Solution State ^{19}F Magnetic Resonance (MR) Account of Molecular Interactions in Solutions

A Thesis submitted by

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Summary

The present Thesis successfully demonstrated that one dimensional (1D) solution-state ^{19}F NMR methods based on relaxation, diffusion, and magnetization transfer are competent enough to offer a complete description of molecular interactions involving small fluorochemicals in the solution. Careful analysis of chemical shift and line-broadening enables preliminary detection of interaction. Compared to ^{1}H NMR spectral lines, ^{19}F NMR peaks exhibit prominent effect of change of chemical environment due to interaction; however, ^{19}F spectral lines are sometimes broadened beyond detection that hinders quantitative spectral analysis. Three different molecular systems are considered: a) fluorinated drug-macromolecule, b) fluorinated agrochemical-natural organic matter and c) fluorinated solvent–solute interaction. The Thesis exploits ^{19}F NMR methods at high magnetic field and introduces the applicability of low field ^{19}F NMR relaxation and ^{19}F Overhauser Dynamic Nuclear Polarization (ODNP) in solution. Further, both ^{1}H and ^{2}H NMR measurements at the high field are employed as supporting NMR experiments addressing the aforementioned molecular systems. In the first part of the Thesis, the existing small molecule-based ^{19}F NMR methods are reviewed in detail besides highlighting the subtle chemical and biological advantages imparted to a molecule due to fluorination. The advantage of low field ^{19}F relaxation measurement has been emphasized for revealing molecular dynamics while describing the theoretical background of the ^{19}F NMR methods.

The first two working chapters (**chapters 3 and 4)** have employed ^{19}F diffusion and transverse relaxation analysis to quantify ligand-macromolecule interactions. It has been demonstrated that analysis of diffusion experiments provides a more robust approach to quantify ligand–protein interactions in terms of the bound fraction of ligand, association constants, and relevant thermodynamic parameters. Determination of self-diffusion coefficients as a function of supramolecular concentrations reveals information related to micellar structure formation in solution in case of natural organic matter. Constant time fast pulsing CPMG pulse sequences similar to relaxation dispersion CPMG method has enabled extraction of the exchange rate of a ligand between its free and bound state. The exchange rate gives direct measurement of ligand residence time in its bound state. In specific, the chapters have revealed the following: **(i)** the test parent organofluorines show significantly higher binding affinity towards serum albumin (SA) and humic acid (HA) compared to the metabolites and their non-fluorinated analogs, **(ii)** fluorochemicals containing carboxylic acid groups (CA) do not show any significant binding interaction with trypsin, **(iii)** structure plays an essential role in modulating the binding of organofluorines with SA and HA, **(iv)** carboxylic acid group of fluorochemicals is found to orient away while interacting with HA/ SA, **(v)** KHA imparts an inhibitory effect (light screening/optical filter effect) by reducing photo–degradation rate of flupyradifurone (FPD), **(v)** careful analysis of 1D spectral changes revealed chemical moiety-wise degradation of FPD, **(vi)** the stepwise disintegration of FPD in the presence of KHA is a result of encapsulation of the molecule within KHA superstructure where the fluorinated aliphatic moiety remained outside. The findings of these chapters provide meaningful insights regarding the fluorochemical-macromolecule binding mechanisms in terms of their binding modes and strength. These parameters are further found dependent on the chemical constituents or structures of the organofluorines, their concentrations (dose), and various physical conditions of the solutions namely, the temperature, pH, solvent conditions, and frequency of exposure. The aforesaid studies may prove useful in predicting the distribution of the drugs and pollutants within the body or in the environment.

The last chapter of the Thesis (**chapter 5**) focuses on the establishment of a general approach to decipher the behavior of fluorinated cosolvent in terms of solvation dynamics by employing low field ^{19}F MR (relaxation and ODNP) and high field ^{19}F and ^{2}H NMR relaxation methods directly monitoring the solvent NMR parameters as a substitute of traditional NOE experiments routinely employed to investigate solute-solvent interactions. The limitations associated with 2D NOE to study such systems have already been discussed in chapter 5. Steady–state ODNP measurements allowed a different and independent window to access motions in the timescale of 10-1000 ps. A point not to be missed that ODNP experiments also require specialized hardware. It is found that a straightforward determination of molecular correlation times from ^{2}H T_1 and low field ^{19}F T_1 simplified the analysis. Also, the relaxation (correlation time) ratio plots depicted a more straightforward overview of the solvent dynamics around peptide and carbohydrates, taking the viscosity effect into account.

Overall the Thesis emphasizes the applicability of ^{19}F 1D MR methods allowing uncluttered and effective interpretation of molecular interactions of fluorinated systems in solution. As the **future scope of the study**, it would be interesting to quantify cross-correlation rates (CCR; ^{19}F dipolar and CSA cross-correlation) for free ligand and target bound ligand in various solvent systems employing spin recovery pulse sequences. CCR rates can be one of the useful probes for quantifying binding events. Also, a similar set of relaxation, diffusion, and STD experiments can be implemented to study the interaction of different organofluorines with other components of natural organic matter (eg., fulvic acid, etc.) and other biologically relevant proteins (e.g. actin, muscle protein). It will help in understanding the overall distribution and fate of organofluorines in the environment and within the body. The design of leading and efficient fluorinated drugs, inhibitors, agrochemicals, bioactive molecules, suitable remediation methods and potential biomarkers can also be undertaken based on the current findings. The established relaxation based approaches to understand the solvation dynamics of model systems in the present study can be employed further to investigate other real systems of interest.

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