

	Page
Abstract	i
Acknowledgements	iii
Contents	v
List of Figures	ix
List of Tables	xv
List of Symbols	xvii
List of Abbreviations	xix
<b>Chapter 1: Introduction</b>	<b>1</b>
1.1 Nuclear Magnetic Resonance: A present day spectroscopic tool	1
1.2 Fluorine: The kingpin	2
1.3 Fluorine NMR: The progress and the pitfall	5
1.3.1 NMR properties of <sup>19</sup> F: The favourables and the unfavourables	6
(i) Chemical shift	7
(ii) Fluorine couplings	7
(iii) Relaxation	8
1.3.2 Applications of <sup>19</sup> F NMR in diverse areas	8
1.4 Molecular Interactions: Assessment by NMR Methods	9
1.4.1 Molecular Interactions: Definition and Occurrence	9
1.4.2 Overview of NMR methods available to probe molecular interactions	10
1.5 <sup>19</sup> F NMR addressing specific interactions under investigation	14
1.5.1 Ligand-Protein interactions in chemical biology: Application of <sup>19</sup> F NMR	14
1.5.2 Host-Guest type interactions in the environment through <sup>19</sup> F NMR	15
1.5.3 Solute-Solvent interactions: Solvation dynamics using <sup>19</sup> F NMR	16
1.6 Scope of the Thesis	17
<b>Chapter 2: Experimental Techniques</b>	<b>21</b>
2.1 Experimental setup	21
2.1.1 NMR measurements	21
2.1.2 Other complementary spectroscopic measurements	21
2.2 NMR parameters relevant for molecular interaction analysis	21
2.2.1 Chemical shift and Linewidth	21
2.2.2 Determination of Stoichiometry: Application of Jobs Plot	24
2.2.3 Determination of Binding Constant: Application of Benesi-Hildebrand Plot	25
2.3 Relaxation: Theoretical Aspects	26
(i) Spectral density and correlation time	28
(ii) Dipole-Dipole (DD) interaction	29
(iii) Chemical Shift Anisotropy (CSA)	30
(iv) Cross correlation: Interference effect	31
2.3.1 Fluorine relaxation at low field (0.34T)	32
2.4 Measurement of relaxation time: Experimental setup	33
2.5 Diffusion NMR: Self-Diffusion Coefficient	34
2.6 Magnetization transfer based methods	37
2.6.1 Saturation transfer difference (STD): Group epitope mapping	37
(i) Heteronuclear STD ( <sup>19</sup> F- <sup>1</sup> H)	39
2.6.2 Overhauser Dynamic Nuclear Polarization (ODNP)	40
(i) Parameters of ODNP	41
<b>Chapter 3: Quantification of Organofluorine-Protein Interactions</b>	<b>45</b>
3.1 Introduction	45
3.1.1 Proteins under investigation: Literature background	45

3.1.2	Organofluorine ligands under investigation: Literature background	46
3.1.3	Methods in focus	47
3.2	Experimental details	49
3.2.1	Sample preparation	49
3.2.2	Details of NMR experiments	49
3.3	Results and Discussion	50
3.3.1	Part-I: Interaction of Organofluorine with Serum Protein	50
	(i) Chemical shift & Linewidth (FWHM): Determination of binding site	50
	(ii) Saturation Transfer Difference (STD): Confirmation of binding interaction	53
	(iii) Diffusion and spin-spin relaxation time ( $T_2$ ): Determination of dissociation constant ( $K_D$ ) & number of binding sites ( $n$ )	54
	(iv) Constant time fast pulsing CPMG: Extraction of exchange rate, residence time	60
3.3.2	Part-II: Organofluorine with Trypsin	63
	(i) Chemical shift and Line broadening	63
	(ii) Relaxation and Diffusion	64
3.4	Conclusions	65
<b>Chapter 4: Interactions of Fluorinated Agrochemicals with Humic Materials</b>		67
4.1	Introduction	67
4.1.1	Structural properties of HS: Possibility of host-guest interaction	67
4.1.2	Photo-degradation of agrochemicals by HA	68
4.2	Experimental details	70
4.2.1	Solution Preparations	70
4.2.2	Details of methods	71
	(a) UV-vis measurements	71
	(b) NMR measurements	71
4.3	Results and Discussion	72
	(a) $^1\text{H}$ NMR spectroscopic measurements	72
4.3.1	Part I: Monoaromatic carboxylic acids with humic acid	74
	(i) Characterization of the interaction between AHA–DFBA and AHA–BA by NMR	74
	(a) $^{19}\text{F}$ and $^1\text{H}$ NMR linewidth (FWHM) and Chemical Shift	74
	(b) Saturation Transfer Difference experiments	75
	(c) Relaxation measurements	76
	(d) Diffusion Measurements	78
	(ii) DFBA interaction with KHA and its comparison with AHA	81
4.3.2	Part-II: Halogenated Agrochemicals with Humic Acid	82
	(i) Molecular behaviour of FPD in presence of HA: NMR evaluation	82
	(a) NMR spectra of FPD in absence and presence of HA	82
	(b) Aqueous photo-degradation of FPD in the absence and presence of KHA	84
	(c) Kinetics of FPD Photo-degradation in absence and presence of KHA	88
	(ii) Molecular interaction of FPD with KHA: Application of NMR relaxation, diffusion and magnetization transfer based measurements	91
	(a) Qualitative evaluation of KHA binding using FPD and DFA $^1\text{H}$ and $^{19}\text{F}$ relaxation rates	91
	(b) Quantitative evaluation of binding through Diffusion measurement	92
	(c) Evaluation of binding mode by chemical shift and STD NMR measurements	93
4.4	Conclusions	95
<b>Chapter 5: Solvent Detected NMR Approach for the Assessment of Solute-Solvent Interactions</b>		97
5.1	Introduction	97
5.1.1	Structural transition of Peptides in TFE cosolvent systems	97
5.1.2	Preferential solvation by TFE	98
5.1.3	Methods in focus	98
5.2	Experimental Details	101

5.2.1	Solution Preparations	101
5.2.2	Technical Details	102
	(i) Circular Dichroism Measurements	102
	(ii) NMR Measurements	102
	(iii) DNP measurements	102
5.3	Results and Discussions	102
5.3.1	Part I: Role of TFE solvent dynamics in inducing conformational transitions in MLT	102
	(i) CD results	102
	(a) Set I: MLT in HEPES Buffer (10 mM) with 0.5 M NaCl, at pH 7.4: Effect of TFE composition	102
	(b) Set II: MLT without buffer in various compositions of TFE	103
	(ii) NMR measurements	105
	(a) Relaxation Measurements	106
	(b) Overhauser Dynamic Nuclear Polarization (ODNP) Measurements	112
5.3.2	Part II: Preferential solvation of carbohydrates in TFE: D <sub>2</sub> O mixture	115
	(a) Analysis of longitudinal relaxation rate ( $R_1$ ) ratio	116
	(b) Determination of correlation time	120
	(c) Supporting experimental data corroborating preferential solvation	122
5.4	Conclusions	122
	<b>Chapter 6: Summary</b>	125
	<b>Annexure A</b>	127
	<b>Annexure B</b>	129
	<b>References</b>	137
	<b>Publications</b>	161

