

Quenching of Dipolar and/or Scalar Connectivities in ^{13}C - ^1H Shift Correlated 2D NMR Spectra of Paramagnetic Metal Complexes

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Several NMR methods have been suggested for delineation of paramagnetic metal-binding sites in ligands in solution. Since the very first discovery of paramagnetic line broadening^{1,2} and of paramagnetic isotropic shift,³ an enormous quantity of theoretical and methodological investigations have appeared.^{4–6} However, several limitations have been outlined,^{4–11} especially for the Solomon–Bloembergen–Morgan theory of paramagnetic relaxation rates.^{12–14}

For these reasons, NMR methods having the potential of delineating metal-binding sites are still sought. It was, for example, reported that paramagnetic labels selectively disrupt connectivities in NOESY^{15–17} as well in COSY 2D ^1H NMR spectra,^{18,19} thus providing a relatively fast qualitative structural delineation.

Here we present evidence that the method also applies in the heteronuclear case and also that the alternative or simultaneous selective disruption of connectivities in COSY and NOESY 2D spectra allows one to separately consider scalar and dipolar contributions to the nuclear relaxation mechanism.

The method takes advantage of the increase in sensitivity in 2D shift correlated heteronuclear spectra and may be helpful, especially in the light of the advances in computer methods for obtaining 2D difference spectra.²⁰ The copper(II) complexes of pyridine and nicotine are considered to test the method.

If we consider a single ^{13}C - ^1H AX spin system (J decoupled), the intensity of the peak in the HOESY experiment is described

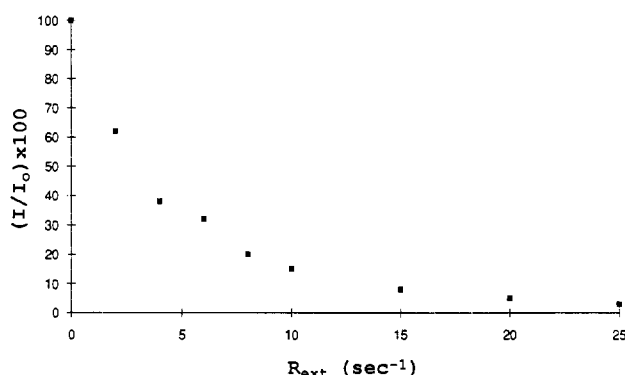


Figure 1. Effect of the value of the external relaxation rate on the peak intensity in a HOESY spectrum as calculated with the following assumed parameters: $r_{\text{CH}} = 1.09 \text{ \AA}$, $\tau_c = 0.1 \text{ ns}$, $B_0 = 4.7 \text{ T}$ (such that $\omega_{\text{H}} = 1.26 \times 10^9 \text{ rad s}^{-1}$ and $\omega_{\text{C}} = 3.16 \times 10^8 \text{ rad s}^{-1}$).

by the mixing coefficient:^{21,22}

$$a_{\text{C-H}}(\tau_m) = -\frac{M_0 R_{\text{CH}}}{2 R_{\text{T}}} \exp(-R_{\text{L}}\tau_m) [1 - \exp(-R_{\text{T}}\tau_m)] \quad (1)$$

where τ_m is the mixing time, R_{CH} is the ^{13}C - ^1H dipolar cross-relaxation rate, R_{T} measures the rate of transfer between the two spins, and R_{L} determines the rate of leakage of magnetization toward the lattice.²² In the absence of external relaxation, the intensity is a function of the mixing time and of the ^{13}C and ^1H dipolar relaxation rates.

In a paramagnetic complex, a potent source of external relaxation is introduced by the presence of the electron magnetic moment.^{4–6,23} If we consider a typical paramagnetic relaxation probe, such as Cu^{2+} ($S = 1/2$), assumption of the metal–nucleus distances allows to calculate such contributions and to demonstrate the effect of the external relaxation rate on the intensity of the peak in the HOESY spectrum, as shown in Figure 1. The peak intensity progressively decreases, approaching zero at values of $R_{\text{ext}}^{\text{H}}$ around 20 s^{-1} . For distances as short as 4 \AA from the metal ion, the external relaxation rates may be estimated at $R_{\text{ext}}^{\text{H}} = 1780 \text{ s}^{-1}$ and $R_{\text{ext}}^{\text{C}} = 116 \text{ s}^{-1}$, such that there is no chance left of detecting the ^{13}C - ^1H dipolar connectivity even at extremely short mixing times.

Finally, in a system containing a paramagnetic probe and a ligand exchanging between the free state and the paramagnetic bound state, the intensity of the cross-peak is a function of the exchange rates and also of the fraction of the ligand in the bound state. It can be calculated that the exchange matrix yields real eigenvalues at exchange rates not necessarily much faster than the paramagnetic spin–lattice relaxation rate. It can be therefore concluded that the disappearance of a peak in a HOESY spectrum is determined by the closeness of the involved nuclei to the paramagnetic center and that such a phenomenon occurs even at exchange rates that are outside the limit of fast exchange, as defined by Swift and Connick.²⁴ In the same HOESY spectrum, as well as in a hetero-COSY 2D spectrum,²⁵ a peak may well disappear because of the efficiency of paramagnetic transverse rates that determine the line widths of the cross-peaks. The consequence is that a comparison of peak behavior in these two 2D spectra provides a unique tool for distinguishing between dipolar and scalar effects of paramagnetic metal ions at very low metal:ligand ratios.

The main drawback of the method is in the low intrinsic sensitivity of heteronuclear shift correlated 2D experiments, but

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- (1) Eisinger, J.; Shulman, R. G.; Blumberg, W. E. *Nature* **1961**, *192*, 963.
- (2) Cohn, M.; Leigh, J. S.; *Nature* **1962**, *193*, 1037.
- (3) McConnell, H. M.; Chesnut, D. B. *J. Chem. Phys.* **1958**, *28*, 107.
- (4) Dwek, R. A. *Nuclear Magnetic Resonance in Biochemistry*; Clarendon Press: Oxford, U.K., 1975.
- (5) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Species in Biological Systems*; Benjamin Cummings: Menlo Park, CA, 1986.
- (6) Niccolai, N.; Tiezzi, E.; Valensin, G. *Chem. Rev.* **1982**, *82*, 359.
- (7) Kowalewski, J.; Nordenskiöld, L.; Benetis, N.; Westlund, P.-O. *Prog. NMR Spectrosc.* **1985**, *17*, 141.
- (8) Benetis, N.; Kowalewski, J.; Nordenskiöld, L.; Wennerström, H.; Westlund, P.-O. *Mol. Phys.* **1983**, *48*, 329.
- (9) Benetis, N.; Kowalewski, J.; Nordenskiöld, L.; Wennerström, H.; Westlund, P.-O. *J. Magn. Reson.* **1984**, *58*, 261.
- (10) Bertini, I.; Luchinat, C.; Mancini, M.; Spina, G. In *Magneto-Structural Correlation in Exchange Coupled Systems*; Willett, R. D., Gatteschi, D., Kahn, O., Eds.; Reidel: Dordrecht, The Netherlands, 1985; p 421.
- (11) Bertini, I.; Luchinat, C.; Kowalewski, J. *J. Magn. Reson.* **1985**, *62*, 235.
- (12) Solomon, I. *Phys. Rev.* **1955**, *99*, 559.
- (13) Bloembergen, N. *J. Chem. Phys.* **1957**, *27*, 572.
- (14) Bloembergen, N.; Morgan, L. O. *J. Chem. Phys.* **1961**, *34*, 841.
- (15) Gaggelli, E.; Tiezzi, E.; Valensin, G. *J. Chem. Soc., Faraday Trans. 2* **1988**, *84*, 141.
- (16) De Jong, E. A. M.; Claesen, C. A. A.; Daemen, C. J. M.; Harmsen, B. J. M.; Konings, R. N. H.; Tesser, G. I.; Hilbers, C. W. *J. Magn. Reson.* **1988**, *80*, 197.
- (17) Bocarsly, J. R.; Barton, J. K. *Inorg. Chem.* **1989**, *28*, 4189.
- (18) Kosen, P. A.; Scheek, R. M.; Naderi, H.; Basus, V. J.; Manogaran, S.; Schmidt, P. G.; Oppenheimer, N. J.; Kuntz, I. D. *Biochemistry* **1986**, *25*, 2356.
- (19) Petros, A. M.; Mueller, L.; Kopple, K. D. *Biochemistry* **1990**, *29*, 10041.
- (20) Fortier, P. L.; Delsuc, M. A.; Guittet, E.; Kahn, P.; Lallemand, J. Y. *J. Magn. Reson.* **1991**, *95*, 161.

(21) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.

(22) Macura, S.; Ernst, R. R. *Mol. Phys.* **1979**, *41*, 95.

(23) Granot, J. *J. Magn. Reson.* **1982**, *49*, 257.

(24) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307.

(25) Maudsley, A. A.; Ernst, R. R. *Chem. Phys. Lett.* **1977**, *50*, 368.

scalar connectivities

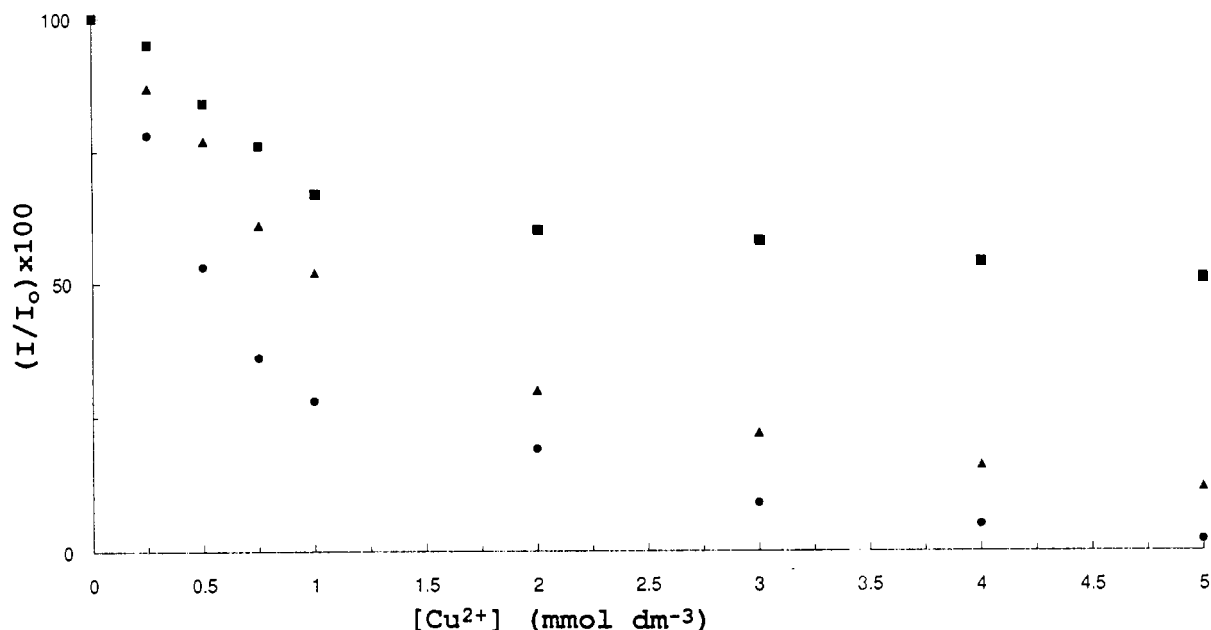


Figure 2. Effect of increasing concentrations of copper ions on the peak intensities of para (full squares), ortho (full triangles), and meta (full circles) carbons in the hetero-COSY 2D spectrum of pyridine (40%) in D_2O at $T = 298$ K.

Table I. ^{13}C NMR Relaxation Rates of Pyridine (40% v/v) in D_2O at $T = 298$ K in the Absence and in the Presence of Copper Ions at a [Metal]/[Ligand] Ratio of 3×10^{-3}

carbon	R_1 (s^{-1})	R_2 (s^{-1})	R_{1p} (s^{-1})	R_{2p} (s^{-1})
ortho	4.31	6.76	1.40	2.31
meta	3.65	6.17	0.12	3.25
para	4.18	6.71	0.02	0.76

the method may derive benefits from progress in the development of technical and methodological tools.

NMR spectra were obtained at 4.7 and 14.1 T at the constant temperature of 294 ± 1 K. ^{13}C - 1H cross-correlated 2D spectra were obtained with conventional pulse sequences.²⁰ The spectral width was 140 ppm in the t_2 dimension and 10 ppm in the t_1 dimension; the data set consisted of 1024×1024 points for a total accumulation time of ca. 10 h. ^{13}C - 1H 2D NOE (HOESY) spectra were obtained by using a previously reported pulse sequence.²⁶ The mixing time was set in the range 100–700 ms while all other parameters were the same as in the hetero-COSY experiment. Intensities were measured by the peak volumes in the 2D contour map.

Pyridine yields heteronuclear 2D spectra (not shown), either scalarly or dipolarly shift correlated, containing three peaks centered at 124.5 (meta), 136.4 (para), and 150.6 ppm (ortho) in the f_2 dimension and at 7.22 (meta), 7.61 (para), and 8.43 ppm (ortho) in the f_1 dimension. The relative intensities reflect the different number of carbons whereas the absolute intensity within the HOESY spectrum is a function of the mixing time that reaches its maximum at $t_m =$ ca. 600 ms.

The addition of copper differently affects the three peaks in the two 2D spectra. The decreases in the peak intensities are shown in Figures 2 and 3, where the percent residual intensity $(100I)/I_0$ is plotted against the actual concentration of copper ions in solution. It is apparent that ortho, meta, and para carbons behave differently as a consequence of the different scalar and dipolar contributions to their longitudinal and transverse relaxation rates. In the case of ortho carbons, being the carbons at the shortest distances from the metal ion coordinated to the nitrogen, where the dipolar paramagnetic contribution is max-

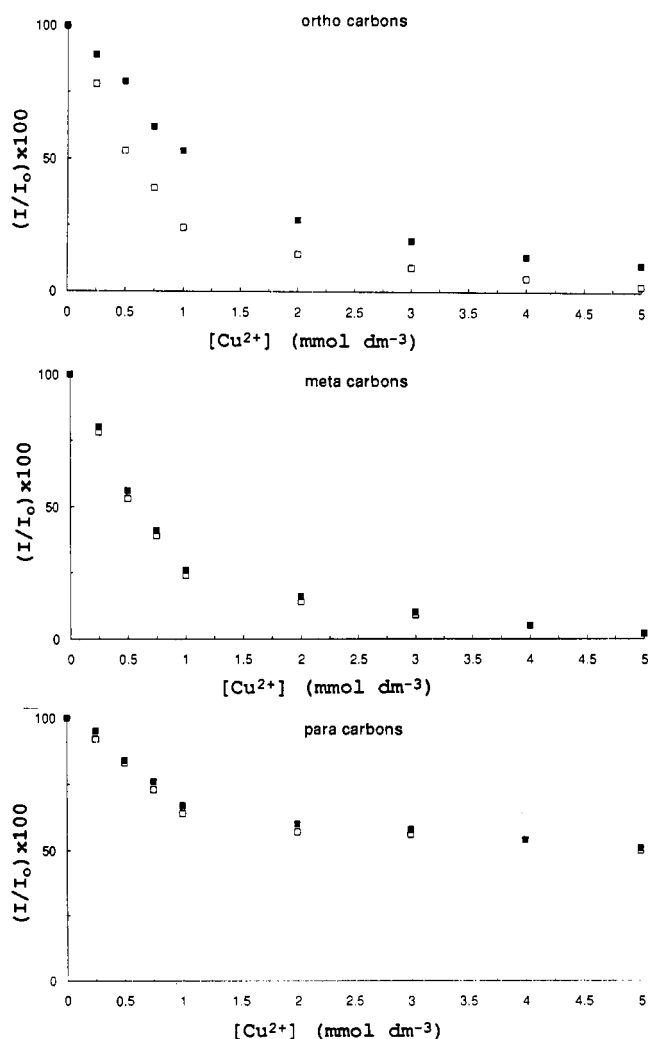


Figure 3. Effect of increasing concentrations of copper ions on the peak intensities in the hetero-COSY (full symbols) and HOESY (open symbols) 2D spectra of pyridine (40%) in D_2O at $T = 298$ K.

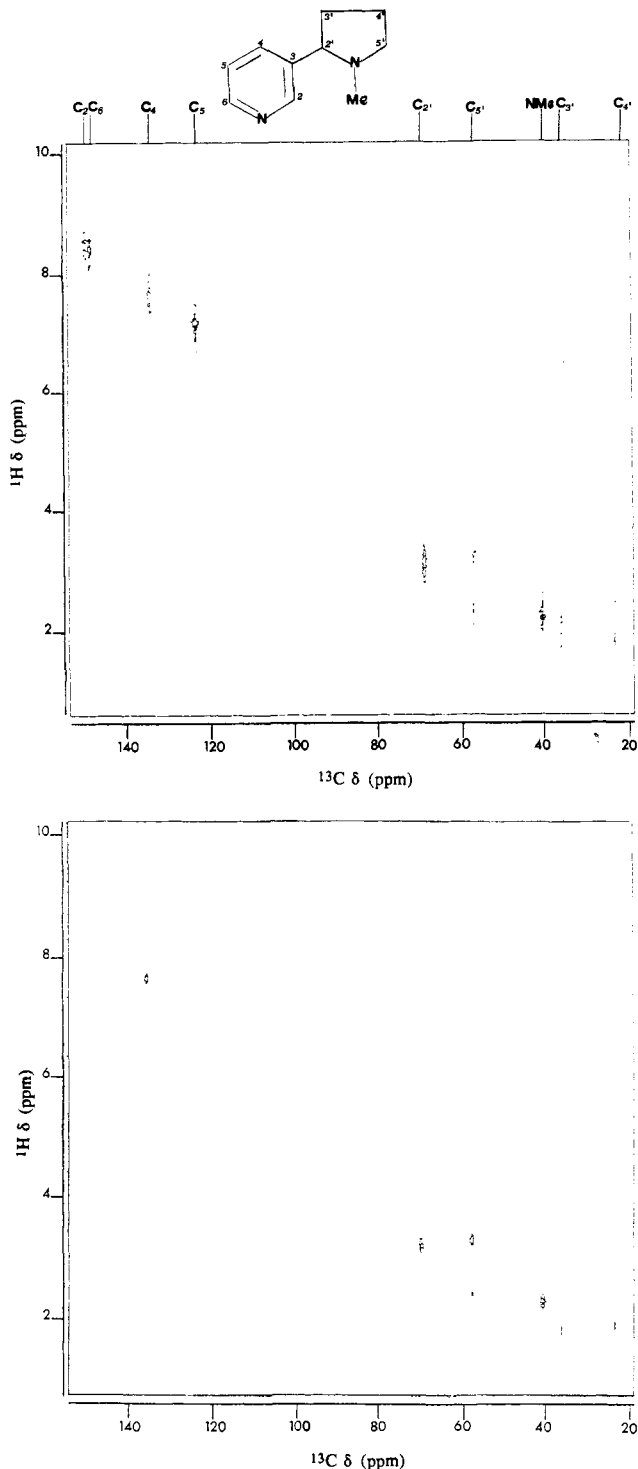


Figure 4. Effect of copper ions at a [ligand]/[metal] ratio of 6×10^3 on the hetero-COSY 2D contour map of nicotine (40%) in D_2O at $T = 298$ K: (top) nicotine; (bottom) nicotine plus copper.

imum, the peak progressively diminishes in intensity at increasing copper concentration and the rate of disappearance is faster in

the HOESY than in the hetero-COSY 2D spectrum. This has to be ascribed to the strong external relaxation that affects the intensity during the mixing period. This is not the case for meta carbons, where the dipolar interaction with the metal ion is much less effective, whereas the scalar one is quite strong due to the unpaired electron spin density in the meta position. In this case, it appears that the peak disappears at almost the same rate in both 2D experiments, which is consistent with a poor external relaxation effect during the mixing period. Finally, in the case of para carbons, the peak is affected exactly in the same way in the two experiments and to a much less pronounced extent with respect to ortho or meta carbons. This last effect is clearly the result of the absence of dipolar interaction with the distant metal ion and of the relatively small electron density on the para carbon.

The results obtained from the 2D spectra are easily confirmed by measuring the paramagnetic relaxation rates, as summarized in Table I. As expected, ortho carbons display the greatest longitudinal and meta carbons the largest transverse paramagnetic contributions, given by:

$$R_{1p} = R_{1obs} - R_{1f} = \frac{p_b}{T_{1b} + \tau_b} \quad (2)$$

$$R_{2p} = R_{2obs} - R_{2f} = \frac{p_b}{T_{2b} + \tau_b} \quad (3)$$

where R_{1obs} and R_{1f} are the relaxation rates measured in solution of the ligand in the presence and in the absence, respectively, of copper ions, p_b is the fraction of bound ligand, T_{1b} is the relaxation time in the ligand bound to the metal ion, and τ_b ($=1/k_b$) is the exchange lifetime in the bound state. It may be noticed that $R_{2p} \gg R_{1p}$, such that, necessarily, the exchange rate cannot dominate the relaxation mechanism and fast-exchange conditions apply. Moreover, the large difference between R_{1p} and R_{2p} can only be consistent with predominance of the scalar contribution to R_{2p} and of the dipolar one to R_{1p} .

The same experiments were then repeated for nicotine. The hetero-COSY and HOESY maps (Figure 4) allowed identification of all the ^{13}C and 1H resonance frequencies. In the presence of the appropriate amount of copper ions, quenching of all the ^{13}C - 1H connectivities in the pyridine moiety but that relative to the para carbon was observed, thus demonstrating that the pyridine nitrogen was the only atom of nicotine directly involved in metal binding.

It can be concluded that paramagnetic species selectively change the appearance of 2D homo- and heteronuclear shift correlated spectra, thus providing a direct means of detecting the binding sites in ligands sufficiently large to allow spectral resolution. 2D difference spectra are then expected to delineate the ligand nuclei directly involved in the interaction with the paramagnetic species. At a more sophisticated level, comparison of such changes in COSY (hetero-COSY) and NOESY (HOESY) experiments has the potential of discriminating between dipolar and scalar interactions with the paramagnetic center, such that mapping of nuclei in the nearest neighborhood of the center may be obtained by NOESY or HOESY 2D difference contour plots.