chemical shift positions of different protons provides $({}^{n}T_{HH})^{R/S}$. The application to another molecule (*R*/*S*)-ibuprofen, which has 17 protons with an

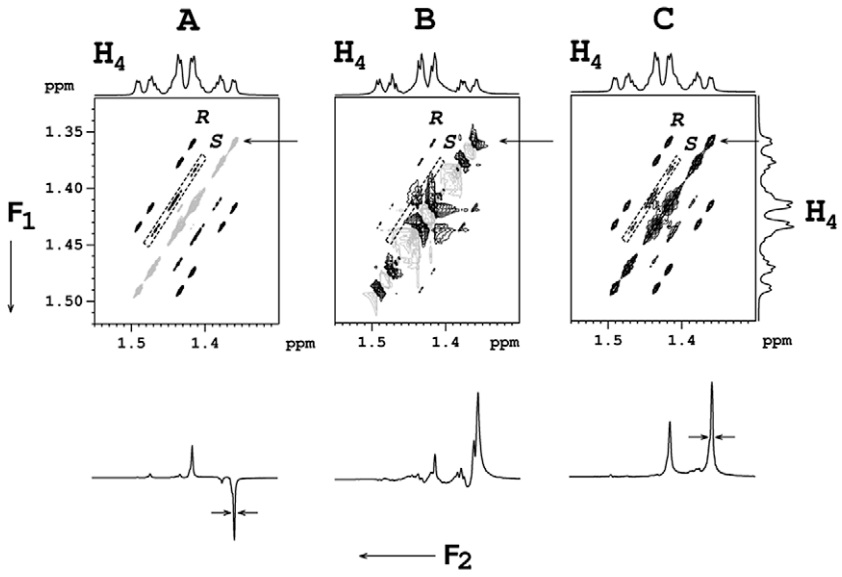

Fig. 4. (A) The 500 MHz 2D methyl group selected BASE-z-COSY spectrum of (*R*/*S*)-propylene carbonate in the solvent PBLG; B and C) The 500 MHz 2D methyl group selected BASE-β-COSY spectra recorded in phase sensitive and magnitude modes. The size of 2D data matrix is 512 × 8192. Spectral widths are 280 and 4562 Hz in F_1 and F_2 dimensions. Number of accumulations for each t_1 increment is 2. The relaxation delay is 2 s. The data was zero filled to 1024 and 16384 points and processed without any window function. The digital resolution in F_1 and F_2 dimensions are 0.5 Hz in both dimensions. (B) The BASE-β-COSY spectrum of (*R*/*S*)-propylene carbonate in the solvent PBLG recorded in phase sensitive mode using TPPI. All experimental and processing parameters are same as A, except for the number of accumulations for each t_1 point, which is 4. (C) All experimental parameters are identical to that given in A except for the spectrum is presented in magnitude mode. The one-dimensional spectra shown below A, B and C are the particular F_2 cross sections depicted by the arrows. Note the pure phase spectrum in A and phase distorted spectrum in B. It is clearly evident that the lines are narrower in A.

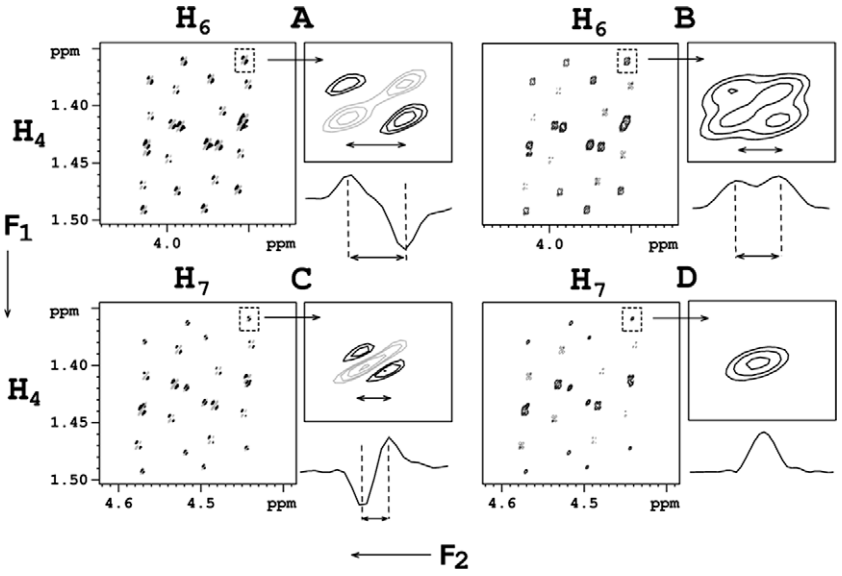

Fig. 5. (A and C) The expanded cross peak multiplet patterns of BASE-z-COSY. (B and D) The magnitude mode BASE-β-COSY spectra pertaining to protons H6 and H7, respectively. One of the cross peak multiplet pattern marked with broken rectangle designated by the arrows are plotted adjacent to each of the 2D data matrix. The cross section pertaining to top doublet pattern of the expanded part is plotted below each of them. The resolution achieved is evident from the well resolved contour in BASE-z-COSY and also their cross sections. *Note:* in H7 a doublet of small coupling is appearing as a broad hump in the BASE-β-COSY spectrum clearly bringing out the advantage of the present experiment.

uncoupled OH group, its spectral complexity and the limitation of the current data in its analysis is given in the Supplementary material.

3. Measurement of enantiomeric excess (*ee*)

Apart from the measurement of couplings the z-COSY spectrum can also be applied for quantitative measurements [23]. An ideal situation for a measurement of couplings and enantiomeric excess (*ee*) would be the significantly larger values of active coupling compared to the line width. When active couplings are comparable or smaller than the linewidths there is partial cancellation of anti-phase components and integration is not precise [34]. The exceptional case being multiplet components with identical couplings, which is uncommon.

We have measured *ee* in a scalemic mixture of 21% excess of *R* enantiomer using the well resolved contours marked 1 and 2 for protons H6 and H7 reported in Fig. 6. For proton H6 (Fig. 6B) the

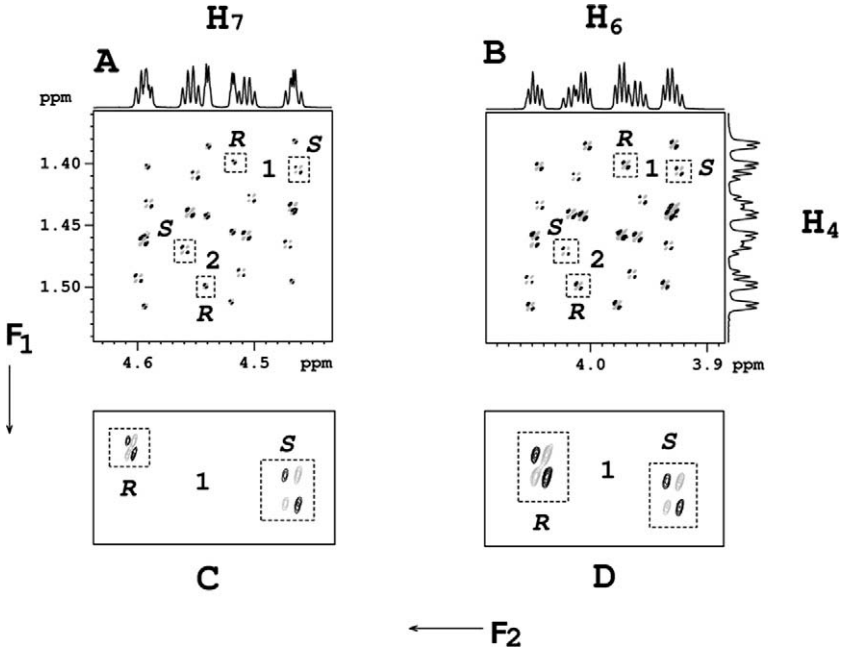

Fig. 6. (A and B) The 500 MHz BASE-z-COSY spectrum of a scalemic mixture (21% excess of *R* enantiomer) of (*R*/*S*)-propylene carbonate oriented in PBLG for protons H6 and H7. Acquisition and processing parameters are same as in Fig. 4 except the delay between successive acquisition, which is 10 s in the present case. The *R* and *S* enantiomers are marked. The contours chosen for the measurement of areas to determine *ee* are denoted by broken rectangles. The numbers 1 and 2 are pairs of contours of the particular multiplet component belonging to each enantiomer. (C and D) One of the contour pairs, 1, is expanded below each of them.

ratiometric analysis provided *ee* of 22% each. The similar measurement using proton H7 (Fig. 6A) provided large errors for both the contours. This is due to the fact that in H6 the coupling strengths and the peak separations for both *R* and *S* enantiomers were nearly same (2.1 Hz) whereas for proton H7 the coupling strength of *R* form is 1.0 Hz and that of *S* form is 2.4 Hz. The large differential values lead to measurement errors, because of the partial cancellation by the anti phase peaks from the *R* enantiomer.

The T_1 values of the protons measured using the standard inversion recovery method ranged from 2.2 to 2.6 s for different peaks. Thus the relaxation delay between successive accumulations of free induction decay should be around 10–11 s to enable quantitative measurement. This implies that in experiments such as, COSY, z-COSY, etc. the total experimental time would be around 14 h, whereas for BASE-z-COSY it was nearly 2 h.

4. Conclusions

In the present work we have demonstrated that the proton detected NMR spectra of chiral molecules aligned in the chiral liquid crystal has plenty of potential for enantiodiscrimination. The BASE-z-COSY experiment which does the selective excitation of the isolated group of coupled protons and correlating them to the entire spectrum provided accurate discrimination of enantiomers. The reduced multiplicity pattern based on the spin state of the passive proton(s) aids in the direct measure of active and passive couplings and also the relative signs of the coupling constants. The significant advantages of the technique are in its pure phase spectra which enable the measure of couplings of negligible strengths, precise measure of enantiomeric excess and also considerable reduction in the instrument time.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2009.11.009.

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