A DNP Investigation of π and σ Transitions for Nitroxide Spin Probes at 1.53 mT

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A weak field DNP study of 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (4-hydroxy-TEMPO), 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy (4-amino-TEMPO), and 2,2-Di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) free radicals in various solvents was performed at 1.53 mT using a double resonance spectrometer. π transitions, in which the RF field has a component perpendicular to the static magnetic field B_0, and σ transitions, in which the RF field has a component parallel to B_0, were recorded. In order to observe the σ transitions a new probe head was designed. The σ transitions were obtained at the frequencies expected from theoretical calculations. However, the results obtained for the observed enhancements deviated from the values expected theoretically.   © 1998 Academic Press

INTRODUCTION

In recent years low field DNP studies have regained importance. The main reason for the low field studies is the necessity for lower frequencies in order to prevent power deposition and sample heating in biological samples (*1–3*). Nitroxides are well-known free radicals and there are studies on the design of new nitroxides for the biological applications of DNP (*4–6*). In recent studies σ transitions of the nitroxide radical PCA were investigated at 6.8 mT and it was proposed that the σ transitions would be more efficient at the 0–1.7 mT field region because of the increase in the transition probabilities with decreasing field (*7, 8*). The aim of this study is to record the π and for the first time the σ spectra of nitroxides at 1.53 mT and to compare them with the theoretical expectations.

THEORY

The DNP study is based on the transfer of magnetization from electrons to protons as a result of cross-relaxation processes. Following the excitation of an EPR transition the NMR

signal is enhanced. The observed enhancement is described as the ratio of the NMR signal amplitudes with and without the excitation of the EPR transition:

$$G(P) = \frac{P_z}{P_0} - 1. \qquad [1]$$

In theory the observed enhancement described in this way is a function of the leakage factor, saturation factor, and transition probability of the EPR line of interest (*9*).

The EPR spectra of the samples can be calculated by solving the time-independent Hamiltonian (*10, 11*):

$$\Re = g\beta\mathbf{B} \cdot \mathbf{S} + \sum_i A_i \mathbf{S} \cdot \mathbf{I}_i. \qquad [2]$$

Two systems were investigated in this study. For an $S = \frac{1}{2}$, $I = 1$ system resonance frequencies and transition probabilities were calculated. At 1.53 mT the theoretical spectrum including both π and σ transitions was obtained (Fig. 1) (*12*). For an $S =$
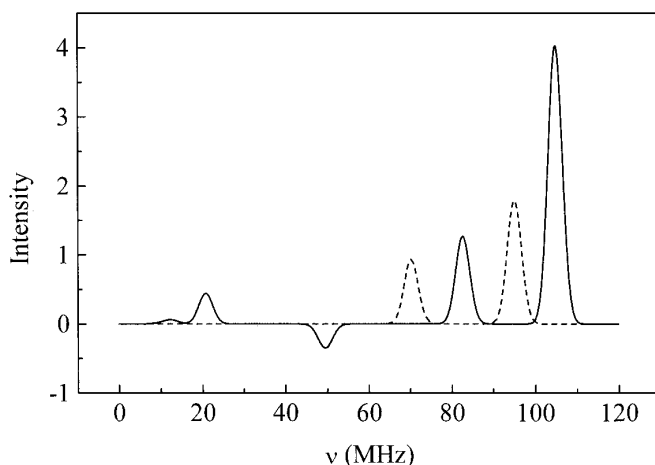

FIG. 1. Theoretical spectrum of 4-amino-TEMPO/water. (—) π-transitions, (– – –) σ-transitions.

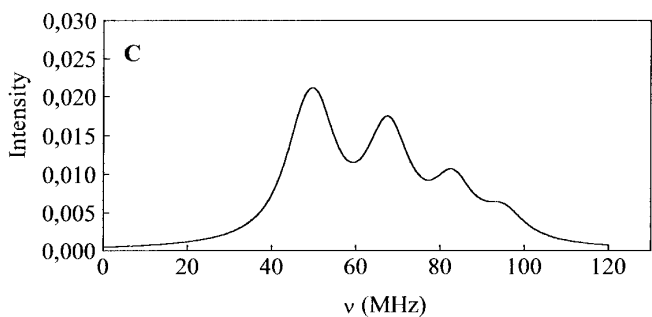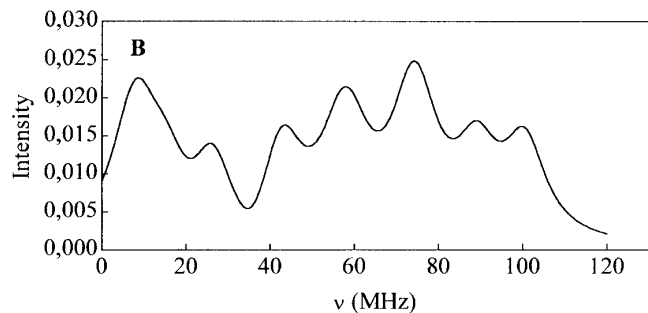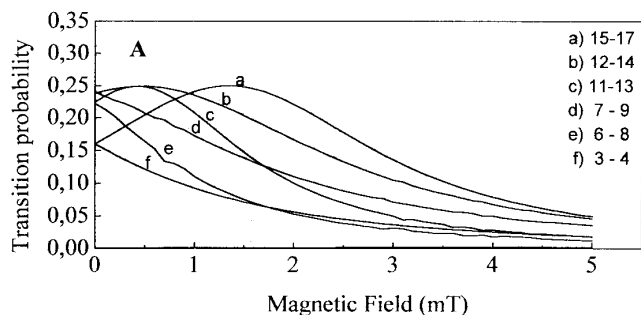[1] To whom correspondence should be addressed. E-mail: maral@eti.cc.hun.edu.tr. Fax: (312) 235 25 50.

FIG. 2. $S = \frac{1}{2}$, $I_1 = I_2 = 1$ system (A) σ-transition probabilities (the energy levels are numbered in decreasing order), (B) π-transitions, (C) σ-transitions.

$\frac{1}{2}$, $I_1 = I_2 = 1$ system, for $A_1 \approx A_2$, the resonance frequencies and transition probabilities were also calculated and the magnetic field dependence of the σ transition probabilities and the theoretical spectra for π and σ transitions were plotted (Fig. 2A, B, C).

EXPERIMENTAL

Studies were performed using a double resonance spectrometer working at 1.53 mT that has a fixed proton resonance frequency of 65 kHz and variable EPR frequencies in the range of 5–120 MHz. In our previous studies in this field (*10, 11, 13, 14*), π transitions were recorded using a probe head in which electron resonance RF field B_1 and proton resonance RF field B_2 were parallel to each other and both were perpendicular to the static field B_0. In order to perform

the measurements related to σ transitions, a new probe head was designed. A variety of coil geometries were examined. At 1.53 mT the best result was obtained when a solenoid (length 4.3 cm, diameter 3.8 cm, wire diameter 0.3 mm) for NMR and a single-turn saddle shaped coil (length 4.2 cm, diameter 3.0 cm, wire diameter 3.0 mm) for EPR was used. The probe head was inserted on a turntable, so that it was possible to record the spectra at an arbitrary angle between B_1 and B_0 (*15*). The inhomogeneity of the RF field, which causes the appearance of the π transitions in the spectrum when this field is kept parallel to main field, was 7%. This value is calculated from the intensity ratios of π transitions obtained when the RF field is perpendicular and parallel to the static field.
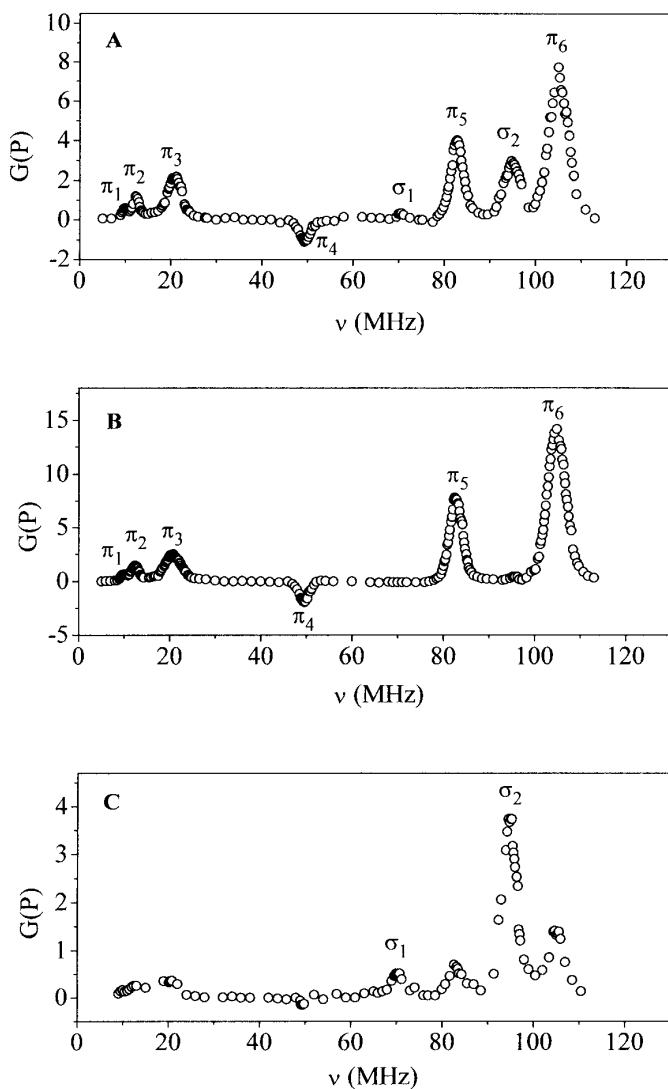
The 1 mM solutions of 4-amino-2,2,6,6-tetramethyl-1-pip-

FIG. 3. The spectra of 4-amino-TEMPO/water samples for an RF field (A) at 45° (π and σ), (B) at 90° (π), (C) at 0° (σ).
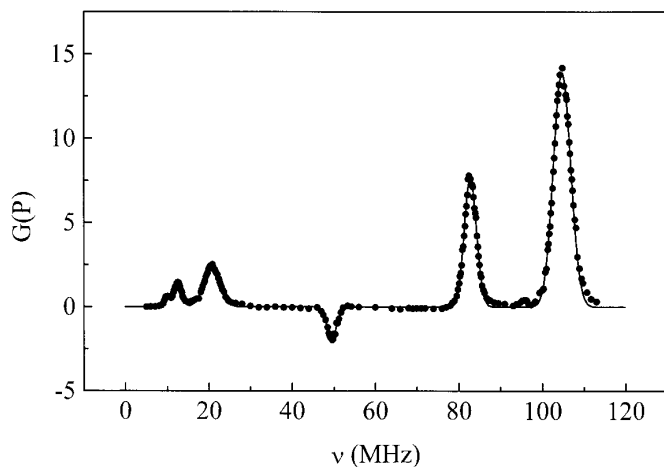
FIG. 4. The fitted spectrum of 1 mM 4-amino-TEMPO/water. (—) fitted line; (●) experimental.

eridinyloxy (4-amino-TEMPO) in water and tetrahydrofuran (THF), 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (4-hydroxy-TEMPO) in water and phosphate buffer, and 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) in benzene were prepared and degassed using the freeze–thaw–pump technique. The sample tubes were 25 mm in diameter and the sample volumes were 20 cm³.

In the spectrometer used, EPR spectra must be obtained by keeping the EPR field amplitude constant and varying the EPR frequency step by step between the required limits. Since field amplitude is proportional to $V(V)/\nu(MHz)$, this ratio is kept constant during the recording of the spectra. In order to avoid saturation, this ratio is chosen as low as possible. The usual value used in our previous studies (*11,*

14) was $\frac{1}{2}$, but with the new probe head, since the coil geometry was changed and the amount of the sample was increased, a reasonable signal-to-noise ratio was obtained for $V/\nu = 1$. The voltage across the EPR coil was measured by a high-frequency AC voltmeter (Rohde & Schwarz, BN1050).

RESULTS AND DISCUSSION

The observed enhancements were calculated using the two methods given in previous studies (*9, 12*). In the method given by Müller-Warmuth (*9*), at first the energy levels, wavefunctions, and transition probabilities of the $S = \frac{1}{2}$, $I = 1$ system were calculated. Then, using the population changes in the energy levels as a result of irradiation one of the permitted transitions, the theoretical enhancement related to the irradiated transition was determined. Calculations were performed for each allowed transition under partial saturation conditions. In the second method, given by Guiberteau and Grucker (*12*), the energy levels, transition probabilities, and wavefunctions were calculated in a similar way, and the same results as in ref. (*9*) were reached. In this case, however; to calculate theoretical enhancements, the total saturation case ($s = 1$) was considered in the population equations and the enhancements obtained for each allowed transition were multiplied by the transition probability of the considered transition.

The experimental spectrum of 4-amino-TEMPO in water is given in Fig. 3. π and σ transitions were obtained at expected frequencies. In the calculated spectra of this sample, the intensity ratios of the resonance peaks follow the correct order in both methods with the experi-

TABLE 1
Fitted and Theoretical Values of Resonance Frequencies and Linewidths of Samples

			Resonance frequencies and linewidths							
	Samples		π_1	π_2	π_3	π_4	π_5	π_6	σ_1	σ_2
4-Amino-TEMPO	Water	Fitted Γ (MHz)	0.73	1.14	2.34	1.34	1.73	2.44	2.50	2.03
	$A = 47.80$ MHz	Fitted ν (MHz)	9.90	12.4	20.7	49.5	82.8	104.8	70.4	94.7
		Theoretical ν (MHz)	10.0	12.4	20.7	49.5	82.8	104.8	70.2	95.1
	THF	Fitted Γ (MHz)	0.66	1.44	2.42	1.33	1.90	2.95	1.14	2.42
	$A = 43.70$ MHz	Fitted ν (MHz)	9.60	12.1	21.5	44.0	77.4	99.1	65.0	89.0
		Theoretical ν (MHz)	9.50	12.1	21.3	44.0	77.4	99.0	65.3	89.5
4-Hydroxy-TEMPO	Water	Fitted Γ (MHz)	0.87	1.22	2.29	1.34	1.79	2.53	1.76	2.26
	$A = 47.96$ MHz	Fitted ν (MHz)	9.87	12.5	20.8	49.5	82.9	104.8	70.2	94.6
		Theoretical ν (MHz)	9.80	12.4	20.7	49.7	82.8	105.1	70.4	95.3
	Phosphate buffer	Fitted Γ (MHz)	0.70	1.10	2.00	1.14	1.63	2.35	1.14	2.14
		Fitted ν (MHz)	9.80	12.4	20.7	49.7	82.8	104.8	70.5	94.8
	$A = 47.96$ MHz	Theoretical ν (MHz)	9.80	12.4	20.7	49.7	82.8	105.1	70.4	95.3

TABLE 2
Example of DNP Calculations, DNP Values of 4-Hydroxy-TEMPO/Water

		π_1	π_2	π_3	π_4	π_5	π_6	σ_1	σ_2
Ref. (9)	s = 1	−5.3	−4.7	−23.7	45.2	−54.3	−131.8	—	—
	s free	—	—	−0.88	0.41	−0.67	−0.66	—	—
Ref. (12)	s = 1	−0.04	−0.17	−1.30	1.00	−3.74	−12.00	−2.82	−5.31

mental ones, but the calculated enhancements are not consistent with the experimental values if the s = 1 approximation is used. Nevertheless, using the theoretical enhancement equations in ref. (9) and leaving the saturation factor s as a free parameter, it was possible to obtain a good fit between the experimental and calculated spectra (Fig. 4). This was performed by fitting the experimental data to the relation

$$y(\nu) = \sum_i (y_{\max})_i \exp\left\{\frac{(-\ln 2)(\nu - \nu_{r_i})^2}{\Gamma_i^2}\right\}, \qquad [3]$$

$$(y_{\max})_i = (\ln 2/\pi)^{1/2}\Gamma_i^{-1}(DNP_{\exp})_i \qquad [4]$$

using the MINSQ packet program (16). Here ν_i are the resonance frequencies, Γ_i are the linewidths, and $(DNP_{\exp})_i$ are the experimental DNP factors, which can be written as

$$(DNP_{\exp})_i = (DNP_{theo})_i * s_i, \qquad [5]$$

where $(DNP_{theo})_i$ are the theoretical DNP factors calculated from the equations in ref. (9) and s_i are the saturation factors. The fitted and theoretical values of the resonance frequencies and the linewidths of the samples are given in Table 1. Because of the overlap of the transition at 9.87 and 12.5 MHz, the s values obtained are not reliable and therefore are not given in Table 2.

The above investigations were repeated for 4-hydroxy TEMPO/water sample and a good fit was reached again by leaving s as a free parameter. For this sample, DNP factors obtained from the fit and from the theoretical calculations using the s = 1 approximation are given in Table 2 for comparison with the results of the methods in refs. (9) and (12).

In order to understand the dependence of observed enhancements on solution and solvent in σ transitions, the solutions of 4-amino-TEMPO in water and THF and of 4-hydroxy-TEMPO in water and phosphate buffer were prepared and their spectra were recorded (Fig. 3 and Fig. 5A, B, C). The observed enhancements of all samples are given in Fig. 6A, B. Consideration of the observed enhancements in Fig. 6A indicates that with the change of solvent from water to THF, there is an increase in the observed

enhancements at all transitions except σ_2 and π_6. The decrease in the observed enhancements for σ_2 and π_6 transitions might be caused by a change in the dominant relaxation process. To understand this behavior, relaxation time measurements are necessary. However, we have not yet performed these measurements with the new probe head
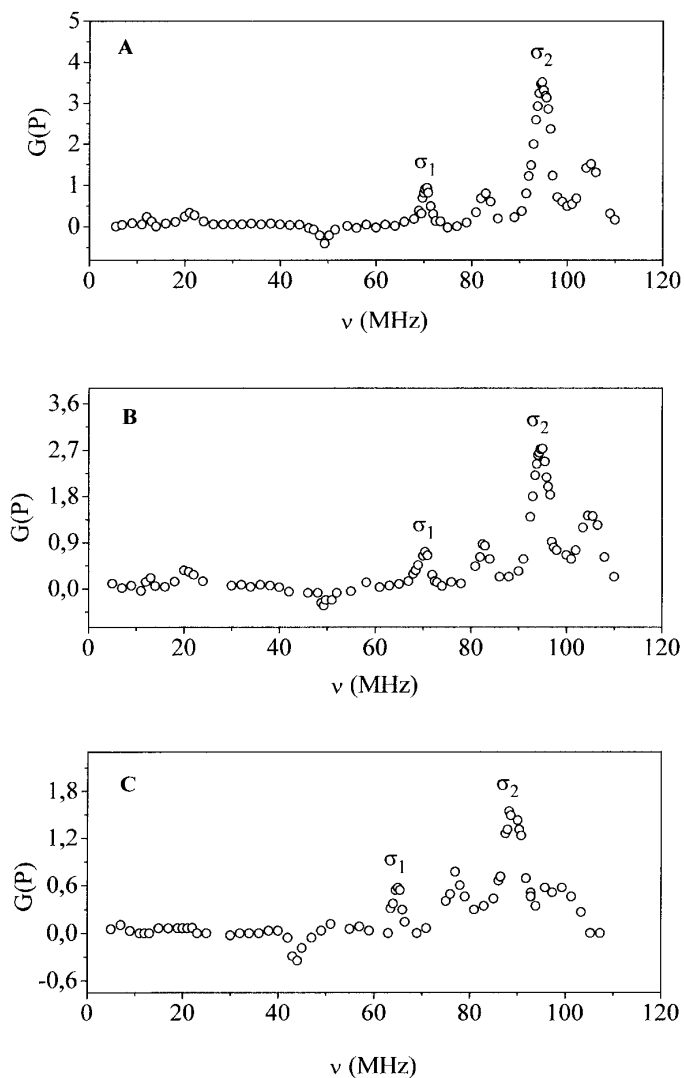

FIG. 5. The σ-spectra of various samples: (A) 4-hydroxy-TEMPO/phosphate buffer, (B) 4-hydroxy-TEMPO/water, (C) 4-amino TEMPO/THF.
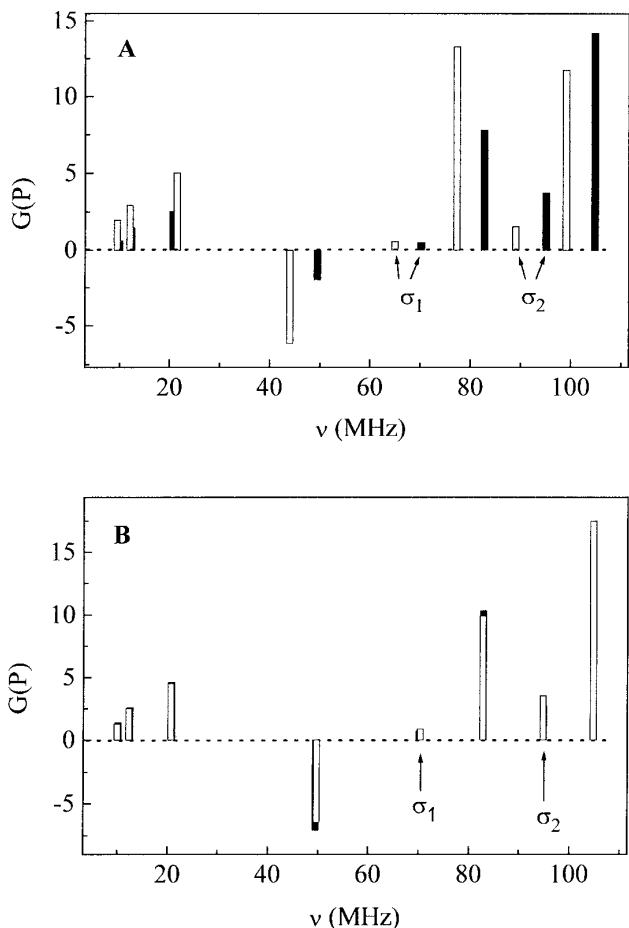
FIG. 6. Observed enhancements of (A) ■ 4-amino-TEMPO/water, □ 4-amino TEMPO/THF, (B) ■ 4-hydroxy-TEMPO/water, □ 4-hydroxy-TEMPO/phosphate buffer.

(Fig. 7A, B). For π transitions the resonances at 22.5 and 37.7 MHz are at opposite polarizations to the other transitions in the spectrum (*17*). An inspection of the experimental σ spectrum obtained for DPPH indicates that all the transitions combined to give an envelope, and from the comparison of this experimental spectrum to the calculated one (Fig. 2C), a shift in the frequency of the transition with highest intensity was observed. This made us think that there might be a transition having opposite polarization. Since it was not possible to determine this fact experimentally, a FORTRAN program was prepared and both π and σ spectra were simulated. The best result was obtained by assuming that the transition at 48 MHz had an opposite polarization (Fig. 7A, B). However, since the signal-to-noise ratio for σ transitions of DPPH was quite small, the standard deviation was found to be greater than that of the other samples. The greatest enhancement for π transitions was 4.7, and it was obtained for the transition at 75 MHz. Similarly, the greatest enhancement for σ tran-
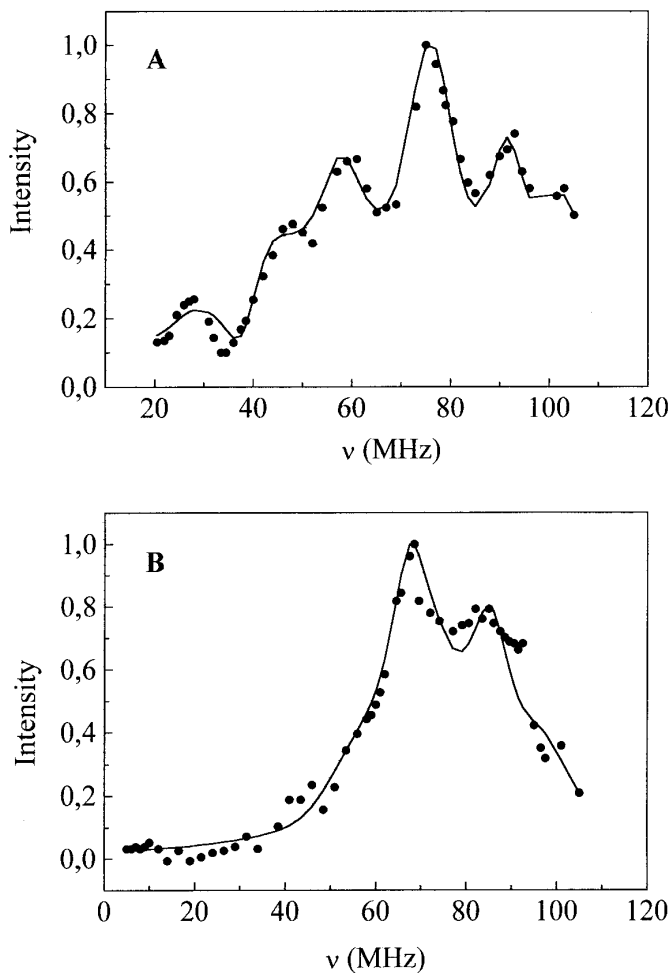
because of the limited power (10 W at 50 Ω) of our RF amplifier. In Fig. 6B, the observed enhancements of 4-hydroxy-TEMPO in water and phosphate buffer exhibit no difference except a slight decrease near 50 MHz and 80 MHz for phosphate-buffered solutions. On the other hand, in the previous work (*13, 14*) the ultimate enhancements of π transitions obtained from saturation measurements using the conventional probe head were reported. In that work, solutions of 4-hydroxy-TEMPO in water had smaller ultimate enhancement at almost all frequencies relative to solutions of the same molecule in ethyl alcohol and THF (*13*); ethyl alcohol solutions had intermediate values, and THF solutions had the greatest values. From these results it can be concluded that ethyl alcohol and THF are more convenient solvents than water for observing π and σ transitions of 4-hydroxy-TEMPO.

The other sample studied was the 1 mM solution of DPPH free radical in benzene, which belongs to the $S = \frac{1}{2}$, $I_1 = I_2 = 1$ system. Both π and σ transitions were recorded

FIG. 7. (A) π-transitions and, (B) σ-transitions of DPPH/benzene. (—) simulated, (●) experimental.

sitions was obtained for the transition at 68.5 MHz and had the value of 1.5.

In conclusion, for the first time at 1.53 mT we observed σ spectra of free radicals. Further studies are necessary using radicals with narrower linewidths to obtain better enhancements for σ transitions; also, relaxation time measurements will complete the information received at this field.

REFERENCES

1. D. J. Lurie, D. M. Bussell, L. H. Bell, and J. R. Mallard, *J. Magn. Reson.* **76,** 366 (1988).

2. D. J. Lurie, I. Nicholson, J. S. McLay, and J. R. Mallard, *Appl. Magn. Reson.* **3,** 917 (1992).

3. M. K. Bowman, T. J. Michalski, M. Peric, and H. J. Halpern, *Pure & Appl. Chem.* **62**(2), 271 (1990).

4. K. Hideg, *Pure & Appl. Chem.* **62**(2), 207 (1990).

5. J. F. W. Keana, L. Lex, J. S. Mann, J. M. May, J. H. Park, S. Pou, V. S. Prabhu, G. M. Rosen, B. J. Sweetman, and Yexin Wu, *Pure & Appl. Chem.* **62**(2), 201 (1990).

6. D. Grucker, T. Guiberteau, B. Eclancher, J. Chambron, R. Chiarelli, A. Rassat, G. Subra, and B. Gallez, *J. Magn. Reson. B* **106,** 101 (1995).

7. T. Guiberteau and D. Grucker, *J. Magn. Reson. A* **105,** 98 (1993).

8. D. G. Gillies, L. H. Sutcliffe, and M. R. Symms, *J. Chem. Soc. Faraday Trans.* **90**(18), 2671 (1994).

9. W. Müller-Warmuth, *Z. Naturforsch.* **15 a,** 927 (1960).

10. M. Sünnetçioğlu, and G. Bingöl, and R. Sungur, *Z. Naturforschg.* **46 a,** 976 (1990).

11. M. Sünnetçioğlu, and G. Bingöl, *Phys. Chem. Liq.* **26,** 47 (1993).

12. T. Guiberteau and D. Grucker, *J. Magn. Reson. B,* **110,** 47 (1996).

13. R. Sungur, *Doğa Tr. J. Phys.* **15,** 63 (1991).

14. N. Horasan, M. M. Sünnetçioğlu, R. Sungur, and G. Bingöl, *Z. Naturforschg.* **52a,** 485 (1997).

15. A. O. Salman, M. Sünnetçioğlu, R. Sungur, and G. Bingöl, *Doğa Tr. J. Phys.,* in press.

16. Micromath Inc. (1991).

17. W. Müller-Warmuth, *Z. Naturforschg.* **19a**(11), 1309 (1964).