Lee, A. G., Birdsall, N. J. M., Levine, Y. K., and Metcalfe, J. C. (1972), *Biochim. Biophys. Acta 255,* 43.

Levine, Y. K., Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., and Robinson, J. D. (1973), *Biochim. Biophys. Acta 291,* 592.

Maclay, W. N. (1956), *J. Colloid Sci. 11,* 272.

Makino, S., Reynolds, J. A., and Tanford, C. (1973), *J. Biol. Chem. 248,* 4926.

Mclaughlin, A. C., Podo, F., and Blasie, J. K. (1973), *Biochim. Biophys. Acta 330,* 109.

Melchior, D. L., and Morowitz, H. J. (1972), *Biochemistry 11,* 4558.

Metcalfe, J. C., Birdsall, N. J. M., and Lee, A. G. (1973), *Ann. N.Y. Acad. Sci. 222,* 460.

Noggle, J. H., and Schirmer, R. E. (1971), The Nuclear Overhauser Effect: Chemical Applications, New York, N.Y., Academic Press, pp 233-235.

Penkett, S. A., Flook, A. G., and Chapman, D. (1968), *Chem. Phys. Lipids 2,* 273.

Phillips, M. C., Williams, R. M., and Chapman, D. (1969), *Chem. Phys. Lipids 3,* 234.

Podo, F., Ray, A., and Némethy, G. (1973), *J. Am. Chem. Soc. 95,* 6164.

Ray, A. (1971), *Nature (London) 231,* 313.

Ray, A., and Némethy, G. (1971), *J. Am. Chem. Soc. 93,* 6787.

Ribeiro, A. A., and Dennis, E. A. (1974a), *Biochim. Biophys. Acta 332,* 26.

Ribeiro, A. A., and Dennis, E. A. (1974b), *Chem. Phys. Lipids 12,* 31.

Ribeiro, A. A., and Dennis, E. A. (1975), *Chem. Phys. Lipids 14,* 193.

Rösch, M. (1967), in Nonionic Surfactants, Surfactant Science Series, Vol. 1, Schick, M. J., Ed., New York, N.Y., Marcel Dekker, pp 753-773.

Schick, M. J., Atlas, S. M., and Eirich, F. R. (1962), *J. Phys. Chem. 66,* 1362.

Seiter, C. H. A., and Chan, S. I. (1973), *J. Am. Chem. Soc. 95,* 7541.

Vold, R. L., Vold, R. R., and Simon, H. E. (1973), *J. Magn. Reson. 11,* 283.

Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E. (1968), *J. Chem. Phys. 48,* 3831.

Woessner, D. E. (1962), *J. Chem. Phys. 36,* 1.

Yedgar, S., Barenholz, Y., and Cooper, V. G. (1974), *Biochim. Biophys. Acta 363,* 98.

Ion Binding by X-537A. Rates of Complexation of Ni^{2+} and Mn^{2+} in Methanol[†]

Hadassa Degani[‡] and Harold L. Friedman*

ABSTRACT: The rates of complexation are studied through the effects of the paramagnetic ions upon the magnetic resonances of three of the proton species in X-537A = XH. For the dissociation of the complex $MX^+ \rightarrow M^{2+} + X^-$ at 25° the rate is $(2.4 \pm 0.4) \times 10^2$ sec^{-1} for Ni^{2+} and in the range from 2×10^4 to 1×10^6 sec^{-1} for Mn^{2+}. For the Ni^{2+} complex the activation parameters are also determined and discussed in terms of the details of the process. The difference in rate constants found here is much greater than the difference in the dissociation constants.

The work reported here is a continuation of an earlier study (Degani and Friedman, 1974) in which the stoichiometries, thermodynamics, and spectra of various complexes of M^+ and M^{2+} ions with the ionophorous antibiotic X-537A (Lasol acid) (Figure 1) were determined. For the reasons discussed in our previous study, the homogeneous solution chemistry of the antibiotic is of basic interest for the interpretation of the membrane phenomena in which the ionophore plays a role. This is as true for rates as for the properties already studied; however, very few rates of complexation of metal ions in solution have been determined for the cyclic ionophores (Diebler et al., 1969; Chock, 1972; Funck et al., 1972) and, until now, none at all for the carboxylic ionophores.

Here the kinetics of the Mn^{2+} and Ni^{2+} complexes in methanol are reported as determined by the effect of the complexation process upon the magnetic resonance of various protons in the antibiotic. While the use of paramagnetic metal ions is dictated by the method chosen here, one's interest in the interaction of X-537A with these ions may be enhanced by the report that in the presence of X-537A the M^{2+} ions all have similar effects upon nerve synapses (Kita and Van Der Kloot, 1974).

As in our previous report (Degani and Friedman, 1974) we write XH for X-537A and $XH \rightarrow X^- + H^+$ for the reaction in which it ionizes. Reference may be made to the earlier study for the determination of the ionization constant of XH in methanol as well as for the equilibrium constants of the reactions:

$$M^{2+} + X^- \rightarrow MX^+$$

which are required in the present study. The latter results are summarized in Table I. They were determined from ex-

---

[†] From the Department of Chemistry, State University of New York, Stony Brook, New York 11794. *Received March 4, 1975.* This work was supported by a grant from the National Institutes of Health (1 R01 HL16474-01). The 220-MHz NMR measurements were made in the facility at Rockefeller University which was supported in part by National Science Foundation Grant No. GB-12278 and by grants from the Research Corporation and Sloan Foundation.

[‡] Present address: Department of Chemistry, Tel-Aviv University, Tel-Aviv, Israel.

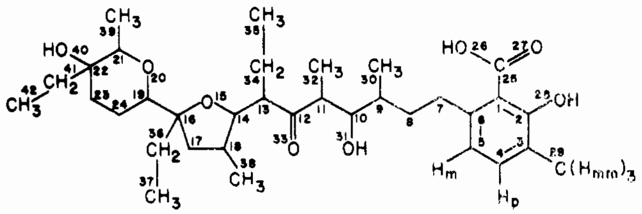

FIGURE 1: X-537A. The special notation for the hydrogens attached to C-4, -5, and -29 is used in the text.

periments in the range of 10^{-6} to 10^{-4} M X-537A, but in the present studies a higher concentration, near 10^{-2} M, is needed. However, the circular dichroic (CD) spectra of solutions containing MnX^+ and X^- in the range 10^{-2} to 10^{-4} M, measured in cells with path lengths of 0.1–10 mm, can be accounted for on the basis of the spectra and formation constants reported in our previous study (Degani and Friedman, 1974). Thus, there is no evidence for the formation of complexes of different formulas than MX^+ in methanol. Moreover, the composition dependence studies of the rate processes reported below give no evidence that additional complexes are required to account for the data either for Mn^{2+} or for Ni^{2+}.

Experimental Section

Methanol-d_4 (99% D) from Stohler Isotope Chemicals was used as solvent. Anhydrous LiOH, $MnCl_2$, and $NiCl_2$ from Alfa Inorganics were dried in a vacuum oven for 24 hr. Solutions were prepared with care to minimize contamination with water from the air. Solutions with compositions in the range studied here stored in a refrigerator were found to be stable for at least 3 months. The concentration of XH was in the range 9×10^{-3} to 4×10^{-2} M and was determined by ultraviolet (uv) absorption. The metal chloride concentrations were low enough to avoid significant formation of the chloro-metal complexes $[M(MeOH)_5Cl]^+$ (Luz, 1969; Levanon and Luz, 1968). Solution pH, measured as described earlier (Degani and Friedman, 1974), was adjusted by adding LiOH in amounts small enough so that appreciable concentrations of LiX were not formed.

Proton relaxation measurements were made at 100 MHz with a Jeolco MN-100 by the usual continuous wave method and at 220 MHz with a Varian HR-220, by a pulse Fourier transform technique.

Longitudinal relaxation times (T_1) in degassed solutions were determined by the Fourier transformed inversion–recovery pulsed sequence $(\pi-\tau-\pi/2-)n$. The effective spin-lattice relaxation rate constants (k_1) defined in the following section are accurate within 50%.

Transverse relaxation times (T_2) were determined by assuming a lorentzian shape for the resonance lines, and are given by:

$$T_2^{-1} = 2\pi\Delta H_{1/2}$$

where $\Delta H_{1/2}$ is the half-width at half-height of the corresponding resonance lines. In cases where, due to broadening, two lines overlap, the T_2 for each line was determined by a computer line-shape simulation program. For a doublet resonance line due to dipolar proton–proton interaction the coupling constants J_{H-H} were determined from nonoverlapping data under similar conditions (Figures 2 and 3). We assumed that $\Delta H_{1/2}$ is the same for the two lines in the doublet. Figure 2 gives an example of the recorded spectrum and the corresponding computer-simulated spectrum.

Table I: Formation Constants of MnX^+ and NiX^+ in Methanol and the Enthalpy and Entropy of NiX^+ Complexation.[a]

Complex	Temp (°C)	pK	ΔH (kcal/mol)	ΔS [cal/(mol deg)]
MnX^+	25	−4.40		
NiX^+	2	−4.10		
	25	−3.96	2.3	26
	46	−3.85		

[a] Data from Degani and Friedman, 1974.

The reduced transverse relaxation rate constants, k_2, defined in the following section are accurate within 20%.

Theory of Interpretation of NMR Relaxation Times and Shifts

We define effective nuclear magnetic resonance (NMR) relaxation rate constants ($k_{1,2}$) and molar shifts (s) which are related to the measured coefficients T_1, T_2, and ω (resonance frequency in radians/second) in solutions in which the concentration of the complex is (MX^+) and which are related to the corresponding coefficients T_{10}, T_{20}, and ω_0 in solutions with the same stoichiometric concentrations ([XH]) of X-537A and the same pH but without the transition metal ion.

$$k_1 = \left(\frac{1}{T_1} - \frac{1}{T_{10}}\right)\frac{[XH]}{(MX^+)} \tag{1a}$$

$$k_2 = \left(\frac{1}{T_2} - \frac{1}{T_{20}}\right)\frac{[XH]}{(MX^+)} \tag{1b}$$

$$s = (\omega - \omega_0)([XH]/(MX^+)) \tag{1c}$$

In general the nine coefficients appearing in eq 1a–c depend on the magnetic field B of the NMR resonance experiment. The ratio $[XH]/(MX^+)$ is determined from the known concentrations of M^{2+} and XH and the MX^+ formation constant. As noted by Eisenstadt and Friedman (1968) the stoichiometric association constant K_c determined by classical methods might differ from the mean stability constants K_1 and K_2 associated with, respectively, the T_1 and T_2 processes. In general, $K_2 \simeq K_1 < K_c$. We have assumed throughout that $K_2 = K_1 = K_c$ numerically; this assumption is discussed later in the Discussion section.

As reported in the following section the coefficients k_1, k_2, and s have been determined for three proton species in X-537A. Next we summarize the theory needed to extract the desired kinetic information from NMR measurements while neglecting certain relatively small proton–proton interactions (Frankel, 1969; Johnson, 1965).

If k_{1m}^{-1} is T_1 for an X-537A proton in the actual MX^+ complex and k_d^{-1} is the mean lifetime of the complex then we have:

$$k_1^{-1} = k_{1m}^{-1} + k_d^{-1} \tag{2}$$

which was derived for a "two-state" model for the relaxing spins (Bloembergen and Morgan, 1961). The corresponding equations for the transverse magnetization and the molar shift are in general much more complicated. We may use the Swift–Connick equations (Swift and Connick, 1962), which are, in our notation:

$$k_2 = k_d\frac{k_{2m}(k_{2m} + k_d) + \Delta\omega_m^2}{(k_{2m} + k_d)^2 + \Delta\omega_m^2} \tag{3a}$$

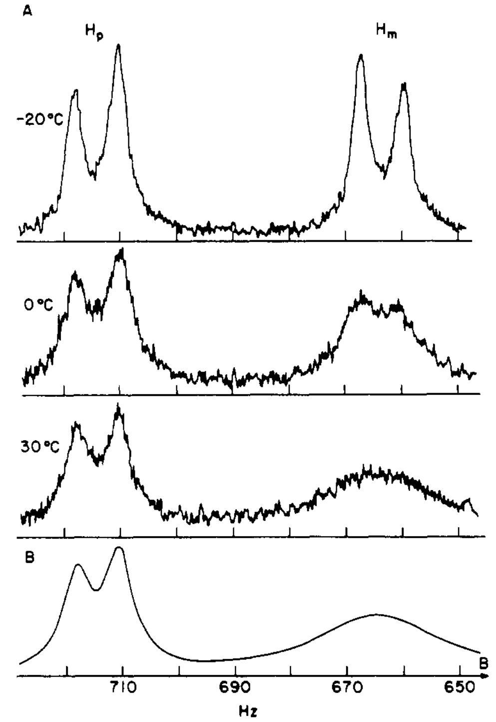

FIGURE 2: (A) Spectra of H_p and H_m in NiX^+ methanol solution at 100 MHz; solution composition: 4×10^{-2} *M* X-537A, 3.7×10^{-3} *M* $NiCl_2$, at pH 6.5; (B) simulated NiX^+ spectrum at 30°.

$$s = \frac{k_d^2 \Delta\omega_m}{(k_{2m} + k_d)^2 + \Delta\omega_m^2} \tag{3b}$$

where k_{2m} is T_2^{-1} for the proton species in the actual MX^+ complex, and $\Delta\omega_m$ is given by:

$$\Delta\omega_m = \omega(\text{in } MX^+) - \omega(\text{in uncomplexed X-537A}) \tag{4}$$

Equations 3a and 3b may be obtained by specializing the result of a more complete theory (Eisenstadt and Friedman, 1968) to the case in which:

$$k_d T_{1e} \ll 1 \tag{5}$$

where T_{1e} is the electron (electron spin resonance, ESR) T_1 in the MX^+ complex. It is fortunate that eq 5 apparently is satisfied in the present case because the theory which allows for $k_d T_{1e} \gtrsim 1$ has only been worked out for spin ½ paramagnetic ions.

Here we also note three limiting cases of eq 3a and 3b: fast exchange limit; $k_d \gg k_{2m}, |\Delta\omega_m|$:

$$k_2 = k_{2m} + k_d^{-1}\Delta\omega_m^2 \tag{6a}$$

$$s = \Delta\omega_m \tag{6b}$$

slow exchange, large shift limit; $|\Delta\omega_m| \gg k_{2m}, k_d$:

$$k_2 = k_d \tag{7a}$$

$$s = k_d^2/\Delta\omega_m \tag{7b}$$

slow exchange, large k_{2m} limit; $k_{2m} \gg k_d, |\Delta\omega_m|$:

$$k_2 = k_d \tag{8a}$$

$$s = k_d^2 \Delta\omega_m / k_{2m}^2 \tag{8b}$$

Finally, we remark that the "intrinsic" relaxation times k_{1m} and k_{2m} of the protons in the MX complex are given by the Solomon-Bloembergen equations (Solomon and Bloembergen, 1956):

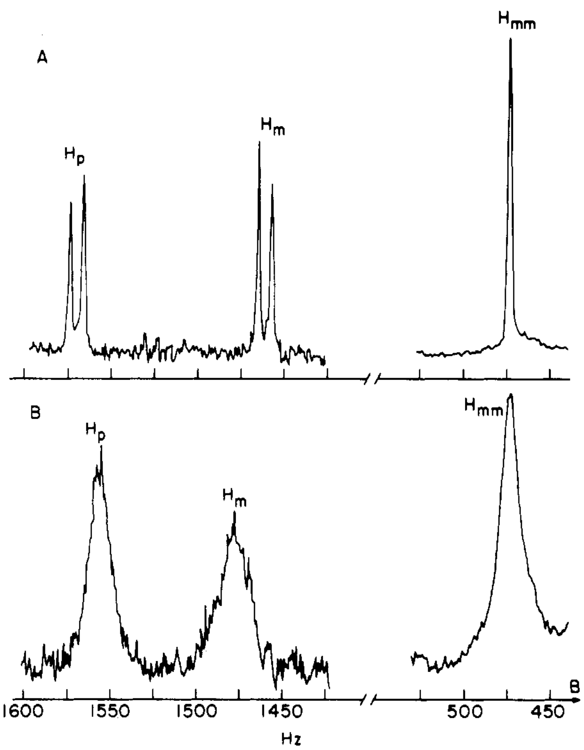

FIGURE 3: (A) NMR spectrum of the salicylic group protons (H_p, H_m, and H_{mm}) of X-537A; (B) of MnX^+ methanol solution, at 23°, at 220 MHz. Solution composition: (A) 2.1×10^{-2} *M* X-537A at pH 7.4; (B) the same as A + 4.2×10^{-4} *M* $MnCl_2$.

$$k_{1m} = \frac{2}{15} S(S+1) A_a^2 \times \left[\frac{\tau_a}{1 + (\omega_I - \omega_S)^2\tau_a^2} + \frac{3\tau_a}{1 + \omega_I^2\tau_a^2} + \frac{6\tau_a}{1 + (\omega_I - \omega_S)^2\tau_a^2}\right] + \frac{2}{3} S(S+1) A_i^2 \left(\frac{\tau_i}{1 + (\omega_I - \omega_S)^2\tau_i^2}\right) \tag{9a}$$

$$k_{2m} = \frac{1}{15} S(S+1) A_a^2 \times \left[4\tau_a + \frac{3\tau_a}{1 + \omega_I^2\tau_a^2} + \frac{\tau_a}{(\omega_I - \omega_S)^2\tau_a^2} + \frac{6\tau_a}{1 + \omega_S^2\tau_a^2} + \frac{6\tau_a}{1 + (\omega_I + \omega_S)^2\tau_a^2}\right] + \frac{1}{3} S(S+1) A_i^2 \left(\tau_i + \frac{\tau_i}{1 + \omega_S^2\tau_i^2}\right) \tag{9b}$$

where S is the magnitude of the electron spin in the complex ($S = 1$ for NiX^+, $S = 5/2$ for MnX^+), ω_I and ω_S are the precession frequencies of the protons and electrons, respectively, in MX^+, A_i is the magnitude of the isotropic (scalar) part of the proton-electron hyperfine interaction tensor, and A_a is the magnitude of the corresponding anisotropic (dipolar) part, and τ_i and τ_a are the corresponding correlation times. They are given by:

$$\tau_i^{-1} = k_d + T_{1e}^{-1} \tag{10a}$$

$$\tau_a^{-1} = \tau_i^{-1} + \tau_r^{-1} \tag{10b}$$

where τ_r is the correlation time for the rotation of the complex.

For a molecule as big as NiX^+ or MnX^+ one quite generally finds that τ_r is adequately given by the Debye equation:

Table II: Reduced Transverse Relaxation Rate Constants for H_m in Methanol Solutions Containing $NiCl_2$ at 25°.

$10^8(H^+)$ (M)	10^3(XH) (M)	$10^{-2}k_2$ (sec^{-1})
32	19	1.9 ± 0.4
32	36	2.5 ± 0.4
3.2	4.9	2.3 ± 0.4

Table III: Formation and Dissociation Rate Data (Eq 13a) for NiX^+ in Methanol at 25°.

Dissociation	k_d	$(2.4 \pm 0.4) \times 10^2$ sec^{-1}
	$\Delta H_d^\ddagger$	4.5 ± 1.5 kcal/mol
	$\Delta S_d^\ddagger$	−32 ± 6 cal/(mol deg)
Formation	k_f	$(2.2 \pm 0.4) \times 10^6$ sec^{-1} M^{-1}
	$\Delta H_f^\ddagger$	6.8 ± 1.5 kcal/mol
	$\Delta S_f^\ddagger$	−8 ± 7 cal/(mol deg)

$$\tau_r = (4\pi/3kT)r^3\eta \qquad (11a)$$

$$= 1.0(r/\text{Å})^3(\eta/\text{cP}) \text{ psec at } 25° \qquad (11b)$$

where r is the mean radius of the complex. It is estimated to be about 8 Å. The units in eq 11b signify that the equation gives τ_r in picoseconds (10^{-12} sec) if r is in angstroms and the viscosity η is in centipoise (for MeOH at 25°, $\eta = 0.55$ cP). Finally, we note that when the Swift-Connick equation is applicable, i.e. when eq 5 is satisfied, then we have (for the usual case $\hbar\omega_S \ll kT$) eq 12.

$$\Delta\omega_m = (1/3)S(S+1)(\hbar\omega_S/kT)A_i \qquad (12)$$

The frequencies ω_S, ω_I, and $\Delta\omega_m$, and the interactions A_i and A_a are all expressed in radians/second. Also, it must be remarked that ω_S and ω_I are proportional to the magnetic field B_0 which is 3 kG in usual (X-band) ESR experiments, 23.5 kG in 100-MHz ^{1}H NMR, and 52 kG in 220-MHz ^{1}H NMR. As a result k_{1m} and k_{2m}, as given by the Solomon-Bloembergen equations, are in general field dependent. Moreover, T_{1e}^{-1} is given by an equation similar to eq 9a so it too is in general field dependent.

We consider the following reactions as possibly determining the lifetime of the MX^+ complex in the solution:

$$MX^+ \xrightarrow{k_a} X^- + M^{2+} \qquad (13a)$$

$$MX^+ + H^+ \xrightarrow{k_b} XH + M^+ \qquad (13b)$$

$$MX^+ + X^- \xrightarrow{k_x} X^- + MX^+ \qquad (13c)$$

$$MX^+ + XH \xrightarrow{k_y} XH + MX^+ \qquad (13d)$$

Then the lifetime k_d^{-1} of the complex is given by the equation:

$$k_d = k_a + k_b(H^+) + k_x(X^-) + k_y(XH) \qquad (14)$$

The dominant contributions to k_d can be determined by studying the variation of k_d when the composition of the solution is changed.

General Features of the NMR Spectra of X-537A

The proton resonance spectra of XH, X^-, MnX^+, and NiX^+ in methanol are very complicated, but one can easily study the protons of the salicylate ring, namely the meta proton (H_m), the para proton (H_p), and the three equivalent meta methyl protons (H_{mm}) (Figure 1) which are shifted by the ring current to lower fields than the other protons. The H_{mm} line can be identified by its threefold greater amplitude than the other two.

The mutual interaction of H_m and H_p gives a splitting $J_{m-p} = 7.6 \pm 0.1$ Hz which is measurable in the spectra given in Figures 2 and 3. Even in the absence of Ni^{2+} and Mn^{2+} the shifts are pH dependent, apparently due to the shift in the $XH \rightarrow X^- + H^+$ equilibrium. Changes in pH do not broaden these lines measurably: the exchange of the carboxylic acid proton is fast on the ^{1}H NMR time scale so that the proton line width, whether for H_p, H_m, or H_{mm}, is the average of the natural widths for XH and X^- and the line center is at the mean frequency:

$$\bar{\omega} = \alpha\omega_{X^-} + (1-\alpha)\omega_{XH} \qquad (15)$$

where α is the fraction of XH ionized. Measurements in the absence of paramagnetic ions show that $T_{10} \gg T_{20}$ and that different protons have different T_{10} values. For H_p, H_m, and H_{mm} the rates $1/T_{10}$ were found to be much smaller than the experimental error of $1/T_1$ measured in the presence of Mn^{2+}. Therefore, they were neglected in calculating the k_1 coefficients. In the presence of $NiCl_2$ or $MnCl_2$ the gross features of the spectrum are little changed but there are obvious broadenings and shifts (Figures 2 and 3).

The NiX^+ Complex in Methanol

The ratio of stoichiometric concentrations $[Ni^{2+}]/[XH]$ was in the range 0.1 to 0.03. The electronic relaxation times T_{1e} and T_{2e} of $Ni(aq)^{2+}$ are 50 and 9 psec, respectively, at a field of 52 kG (Hwang, 1973). The fast electron relaxation is due to the very large zero-field splitting in Ni^{2+}. It is expected that the zero-field splitting (zfs) also is large in methanol solution and therefore that T_{1e} and T_{2e} are again very short, so it is assumed that eq 3a and 3b are applicable to the data of NiX^+ data in methanol at 23.5 and 52 kG, provided only that k_d^{-1} exceeds 100 psec.

Measurements at 100 and 220 MHz below 0° show no measurable field dependence of T_2; this requires that below 0° we are in one of the slow exchange limits described by eq 7a or 8a. This conclusion is consistent with data for the temperature dependence at 100 MHz (Figure 3). As we see, the lines broaden (k_2 increases) with increasing temperature as expected if the temperature dependence of the equilibrium constant is small (Table I), while k_d has the usual Arrhenius form with an appreciable activation energy. Studies at different pH values and XH concentrations are summarized in Table II. The results show that eq 13a governs the line width and other reactions can be neglected.

The resulting kinetic coefficients are given in Table III. At a field of 23.5 kG both H_p and H_{mm} reached the slow exchange limit below 0°, while H_m at the same field was found to be still in this limit up to 30° (Figure 2). Also, the measurements at 52 kG for the three lines were found to be in the slow exchange limit in the range from −10 to +23°. Together with the conditions for the fast exchange and slow exchange, these data enable us to calculate the following limits for A_i of each line: $20 < |A_i(H_p)| < 30$, $40 < |A_i(H_m)| < 50$, $50 < |A_i(H_{mm})|$.

The MnX^+ Complex in Methanol

In these experiments the ratio of stoichiometric concentrations $[Mn^{2+}]/[XH]$ was in the range 0.02 to 0.006. The 52-kG data for k_1, k_2, and s together with k_2 data at 23.5

Table IV: Reduced Relaxation Rate Constants and Molar Shifts for MnX^+ in Methanol.

Conditions							
Temp (°C)	B_0 (kG)	100(MnX^+)/[XH]	pH[a]	Coefficients	H_p	H_m	H_{mm}
23	52	1.5	7.0	k_1, msec^{-1}	0.7 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
23	52	1.7	6.3	k_2, msec^{-1}			1.3
23	52	2.0	7.4	k_2, msec^{-1}	1.2	2.2	1.5
23	52	3.3	7.0	k_2, msec^{-1}	1.2	2.6	1.3
23	52	3.8	7.75	k_2, msec^{-1}	1.7	2.8	1.5
−40	52	2.0	7.4	k_2, msec^{-1}	3.3	5.1	4.6
23	23.5	2.0	7.4	k_2, msec^{-1}	1.4	2.4	1.4
23	52	2.0	7.4	s, rad/msec	−3.9	+5.6	−1.3[b]
−40	52	2.0	7.4	s, rad/msec			−1.0
				A_i, rad/msec	57	−82	+19
23	52			$bT_{1e} + k_d^{-1}$, μsec	35 ± 25	40 ± 15	100 ± 100
				$\frac{k_2\ (23.5\ \text{kG})^c}{k_2\ (52\ \text{kG})}$	0.75	0.60	0.90

[a] At 25°. [b] Estimated from results at −40°. [c] Calculated as described in the text.

kG are given in Table IV.

To find whether eq 5 applies to this system reference may be made to the $Mn(aq)^{2+}$ octahedral complex. It is generally agreed that the dominant relaxation mechanism in this case also depends on the zfs which comes from fluctuating distortions of the $Mn(H_2O)_6{}^{2+}$ from octahedral symmetry with a correlation time $\tau_\nu = 1.9$ psec at 30° (Bloembergen and Morgan, 1961). At a field of about 3 kG one has $T_{2e} \simeq 3 \times 10^{-9}$ sec (Bloembergen and Morgan, 1961), from which the relevant theory (Rubinstein et al., 1971; Luckhurst, 1972; Hwang, 1973) enables one to estimate $T_{2e} \simeq 10^{-8}$ sec and $T_{1e} \simeq 3 \times 10^{-8}$ sec at 52 kG. For the MnX^+ complex in methanol, the effect upon the relaxation is expected to be larger than in $Mn(aq)^{2+}$ owing to the lower symmetry. Similar larger effects were reported for other Mn^{2+} complexes with lower symmetry (Levanon and Luz, 1968; Reed et al., 1971; Grant et al., 1971). In addition, modulation of the zfs in MnX^+ is no doubt mainly due to the rotational diffusion of the complex with correlation time, $\tau_r \sim 280$ psec (see below), which is much longer than τ_ν. Thus, it seems safe to assume that for MnX^+ in methanol at 52 kG one has:

$$T_{1e} \leq 3 \times 10^{-8}\ \text{sec} \tag{16}$$

since even for $Mn(aq)^{2+}$ this condition is met. Consistent with eq 16 is the report (Hamilton et al., 1974) that the electron paramagnetic resonance (EPR) spectrum of MnX^+ in methanol cannot be observed. The results to be described provide additional confirmation.

The rate constant for association of Mn^{2+} and X^- in methanol at 25° is not more than $3 \times 10^{10}\ M^{-1}\ \text{sec}^{-1}$, the estimate for diffusion-controlled association (Diebler et al., 1969). Thus, in view of the pK value in Table I, one has:

$$k_d < 1 \times 10^6\ \text{sec}^{-1},\ 25° \tag{17}$$

and eq 5 is satisfied at 25° and 52 kG and at lower temperatures and fields.

It is concluded that the Swift-Connick equations apply. The next step is to see whether any of the limiting forms (eq 6–8) apply.

In the case that $k_{1m} \gg k_d$, eq 2 reduces to $k_1 = k_d$ and, since $k_{2m} \geq k_{1m}$, one also has $k_{2m} \gg k_d$ and either eq 7 or 8 applies. Then $k_2 = k_d$ would be the same for all three proton species. The data in Table IV are not consistent with this hypothesis. The k_2 values depend on proton species and differ from the k_1 values. Furthermore, the observed negative temperature dependence of k_2 of a given proton species is contrary to what one expects for k_d.

In the case that $k_{1m} \ll k_d$, eq 2 reduces to:

$$k_1 = k_{1m} \tag{18}$$

and, if eq 6 applies, then $k_1 \leq k_2$. Moreover, the temperature coefficients of k_1 and k_2 are now characteristic of magnetic relaxation processes rather than chemical processes and need not be given by an Arrhenius equation with positive activation energy. So this case seems consistent with the data in Table IV and will be further explored. The first step is to specialize the Solomon-Bloembergen equations to this case.

Estimating 8 Å as the radius of MnX^+ one has, from eq 11:

$$\tau_r = 280\ \text{psec} \tag{19}$$

and in this case $\tau_a \simeq \tau_r$; also one has $k_d \leq 1 \times 10^6\ \text{sec}^{-1} < 1 \times 10^7\ \text{sec}^{-1} \leq T_{1e}{}^{-1}$, and then $\tau_i \simeq T_{1e}$. At $B_0 = 52$ kG one also has: $\omega_S = 9.1 \times 10^{11}$ rad/sec; $\omega_I = 1.4 \times 10^9$ rad/sec; $\omega_I\tau_r = 0.39$; $\omega_S T_{1e} < 10^5$; $T_{1e}/(1 + \omega_S{}^2 T_{1e}{}^2) \leq 5 \times 10^{-13}$ sec. Now eq 6 and 9 may be combined to get:

$$k_1 = (2/5)S(S + 1)A_a{}^2\,[\tau_r/(1 + \omega_I{}^2\tau_r{}^2)] \tag{20}$$

$$k_2 = 1.27k_1 + s^2(bT_{1e} + k_d{}^{-1}) \tag{21}$$

$$b \equiv (1/3)S(S + 1)(A_i{}^2/s^2) = 600 \tag{22}$$

So for this case we may use these equations to calculate $bT_{1e} + k_d{}^{-1}$ from the data for each proton species, with the results given in Table IV. Since k_2 for H_{mm} is mostly determined by the k_1 term, the error in the calculation of $bT_{1e} + k_d{}^{-1}$ is very large. Based upon the data for H_p and H_m it can be concluded that at $B_0 = 52$ kG one has:

$$600T_{1e} + k_d{}^{-1} = 40 \pm 30\ \mu\text{sec} \tag{23}$$

This equation fixes an upper bound for $1/k_d$, i.e. for $T_{1e} = 0$. This bound together with eq 17 gives the result:

$$1.4 \times 10^4\ \text{sec}^{-1} \leq k_d \leq 1 \times 10^6\ \text{sec}^{-1} \tag{24}$$

Equations 23 and 24 also determine bounds for T_{1e} at 52 kG, namely:

$$1.6 \times 10^{-8}\ \text{sec} < T_{1e} < 2.5 \times 10^{-7}\ \text{sec} \qquad (25)$$

which is consistent with eq 17.

According to a more detailed analysis of the theory, if $(k_1/s)^2$ were not small compared to the fractional experimental uncertainties in k_1, k_2, and s for each proton species, one could determine both k_d and T_{1e} from the trend in values of $bT_{1e} + k_d^{-1}$ in Table IV. However, since, in fact, the trend is nil within experimental error, the data only serve to give the combination in eq 23.

Some confirmation of these results is provided by less complete data at $B_0 = 23.5$ kG. Considering the right side of eq 21 term by term, in passing from 52 to 23.5 kG, the k_1 term is expected to increase by 10% and the $s^2k_d^{-1}$ term is expected to decrease by a factor of 4.84. The remaining term is more complicated. It is:

$$s^2bT_{1e} \propto \left(\frac{\tau_\nu}{1 + \omega_S^2\tau_\nu^2} + \frac{4\tau_\nu}{1 + 4\omega_S^2\tau_\nu^2}\right)^{-1} \equiv C_e \qquad (26)$$

where the theory (Bloembergen and Morgan, 1961) for T_{1e} has been introduced with the suppression of various field-independent factors. Similar results come from the more recent theories of T_{1e} referred to above. For $\omega_S\tau_\nu \ll 1$, we have $C_e(52\ \text{kG}) = C_e(23.5\ \text{kG})$, while for $\omega_S\tau_\nu \gg 1$, $C_e(52\ \text{kG})/C_e(23.5\ \text{kG}) = 4.84$. Assuming the latter case, which would give the greatest dependence of k_2 upon the field, we obtain the calculated ratios $k_2(23.5\ \text{kG})/k_2(52\ \text{kG})$, given in the bottom row of Table IV. The ratios indicate that within the experimental error we cannot distinguish conclusively between the two cases.

Discussion

The rate constants found for the formation and dissociation of NiX^+ are two to four orders of magnitude smaller than for MnX^+. Usually rates of solvent exchange on Ni^{2+} are much slower than on Mn^{2+} (Swift and Connick, 1972; Luz and Meiboom, 1964; Levanon and Luz, 1968; Pearson and Lanier, 1964; Pearson et al., 1960). Rates of substitution of solvent on Ni^{2+} and Mn^{2+} by other ligands differ in the same order (Melson and Wilkins, 1963; Holyer et al., 1965, 1966). This is in agreement with the crystal-field theory applied to $\text{d}^5(\text{Mn}^{2+})$ and $\text{d}^8(\text{Ni}^{2+})$ weak field systems (Basolo and Pearson, 1967).

It is sometimes possible to suggest a mechanism for a ligand replacement reaction by analyzing the overall formation and dissociation rate parameters. (For examples for octahedral Ni^{2+} complexes, see: Wilkins, 1970; Kustin and Swinehart, 1970; Coetzee and Hsu, 1975.) The similarity in the rate constants and in the enthalpy and entropy of activation ($\Delta H_f^\ddagger = 9$-12 kcal/mol and $\Delta S_f^\ddagger \simeq 0$ cal/(mol deg)) for the formation of Ni^{2+} complexes in water by a variety of ligands of the same charge tends to discount an associative (SN2) mechanism and supports a dissociative one (SN1). An interchange dissociative mechanism (I_d) in which the rate-determining step is the interchange of ligand and solvent in an initially outer sphere complex resulting in an inner sphere complex is often accepted as a mechanism for ligand substitution reactions for Ni^{2+} octahedral complexes (Eigen, 1960, 1962; Eigen and Tamm, 1962). Calculations of formation rate constants based on this mechanism, on the Fuoss equation for outer sphere ion pair formation constants (Fuoss, 1958), and the experimental water exchange rate constant (Swift and Connick, 1962) gave usually satisfactory agreement with the measured formation rates. A similar calculation for the NiX^+ formation rate constant using the known methanol exchange rate for Ni^{2+} ($1 \times 10^3\ \text{sec}^{-1}$; Luz and Meiboom, 1964) and the Fuoss equation for ion pair formation in methanol is easily made; it leads to a calculated rate constant that is about two orders of magnitude smaller than the measured one. This striking disagreement may indicate that the I_d mechanism does not apply to the Ni^{2+}-X^- association but it may equally likely indicate that the Fuoss equation is not adequate for estimating the formation constant of the outer sphere complex of Ni^{2+} with a very large and complicated structure such as X^- (Evans and Matesich, 1973); therefore, we cannot suggest a definite mechanism for the interaction between solvated ions and X-537A. The relaxation studies of complex formation between monovalent cations and valinomycin (Funck et al., 1972) and dibenzo-30-crown 10 (Chock, 1972) in methanol indicated that a slow conformational change occurs in the ionophore as it replaces solvent molecules on the cation. For valinomycin the rate-limiting step is believed to be a conformational change in the cation-valinomycin ion pair, while a fast conformational change is suggested for the free crown ether, prior to the final step of complex formation. In the case of X-537A and its complexes, it has been already shown in an earlier study (Degani and Friedman, 1974) that in solution there are at least three different conformational tautomers in equilibrium. The large difference between the formation rate constants of NiX^+ and MnX^+ rules out the possibility of a rate-limiting conformational change of X^- prior to the complexation process, but leaves undecided the possibility for a conformational change in a transient complex to be the rate-limiting step.

As mentioned in the theory section, we have assumed that $K_2 = K_1 = K_c$. K_c for the formation of the NiX^+ and MnX^+ complexes in methanol is the average value for the formation of all the three tautomers present. It is possible that different tautomers have different formation constants, and one of them with a formation constant different than the average K_c causes the broadening of the proton resonance lines. If the exchange rate between the different tautomers is faster than the dissociation rate k_d, then our assumption is still valid. If, on the other hand, the exchange rate between the tautomers is slower than k_d, this assumption may increase the uncertainty of our kinetic results. However, the large ratio between the formation rate constant for MnX^+ and NiX^+ would be only slightly affected, since the average K_c and the concentration ratios between the tautomers are very similar for both complexes. Therefore, the kinetic data reported here can be used in membrane transport studies with X-537A and Ni^{2+} and Mn^{2+} to determine whether the process is kinetically controlled by a reaction like 13a, or by diffusion of the free or ion-bound ionophore in the membrane.

Acknowledgment

We thank Dr. J. Berger of Hoffmann-La Roche for supplying the X-537A and Professor S. R. Simon for advice and helpful discussions. We are grateful to Mr. Peter Ziegler at Rockefeller University for help with the NMR measurements.

References

Basolo, F., and Pearson, R. G. (1967), in Mechanisms of Inorganic Reactions, New York, N.Y., Wiley, pp 145-158.

Bloembergen, N., and Morgan, L. O. (1961), *J. Chem. Phys. 34,* 842.
Chock, P. B. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1939.
Coetzee, J. F., and Hsu, E. (1975), *J. Solution Chem. 4,* 45.
Degani, H., and Friedman, H. L. (1974), *Biochemistry 13,* 5022.
Diebler, H., Eigen, M., Ilgenfritz, G., Maas, G., and Winkler, R. (1969), *Pure Appl. Chem. 20,* 93.
Eigen, M. (1960), *Z. Elektrochem. 64,* 115.
Eigen, M. (1962), in Advances in the Chemistry of the Coordination Compounds, Kirshner, S., Ed., New York, N.Y., MacMillan, p 371.
Eigen, M., and Tamm, K. (1962), *Z. Elektrochem. 66,* 93, 107.
Eisenstadt, M., and Friedman, H. L. (1968), *J. Chem. Phys. 48,* 4445.
Evans, D. F., and Matesich, M. A. (1973), *J. Solution Chem. 2,* 193.
Frankel, L. S. (1969), *J. Mol. Spectrosc. 29,* 273.
Funck, Th., Eggers, F., and Gress, E. (1972), *Chimica 26,* 637.
Fuoss, R. M. (1958), *J. Am. Chem. Soc. 80,* 5059.
Grant, M., Dodgen, H. W., and Hunt, J. P. (1971), *Inorg. Chem. 10,* 71.
Hamilton, R., Degani, H., and Friedman, H. L. (1974), unpublished work.
Holyer, R. H., Hubbard, C. D., Kettle, S. F. A., and Wilkins, R. G. (1965), *Inorg. Chem. 4,* 929.
Holyer, R. H., Hubbard, C. D., Kettle, S. F. A., and Wilkins, R. G. (1966), *Inorg. Chem. 5,* 622.
Hwang, L. P. (1973), Ph.D. Dissertation, State University of New York at Stony Brook.
Johnson, C. S. (1965), *Adv. Magn. Reson. 1,* 37.
Kita, H., and Van der Kloot, W. (1974), *Nature* (*London*) *250,* 558.
Kustin, K., and Swinehart, J. (1970), *Prog. Inorg. Chem. 13,* 107.
Levanon, H., and Luz, Z. (1968), *J. Chem. Phys. 49,* 2031.
Luckhurst, G. R. (1972), in Spin Relaxation in Liquids, Muss, L. T., and Atkins, P. W., Ed., New York, N.Y., Plenum Press, p 313.
Luz, Z. (1969), *J. Chem. Phys. 51,* 1206.
Luz, Z., and Meiboom, S. (1964), *J. Chem. Phys. 40,* 2686.
Melson, G. A., and Wilkins, R. G. (1963), *J. Chem. Soc.,* 2662.
Pearson, R. G., and Lanier, R. D. (1964), *J. Am. Chem. Soc. 86,* 765.
Pearson, R. G., Palmer, J., Anderson, M., and Allred, A. L. (1960), *Z. Elektrochem. 64,* 110.
Reed, G. H., Leigh, J. S., Jr., and Pearson, J. E. (1971), *J. Chem. Phys. 55,* 3311.
Rubinstein, M., Baram, A., and Luz, Z. (1971), *Mol. Phys. 20,* 67.
Solomon, I., and Bloembergen, N. (1956), *J. Chem. Phys. 25,* 261.
Swift, T. J., and Connick, R. E. (1962), *J. Chem. Phys. 37,* 307.
Wilkins, R. G. (1970), *Acc. Chem. Res. 3,* 408.

Relation between Redox Potentials and Rate Constants in Reactions Coupled with the System Oxygen–Superoxide[†]

Yukiharu Sawada, Takashi Iyanagi,[‡] and Isao Yamazaki*

ABSTRACT: Univalent oxidation–reduction reactions coupled with the oxygen-superoxide system were investigated in the reactions shown in eq 3 and 8, where Q and $Q\cdot^-$ stand for *p*-benzoquinone and *p*-benzosemiquinone, respectively. From kinetic experiments the following rate constants were obtained at pH 7.0: $k_3 = 4.5 \times 10^4\ M^{-1}\ \mathrm{sec}^{-1}$ and $k_8 = 3 \times 10^{-2}\ M^{-1}\ \mathrm{sec}^{-1}$. With known values of k_{-3} and k_{-8}, and of E_0' for the systems Q-$Q\cdot^-$ (0.10 V) and Cyt c^{3+}-Cyt c^{2+} (0.255 V), the calculated values of $E_0(O_2\text{-}O_2\cdot^-)$ were found to lie in the range between −0.27 and −0.33 V.

The formation of superoxide radical was suggested in reactions of xanthine oxidase (Fridovich and Handler, 1958) and of peroxidase-oxidase (Yamazaki and Piette, 1963). Accumulation of the radical during the above reactions has been confirmed by means of electron spin resonance (ESR) spectroscopy (Knowles et al., 1969; Nilsson et al., 1969). Since the previously known copper proteins, erythrocuprein and hemocuprein, were found to have a catalytic function accelerating dismutation of superoxide anion radicals (McCord and Fridovich, 1969) a large number of papers have been devoted to the study of the formation and reactivity of the superoxide radical. Stepwise oxidation, as formulated by Michaelis (1951), becomes of fundamental significance in the kinetic analysis of reactions involving the superoxide radical. Experimental evidence which shows the relation between one-electron redox potentials in a bivalent system and rate constants has been reported by Yamazaki and Ohnishi (1966).

A reliable value for the O_2-$O_2\cdot^-$ redox potential is needed in order to analyze reactions coupled with its redox system. By thermodynamic calculation the redox potential was reported as being −0.56 V by Latimer (1952). The similar-

---

[†] From the Biophysics Division, Research Institute of Applied Electricity, Hokkaido University, Sapporo 060, Japan. *Received March 18, 1975.*

[‡] Present address: The School of Medicine, Tsukuba University, Ibaragi 300-31, Japan.