Abstract

The NMR spectroscopy as usually practiced in isotropic achiral solvents is unable to differentiate enantiomers, unless the intermolecular diastereomeric interactions are imposed with an enantiomerically pure reagent. Chiral auxiliaries such as chiral solvating agents (CSAs), chiral derivatizing agents (CDAs), chiral lanthanide shift reagents (CLSRs) in isotropic solvents and chiral liquid crystals are utilized to distinguish enantiomers. Chiral auxiliaries make use of chemical shift difference between the enantiomeric resonances to visualize enantiomers. On the other hand, chiral liquid crystals (CLCs) give access to different order sensitive NMR parameters for the enantiomers because of differential ordering effect on them. Among all the NMR active nuclei available for the investigation of chiral molecules, ^{1}H and ^{13}C at natural abundance are largely explored. However, the high sensitivity of proton and its ubiquitous presence in all organic molecules renders proton NMR as one of the foremost analytical tools for the study of enantiomers dissolved in chiral isotropic and anisotropic solvents. Despite its merits, proton detection has not been a preferred method for chiral discrimination in CLC, due to overcrowding of the spectra, resulting in non-resolution of coupling fine structures even for a molecule with few interacting protons. This is because of the presence of numerous couplings yielding large number of transitions in addition to doubling of signals from both the enantiomers. Nevertheless, the ^{1}H NMR spectrum is rich in information content and the errors associated in the measurement of enantiomeric excess (*ee*) is reported to be relatively less (< 5%). Therefore, in this thesis, we have focused our attention to the use of proton NMR for chiral analysis. Selective excitation in 2D NMR techniques is a general strategy to simplify unresolved proton NMR spectra of enantiomers. Though other methodological developments for chiral liquid crystal NMR are progressing well, the work reported herein is the development of novel strategies using selective excitations for the resolution of overlapped-crowded ^{1}H NMR spectra of enantiomers. The thesis is organized in six chapters and brief discussions of the contents of the individual chapters are given below.

Chapter 1 covers the theoretical preliminaries required for experimental works described in the rest of the thesis. After a brief discussion about NMR, its basic principle, and the interaction Hamiltonians responsible for yielding NMR spectra are discussed. Subsequently, it is followed by an introduction to product and polarization operator formalisms that gives an insight into the spin dynamics for designing two-dimensional NMR experiments. This introduction sets the foundation to understand the spectral patterns obtained from the experiments presented in this thesis. Since this thesis work involves the study of enantiomers embedded in CLC, a brief introduction is provided about chirality and liquid crystals. In the following sections, mechanism and spectral parameters used for the chiral discrimination process are discussed in detail.

Chapter 2 deals with a method based on transition selective one-dimension proton-proton COSY experiment for the selective detection of a single-enantiomer spectrum. The presence of numerous long-range couplings is considered as a hindrance for proton detection. On the other hand, in this method, the benefit is derived from the presence of numerous couplings among the various protons to single out the single-enantiomer or enantiopure spectrum from the severely overlapped spectrum of a racemic or scalemic mixture. The distinct advantage of the method is the determination of *ee* with an error of less than 3%. The method also finds significant advantage in the selective and systematic determination of proton-proton couplings of one of the enantiomers.

In **chapter 3,** two correlation experiments using ^{13}C as a spy nucleus are described. It is divided in three parts. **Part-I** deals with the CESS-COSY experiment, where a single ^{13}C spin edited selective proton-proton correlation is used to decipher overcrowded carbon coupled ^{1}H NMR spectra of enantiomers embedded in CLC. The experiment unravels the masked ^{13}C satellites in proton spectrum and permits the measurement of one bond carbon proton total (sum of indirect and direct) couplings in methyl group and for each diastereotopic proton in the methylene group. It also provides homonuclear proton-proton total couplings among the selectively excited protons for each enantiomer which are otherwise difficult to extract from the broad and featureless one dimensional ^{1}H NMR spectrum. Employment of heteronuclear (^{13}C) decoupling in the indirect dimension results in complete resolution of overlapped signals. The anomalous intensity pattern, approximately in the ratio 1:2:3 for the dipolar coupled methyl protons observed in methyl selective CESS-COSY spectrum has been explained using polarization operator formalism.

In Part-II, another spin selective correlation experiment, designated as C-HetSERF is discussed not only for enantiodiscrimination, but also for the measurement of short and long range homonuclear and heteronuclear total couplings from the broad and featureless carbon-coupled ^{1}H NMR spectra. The method employs a single natural abundant ^{13}C spin as a spy nucleus to probe all the coupled protons and permits the determination of couplings of negligible strengths. This technique has been demonstrated for the study of organ soluble chiral molecules aligned in CLC where additional challenge is to unravel the overlapped spectrum of enantiomers. The significant advantage of the method in better chiral discrimination using homonuclear total couplings as additional parameters is described. The method also finds significant advantage in the measurement of relative signs of long-range heteronuclear total couplings. The merits and demerits of this method over the HetSERF experiment are highlighted.

In **Part-III**, C-HetSERF experiment is applied to the isotropic systems, especially for the measurement of long-range heteronuclear *J*-coupling. This extension of C-HetSERF experiment and its utilization signifies the importance. The measurement of these couplings is difficult because they are small in magnitudes and their measurement is associated with the low □ dilute nuclei such as ^{13}C, ^{15}N etc. A salient feature about C-

HetSERF experiment is that ^{13}C α/β cross peaks appear in different cross-sections instead of a single cross-section in 2D. Therefore, the displacement between them pertains to the long-range couplings. This application has been demonstrated on an alkaloid and an undecapeptide.

Chapter 4 describes *J/D*-resolved techniques, cited as CH-SERF and CH-DQSERF for the visualization of enantiomers. These techniques utilize ^{13}C-bound proton signals for the selective refocusing of the single and double quantum coherences of the methyl protons. Both methods yield distinct proton-proton couplings among the selectively excited protons and carbon-proton couplings to their directly attached carbon for each enantiomer thereby simplifying the spectrum in indirect dimension. In the CH-DQSERF, each cross-section taken along the direct dimension represents the enantiopure spectrum of the methyl group and provides all proton-proton total couplings. CH-SERF also overcomes the problem of overlap of central transitions of the methyl selective refocusing (SERF) experiment resulting in better chiral discrimination.

In **chapter 5**, several protons detected ^{13}C-filtered ω_1-heterodecoupled resolved experiments and homonuclear multiple-quantum NMR experiments developed for the accurate measurement of *ee* are described. These methods retain the differential values of both ^{1}H-^{1}H and ^{13}C-^{1}H dipolar couplings for the enantiomers in the direct dimension and only ^{1}H-^{1}H dipolar couplings in the indirect dimension enabling complete unraveling of overlapped enantiomeric peaks. The creation of unequal ^{13}C-edited proton signal because of average delay used in the INEPT block in resolved experiments and non-uniform excitation of coherences in homonuclear multiple quantum experiments for the enantiomers do not yield accurate measurement of *ee*. In order to combat these difficulties, a coupling dependent intensity correction factor has been invoked which substantially reduces the errors providing the accurate information. The error in the measurement of the *ee* is demonstrated to be within 2%. The phase sensitive DQ-SERF experiment using an adiabatic z-filter and its utility in the accurate measurement of *ee* is also demonstrated.

Chapter 6 discusses the study of chiral molecules in isotropic phase employing either a CLSR or a CSA as chiral auxiliaries. The higher substrate and chiral auxiliary concentration is a pre-requisite to obtain efficient separation of ^{1}H NMR signals of enantiomers. The higher concentration of CLSRs causes spectral lines to broaden due to paramagnetic relaxation of lanthanide ions resulting in severe loss of resolution between the enantiomer resonances. To circumvent such difficulties, the application, and the usefulness of a selective F_1 decoupled correlation (COSY) experiment which yields proton decoupled proton spectrum in the indirect dimension have been demonstrated. Use of this methodology overcomes the effect of paramagnetic relaxation online broadening at lower shift reagent concentration. The potential of the experiment is demonstrated on several chiral compounds possessing different functional groups.