Removal of Nuclear Magnetic Resonance Proton Signals from Residual Water in D_2O Solutions using a Double Resonance Technique

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Summary A method is described for removal of n.m.r. proton signals from residual water in D_2O solutions by strong irradiation at the resonance frequency of exchangeable protons present in small amounts in the system.

NUCLEAR magnetic double resonance techniques have been used previously to study intermolecular chemical exchange in systems where the exchange rate is such that separate n.m.r. absorption bands are observed for the exchanging nuclei in the two different sites.[1] If the relaxation rate of the observed nucleus is slower than the exchange rate, irradiation at the absorption frequency corresponding to one of the sites causes a decrease in intensity at the frequency corresponding to the other site. This is due to transfer of saturated nuclei from one site to the other by chemical exchange. We have recently used this technique

(i) to remove almost completely residual HDO ^{1}H absorption bands in D_2O solution to reveal bands previously obscured by the water absorption band and (ii) to measure chemical shifts of exchanging protons in D_2O solution even when their absorption bands cannot be observed directly in the normal spectrum because of extensive deuteriation at these sites resulting from exchange with the solvent.

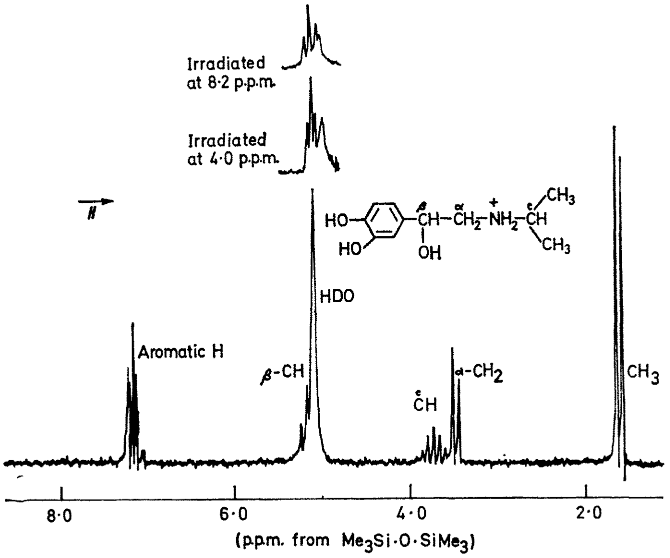

FIGURE. *The* ^{1}H *n.m.r. spectrum at* 100 *MHz of isoprenaline sulphate in* 99·86% D_2O *solution.*

The Figure shows the ^{1}H spectrum of isoprenaline sulphate (80 mg in 0·5 ml D_2O) recorded at 100 MHz: the assignments are indicated on the Figure. No absorption bands are observed in the spectrum for the labile NH_2^+ nor for the aliphatic and phenolic OH protons. However, in 99·86% D_2O, a very small fraction of the exchangeable groups in isoprenaline will still bear protons which can be involved in slow exchange with the residual protons of the solvent. Systematic double resonance experiments throughout the spectral region from δ 2—10 reveal that such a situation exists: on irradiation at δ 8·2 ($\pm$0·05) there is a dramatic decrease in intensity of the HDO band which allows us to observe the previously obscured β-CH triplet and provides us also with the chemical shift of the NH_2^+ proton absorption band which is not visible in the normal spectrum. Examination of the 100 MHz ^{1}H spectrum of isoprenaline in H_2O confirms the presence of a broad absorption band at 8·21 $\pm$ 0·02 p.p.m. It was not possible to measure the chemical shifts of the other labile protons in isoprenaline with any accuracy because their exchange rates are such that they have very broad absorption bands roughly centred about the HDO signal: collapse of the HDO can be achieved by irradiation anywhere in the range δ 3·5—6·5 and irradiation at δ 4·0 results in almost total collapse of the water absorption. This latter irradiation is not too far from the α-CH_2 resonance frequency and thus it also causes some collapse of the β-CH triplet.

This effect could thus prove of use not only in removal of unwanted water signals but also in characterising chemical shifts of exchangeable protons in D_2O solution. We estimate that such chemical shifts can be measured to $\pm$5 Hz in favourable cases using this technique.

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[1] R. A. Hoffman and S. Forsen, *J. Chem. Phys.*, 1963, **39**, 2892; J. Feeney and A. Heinrich, *Chem. Comm.*, 1966, 295.