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Unprecedented Concentration Dependent Chemical Shift Variation in ^{1}H-NMR Studies: A Caveat in the Investigations of Molecular Recognition and Structure Elucidation

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This paper is dedicated to Prof. Gilbert Stork for his fifty years of excellence in teaching and research.

Abstract:

Our studies show that changes in concentration can lead to significant changes in the ^{1}H chemical shifts of non-exchangeable hydrogens, and thus, in the NMR spectra. An important observation, as described for quinoline and other aromatic compounds, is that different protons shift to different extents in the same solvent as the concentration of the solute is varied, leading to overlap/coalescence or cross over of proton signals. Chemical shift variations can be so pronounced that they lead to misleading 1D and 2D spectral signatures. Such concentration dependent spectral pattern changes have far reaching implications in the use of ^{1}H-NMR spectroscopy for structure elucidation, spectral characterization, determination of purity and the study of molecular recognition.

Keywords: NMR, Quinoline, Acridine, Indole, Benzofuran

Introduction

The conventional practice in the elucidation of structural features of a molecule usually involves recording and interpreting its ^{1}H-NMR spectrum in addition to other spectroscopic and analytical studies. However, examination of the NMR spectrum of a molecule as a function of its concentration is seldom done. We have found that the chemical shifts of different non-exchangeable protons change remarkably as the concentration of the molecule (solute) is varied.[1] Sometimes, the chemical shift variations are so pronounced that they lead to 1D and 2D spectral signatures that may be misleading. Lack of consideration of the importance of such concentration effects can also lead to unwanted controversies in the design of self replicating systems.[2]

In view of the exceptional importance of the use of NMR spectra as a tool for structure elucidation, spectral characterization, determination of purity and in the study of molecular recognition, we sought to carefully examine the ^{1}H-NMR spectra of a range of small organic molecules in a number of solvents. We are pleased to report the evidence of exceptionally large concentration dependencies in the chemical shifts of various nonexchangeable protons in a given molecule in a variety of solvents commonly used in NMR experiments. We observe that different nonexchangeable protons shift to different extents and can result in coalescence[3], overlap, or

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complete cross over of signals, thus changing the spectrum. Such coalescence or overlap of signals also lead to loss of connectivity in the ^{1}H-^{1}H COSY spectra. Although solvent, concentration, and temperature dependent chemical shift changes are known,[4-6] such as the well known concentration dependent chemical shift changes of exchangeable hydrogens,[6] concentration dependent chemical shift changes which lead to cross over or coalescence of ^{1}H-NMR signals[1] of nonexchangeable hydrogens in the same solvent have not been reported.[6] These changes may not be solely due to aromatic solvent induced shifts (ASIS) at least in dilute solutions *(vide infra)*, simple dimerization or conventional π - π stacking of solute molecules.[5] We have studied 20 different compounds to verify the generality of such phenomenon.

Results and Discussion

As a representative example we first discuss the ^{1}H-NMR spectra[7] of the electron poor quinoline **1** molecule, measured in $CDCl_3$, a weakly hydrogen bond donating solvent ($\varepsilon = 4.70$, $\mu = 1.87$).[8] We observed that the chemical shifts of the quinoline hydrogens are concentration dependent and change to different extents (Figures 1 and 2). The change in the chemical shift of

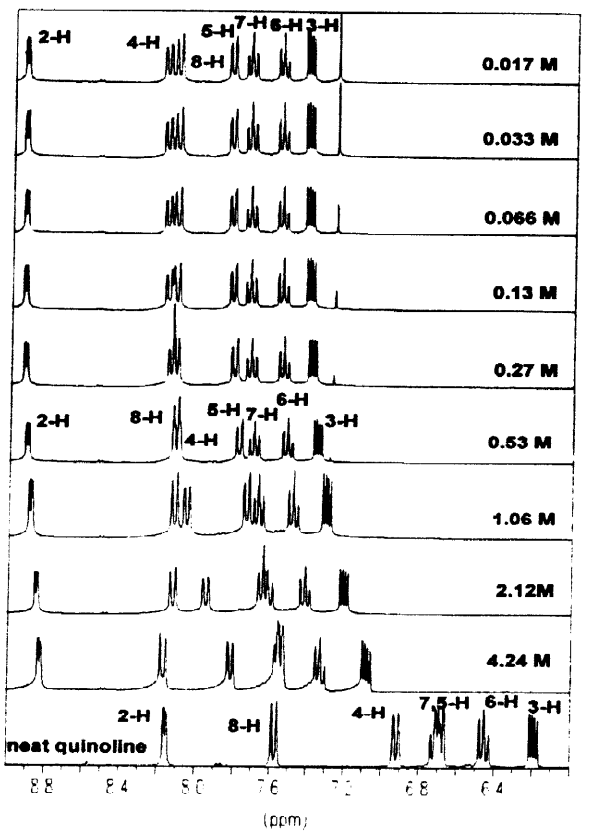


Figure 1. The ^{1}H-NMR spectra of quinoline in $CDCl_3$ at different concentrations (M). The numbers on the signals refer to the hydrogen atoms.

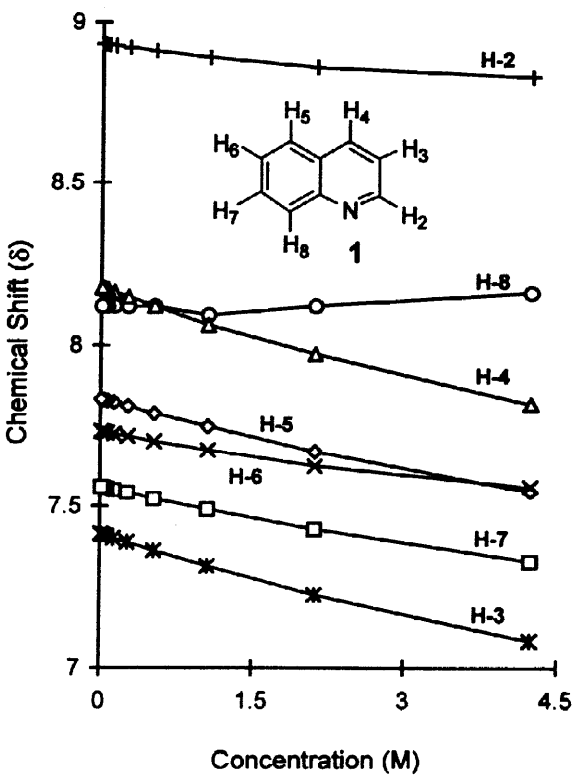


Figure 2. Chemical shift (δ, ppm) vs. concentration (M) for the protons of quinoline.

4-H (Δc = 4.22, $\Delta\delta$ = -0.375)[9] is the largest, leading to an overall change in the spectrum by its cross over of 8-H (Figure 1) at ~0.5 M. It is interesting to note that, contrary to the common belief, sometimes a better dispersed spectra is obtained at a higher solute concentration (Figure 1, e.g., spectra taken at 1.06M vs. 0.13M; Figure 8, e.g., spectra taken at 1.06M vs 0.066M). The 4-H and 8-H signals also cross (Figure 3) at 2.75 M in CD_3CN, a weakly hydrogen bond accepting solvent (Table 1) and at 3.37 M in CD_3OD, a hydrogen bonding solvent. In DMSO-d_6, a hydrogen bonding accepting solvent, there is no cross over and the chemical shift changes are positive between 0 and 2.0 M concentrations. In the nonpolar solvent CCl_4, the 4-H and 8-H coalesce and could be mistaken as a two proton doublet at concentrations below 0.13 M. In other nonpolar solvents like cyclohexane - d_{12} and benzene - d_6 (Δc= 4.11, 8-H, $\Delta\delta$ = +0.077; 4-H, $\Delta\delta$ = + 0.165) these signals also change to different extents with concentration, but do not cross over. The concentration dependent chemical shift changes in benzene-d_6 are similar to those in the nonaromatic solvent DMSO-d_6 and show positive slopes. These findings indicate that these concentration dependent shifts are not solely due to aromatic solvent induced shifts (ASIS).

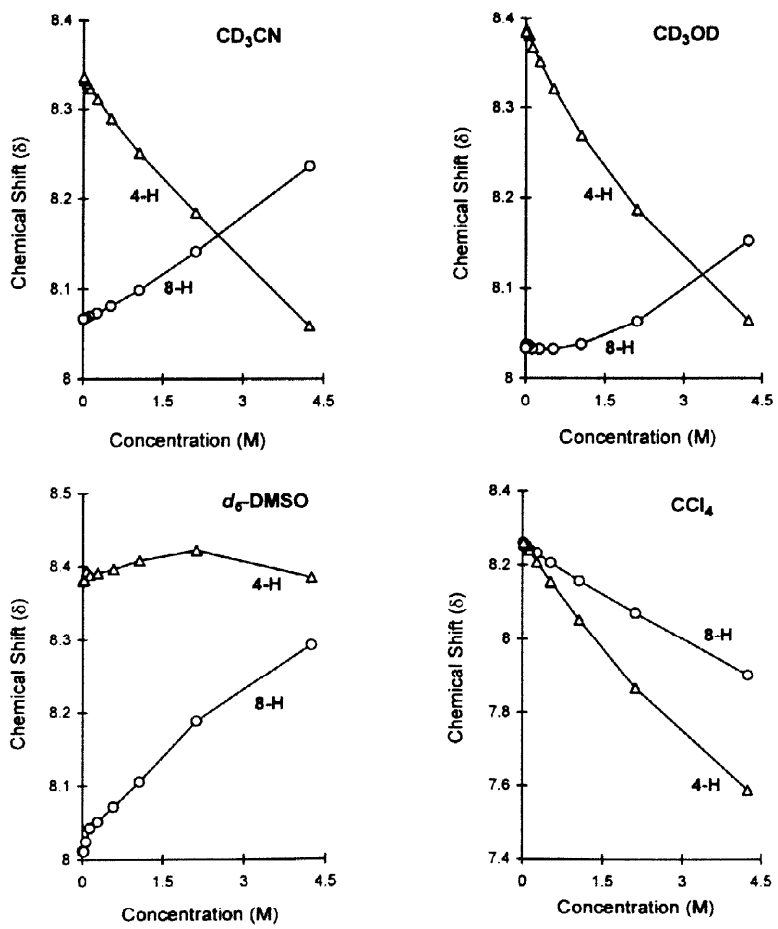


Figure 3. Concentration dependent cross over of 8-H and 4-H of quinoline in different solvents.

Table 1. Solvent parameters[8]

Solvent	Dielectric Constant ε	Dipole Moment μ	Volume Susceptibility ($-\chi_o \times 10^6$)
$CHCl_3$	4.70	1.87	0.735
CH_3CN	36.2	3.92	0.534
CH_3OH	32.6	1.70	0.515
CCl_4	2.23	0	0.684
DMSO	49	3.96	
CH_2Cl_2	8.9	1.60	0.733
Cyclohexane	2.02	0	0.631

The observed concentration dependent chemical shift changes are different from those caused by paramagnetic shift reagents and temperature changes. In the presence of the shift reagent, $Eu(thd)_3$, the chemical shifts of the 2-H and 8-H of quinoline, which are proximal to the coordinating nitrogen, move to higher ppm values with increasing concentrations of the shift reagent (Figure 4). In the absence of shift reagents, the 2-H and 8-H of quinoline show insignificant chemical shift changes as the concentration of quinoline is increased (Figure 1). On the other hand, the 4-H signal changes most significantly with temperature. With an increase in temperature, we would expect that the chemical shift changes should parallel dilution. However the observed shifts are opposite. The 4-H moves to lower ppm values with increasing temperature (Figure 5), in a manner opposite to those observed for dilution (Figures 1-3).

Pi-π stacking has been attributed to be one of the causes of chemical shift changes.[10] However, the role of concentration on the extent and nature of π-π stacking is not clear. Consequently, substituted quinolines were studied to examine the effect of substituents on π-π stacking, if any, on the concentration dependent chemical shift changes. Methyl quinolines showed concentration dependent chemical shift changes. The change in the chemical shift of 4-H of 6-methylquinoline **2** in $CDCl_3$ is the greatest ($\Delta c = 3.70$, $\Delta\delta = -0.377$) and at ~0.6 M not only do the 4-H and 8-H cross over, but the 5-H and 7-H cross over as well (Figure 6). The chemical shifts of the nonaromatic methyl (benzylic) hydrogens of methylquinolines also change. The methyl hydrogens of 4-methylquinoline show the greatest shift ($\Delta c = 4.223$, $\Delta\delta = -0.464$).

The 6- and 8-*tert*-butylquinoline[11] were studied to check the effect of a bulky group on conventional face to face π - π stacking on concentration dependent chemical shift changes. The trend in the changes of chemical shifts of ring hydrogens in both 6- and 8-*tert*-butylquinoline are similar to those observed in quinoline and in 6- and 8-methylquinoline. In 6-*tert*-butylquinoline the 4-H ($\Delta c = 1.17$, $\Delta\delta = -0.112$) and 8-H ($\Delta c = 1.17$, $\Delta\delta = +0.024$) also cross over at ~0.73 M, indicating that the bulky *tert*-butyl group exerts little effect on the chemical shift changes.

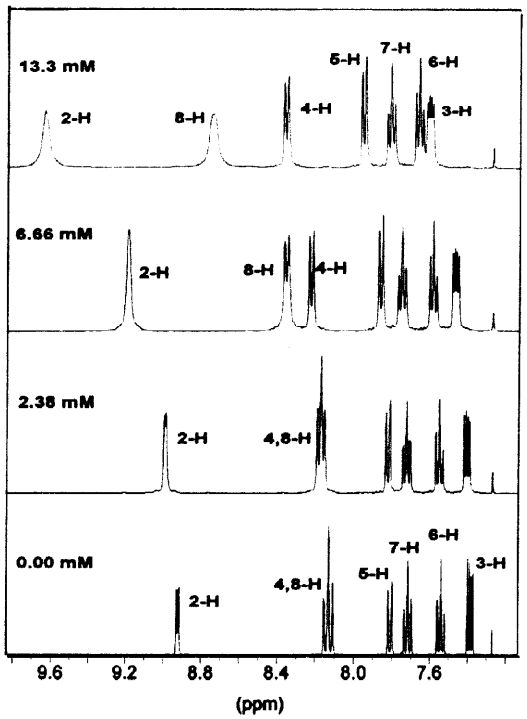


Figure 4. ^{1}H-NMR spectra of quinoline in $CDCl_3$ (0.29M) with increasing concentration of the shift reagent, Eu(thd)$_3$,europium tris(2,2,6,6-tetramethyl-3,5-heptadienoate).

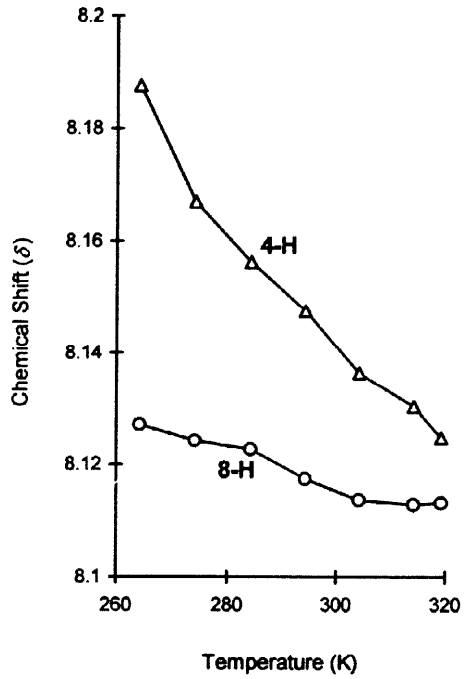


Figure 5. Temperature dependent ^{1}H-NMR chemical shifts of 4-H and 8-H of quinoline (0.29 M) in $CDCl_3$.

Therefore, conventional π - π stacking, if any, does not have any effect on the concentration dependent chemical shift changes.

The electronic effect of substituents on concentration dependent chemical shift changes was next studied using an electron donating methoxy group. In 6-methoxyquinoline **3,** the 4-H and 8-H cross ~ 0.5 M in $CDCl_3$ and 3-H and 7-H coalesce at low concentrations (Figure 7). The nonbenzylic methoxy protons of 6-methoxyquinoline also shift (Δc = 3.60, $\Delta\delta$ = -0.28). Changing the electron density of quinoline by protonating the nitrogen with TFA (1:1 mixture) also caused the quinoline protons to shift with concentration. In this case, the 3-H signal shifted the most (Δc = 0.87, $\Delta\delta$ = 0.082) whereas, the 3-H of quinoline, over a similar concentration range showed an *opposite* shift (Δc = 0.93, $\Delta\delta$ = -0.103).

Electronic effects on concentration dependent chemical shift changes were also investigated in other ring systems. In electron rich acridine [12] **4**, 9-H (Δc = 2.103, $\Delta\delta$ = -0.453), which is equivalent to 4-H in quinoline, (Δc = 2.103, $\Delta\delta$ = -0.230) shifts the most in $CDCl_3$. The 4-H/5-H (Δc = 2.103, $\Delta\delta$ = -0.352) and 2-H/7-H signals (Δc = 2.103, $\Delta\delta$ = -0.170) cross over at (2.12 M) in $CDCl_3$. In CD_3OD the 4-H/5-H signal moves (Δc = 2.103, $\Delta\delta$ = -0.499) from the 2-H/7-H signal and coalesces with the 1-H/8-H signal (Figures 8, 9).

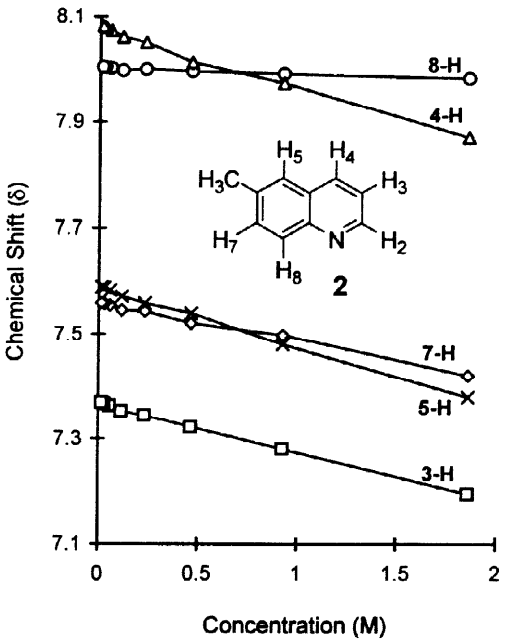


Figure 6. Chemical shift (δ, ppm) vs. concentration (M) of 6-methylquinoline in $CDCl_3$. H-2 showed only very small shifts (δ = 8.78 at 0.015 M and δ = 8.71 at 1.81 M) and is not included in this plot.

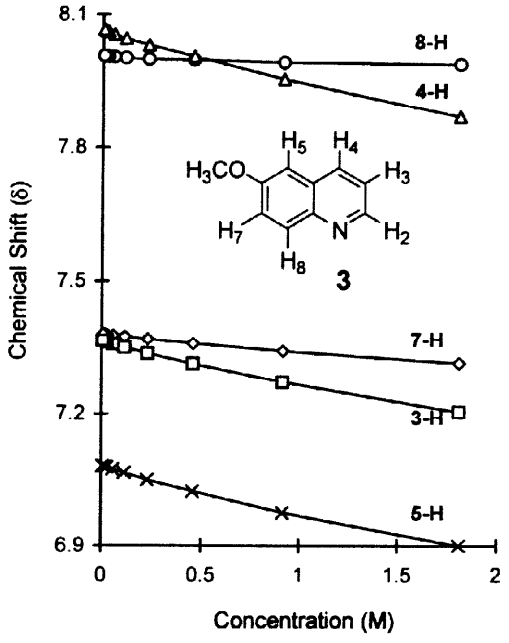
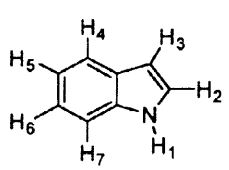


Figure 7. Chemical shift (δ, ppm) vs. concentration (M) of 6-methoxyquinoline in $CDCl_3$. H-2 showed only very small shifts (δ = 8.85 at 0.015 M and δ = 8.78 at 1.81 M) and is not included in this plot.

In indole[13] **5**, another electron rich system, 2-H and 7-H shift dramatically (Δc = 3.71; 2-H, $\Delta\delta$ = -0.376; 7-H, $\Delta\delta$ = -0.383). The 7-H signal crosses over and the 2-H coalesces with the 4-H, 5-H, 6-H multiplet. It is very interesting to note that these shifts are so dramatic that the connectivity in COSY spectrum is lost at lower concentrations of indole. Benzofuran[13b] **6**, an oxygen containing system, also showed chemical shift changes with concentration (Δc=4.26; 2- H, $\Delta\delta$ = 0.211; 3-H, $\Delta\delta$ = 0.222). Naphthalene,[14] a nonpolar electron rich system, showed small concentration dependent chemical shift changes in $CDCl_3$ (Δc = 2.977; 1-H, $\Delta\delta$ = -0.137; 2-H, $\Delta\delta$ = -0.132). In contrast, the electron poor 1-fluoronaphthalene **7**, showed larger chemical shift changes (Δc = 2.977; 4-H, $\Delta\delta$ = -0.193; 8-H, $\Delta\delta$ = -0.193). Similarly, the ^{1}H-NMR of the electron poor molecule isoquinoline[15] **8** showed significant shifts with concentration in $CDCl_3$ (Δc = 4.223; 4-H, $\Delta\delta$ = -0.262; 8-H, $\Delta\delta$ = -0.303) and in cyclohexane-d_6, the 4-H crosses the 6-H at a higher concentration (~4.24 M) and the 7- H at a lower concentration (~0.13 M).



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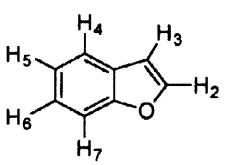

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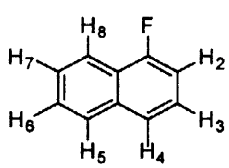

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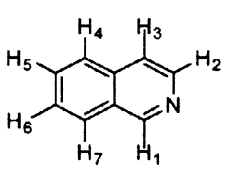

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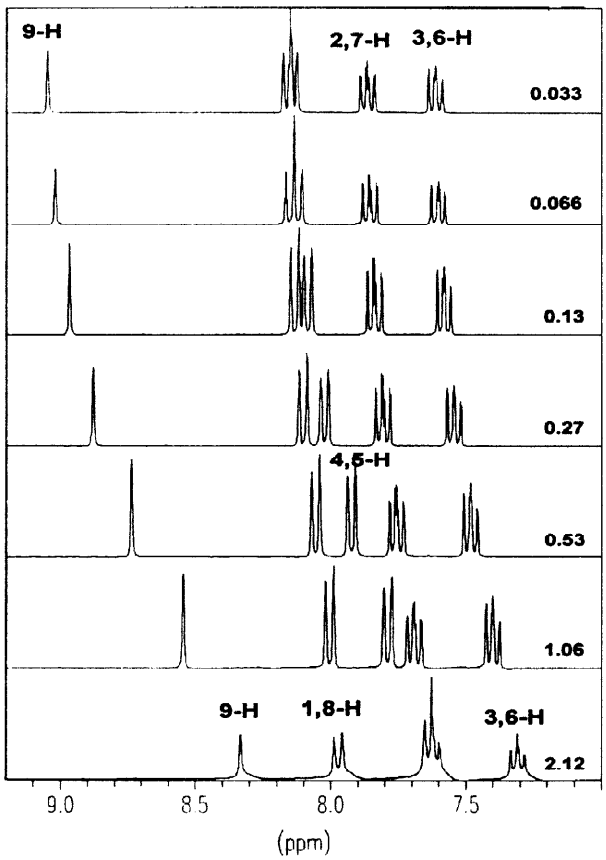

Figure 8. The ^{1}H-NMR spectra of acridine in CD_3OD at different concentrations (mol L^{-1}). The numbers on the signals refer to the hydrogen atoms.

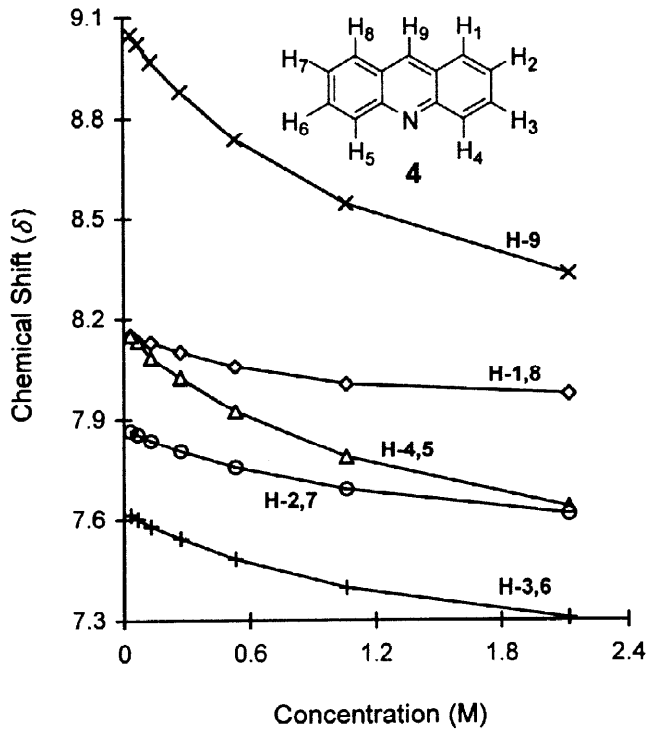

Figure 9. Chemical Shift (δ, ppm) vs concentration (M) of the protons of acridine in CD_3OD.

Since we have examined this phenomena in a range of solvents, including H-bond donors and acceptors, polar and nonpolar solvents, protic and aprotic solvents as well as hydrocarbons, the present observations do not originate from classical[4] solvent induced effects. We have also found that these solvent dependent concentration dependent chemical shift changes are quite different from chemical shift changes resulting from shift reagents and temperature variation.

Solvent dependent changes in liquid/solution structures[16] of compounds, might also manifest themselves in other spectral properties such as $^3J_{H\text{-}H}$ coupling constants,[4] ^{13}C-NMR chemical shifts and ^{1}H longitudinal relaxation times. Preliminary observations show that there are insignificant changes (< 0.05 Hz) in the proton ($^3J_{H\text{-}H}$) coupling constant values of the compounds studied with changes in concentration. In addition, the ^{13}C chemical shift changes of quinoline with change in concentration are small (1-2 ppm) and do not result in a spectral pattern change. The ^{1}H longitudinal relaxation times (T_1 in secs) for the various protons decrease with increasing concentrations of quinoline, but to different extents for different protons.[17] These changes in T_1 values do not parallel the corresponding concentration dependent changes in the chemical shifts.

Conclusion

Chemical shift changes due to solute-solvent/solvent-solvent interactions,[18] host-guest complexation[19] and hydrophobicity of organic molecules[20] have been reported. Although chemical shift changes due to the stacking of DNA/RNA bases in helical structure[21] have been reported, such chemical shift changes may be due to the changes on the conformation of these molecules at different concentrations. Obviously in our case, chemical shift changes are not taking place due to the change in conformation (with concentration) of the molecule. Chemical shift changes due to concentration dependent intramolecular conformational changes in the rigid systems that we have studied are impossible. The dramatic variation of the chemical shifts observed in the present study could be a consequence of molecular aggregation leading to the formation of differently packed supramolecular assemblies. In such assemblies, the number of molecules, the orientations in the aggregate and their mutual interactions and "tightness" of association should vary as a function of concentration which in turn should manifest in the altered chemical shifts. Lack of the consideration of the importance of concentration effects in the study and thus design of self replicating systems have led to unwanted controversies.[2]

Our observations of such pronounced variation of chemical shifts as a function of concentration is hitherto not reported in the literature. It is obvious that the implications of such findings are extremely significant, given the resurging interest in the use of NMR spectroscopy for the determination of binding constants in molecular recognition phenomena. In these studies a solution of a guest molecule is added in incremental amounts to a solution containing the host. The observed changes in the chemical shifts of the highest affected proton is plotted against the concentration of the host in a typical molecular recognition experiment for the determination of the association constant and the stoichiometry of binding between the host and the guest. However, verification of any changes in the chemical shift as a function of concentration of either the host or the guest is not done. In view of the findings presented in this communication, it is clear that such conclusions on the binding strength and nature of association could often be very misleading, if not incorrect.[2]

One may conclude from the forgoing experiments that the concentration of the molecule under study has an important bearing on the kind of NMR signature it produces. Such changes in spectral pattern become even more important in view of the fact that higher sample concentrations are required for spectra taken at lower external magnetic field strengths.[22] Therefore, lack of consideration of the concentration of the NMR sample could lead to incorrect conclusions pertaining to the structural identity of a given molecule. It is also apparent that interpretations pertaining to host-guest binding involving pronounced changes in concentration could result in grossly conflicting or misleading conclusions. It is thus, important to give the solute concentration when reporting NMR spectra.[23]

Experimental

All spectra were taken on a Bruker AM-300 spectrometer, operating at 300.13 MHz Larmor frequency for proton and at 291K temperature. All assignments were checked by homonuclear decoupling and ^1H-^1H COSY experiments. The T_1 values were measured in $CDCl_3$ by the inversion recovery method. Our studies show that the chemical shift of the $CHCl_3$ (in $CDCl_3$) signal, as well as other solvent signals, change with concentration. Consequently, in all cases the TMS signal was taken as the reference.

Quinoline and isoquinoline were freshly distilled over CaH_2 under argon. The 6- and 8-*tert*-butylquinolines were synthesized from 4-and 2-*tert*-butylanilines respectively using the Skraup's quinoline synthesis.[11] The details of this synthesis will be published elsewhere.

Variable temperature studies were made at two different concentrations, 0.29 M and 1.06 M in $CDCl_3$ from 265.2 K to 314.2 K at 5 K increments. ^1H -NMR spectra of 1.06 M and 0.29M quinoline solution in $CDCl_3$ were taken with different amounts of Resolve-Al (Aldrich Chemical Co.), $Eu(thd)_3$,europium tris(2,2,6,6-tetramethyl-3,5-heptadienoate), CAS Registry number: 15522-71-1.

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9. Δc Refers to change in concentration (M) and $\Delta\delta$ to change in chemical shift in ppm. If the chemical shift changes to higher ppm value we have assigned $\Delta\delta$ as positive, whereas if the shift is to lower ppm value we have assigned $\Delta\delta$ as negative.
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16. We are currently doing M.O. and molecular dynamics calculations to explore potential models to explain our observations and their implications. We are also doing NMR expts. to study the effect of orientation of the molecule at different concentrations on the chemical shift changes, as noted by the authors in reference 5(b).
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22. These observations have been made irrespective of the external magnetic field strength (B_0). ^{1}H-NMR spectra of various compounds were also measured at 90 MHz JEOL NMR Spectrometer at UNCW, NC, and 500 MHz, Bruker Avance-DRX NMR Spectrometer at the Department of Chemistry, Columbia University, NY.
23. Partial support for this research was provided by the PEW consortium, NSF in the form of an instrument grant to AM (USE-9052233) and in the form of a research grant to PS (CHE-9408755).