

Cadmium-113 Nuclear Magnetic Resonance Studies of Cadmium–Ethylenediaminetetraacetic Acid Complexes

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Received September 8, 1980

Abstract: The pH dependences of the ¹¹³Cd and ¹³C NMR parameters for cadmium–ethylenediaminetetraacetic acid (Cd–EDTA) complexes have been investigated. The variation of the ¹¹³Cd chemical shifts for Cd–EDTA as a function of pH, over the range pH 3–14, is interpreted in terms of an acid/base equilibrium with an associated pK_a of approximately 12.5. The origin of this ionization was determined by examining the field dependence (2.3, 4.7, and 9.4 T) of the ¹¹³Cd spin–lattice relaxation time, T₁, and its associated ¹³Cd–{¹H} nuclear Overhauser effect, NOE = 1 + η, at selected pH values. These data strongly support the existence of hydrated form(s) of the Cd(EDTA)²⁻ complex within the pH range of pH 4–11. Further, combining these parameters with the field dependence of the ¹¹³Cd and ¹³C line widths, these data are indicative of the existence of complex equilibria involving different hydroxyl forms of Cd(EDTA)²⁻, i.e., Cd(EDTA)(OH)_n⁽²⁺ⁿ⁾⁻, at pH values in excess of pH 11. By properly accounting for the frequency dependence of η_{max}, it is shown that the ¹¹³Cd relaxation data of the low pH form(s) can be accounted for by a dominance of dipolar processes at 2.3 T and by the mechanism of chemical shift anisotropy at 9.4 T. Further, it has been demonstrated that solvent water protons make a significant contribution to the dipolar mechanism. Finally, it is argued that T₁(¹¹³Cd) for the high pH form(s) is dominated by chemical exchange processes.

Introduction

Since the early applications of Fourier transform methods to the direct observation of high-resolution ¹¹³Cd NMR spectra,² there has been a significant interest in the use of this nuclide as a probe to study the metal binding sites in a variety of metalloproteins³ and metal-dependent proteins.⁴ From these and other studies⁵ it is known that cadmium in these systems is coordinated to a combination of nitrogen-, oxygen-, and sometimes sulfur-containing ligands. In order to properly interpret the ¹¹³Cd NMR parameters of these biological systems, it is imperative to investigate the corresponding parameters of appropriate model systems in both the liquid and solid phase. An excellent ligand for the binding of Cd(II) is ethylenediaminetetraacetic acid (H₄EDTA). Thus Cd–EDTA, which has a stability constant K = 10^{16.5,6} provides a convenient model system for the interactions of amino acids and proteins with heavy metal ions.

Parameters obtained from ¹H,⁷ ¹³C,⁸ ¹⁵N,⁸ and ²³Na NMR

spectra⁹ of EDTA and its metal complexes have been extensively examined and have contributed much to the information available about these complexes. These studies also include investigations on Cd–EDTA.^{7c,e,h,k,8} In addition the ¹¹³Cd chemical shift and line width for this complex (δ 85.2 ppm and Δν_{1/2} 60 Hz, pH 5) was included in a recent report^{5b} on ¹¹³Cd NMR of representative Cd(II) complexes.

The solution structure of bivalent metal complexes of EDTA has long been investigated using several physical methods. Although EDTA is a hexadentate ligand, the results of a number of these investigations conflict as to the number of EDTA coordination sites utilized in the binding of metal ions. Evidence for both a pentacoordinated structure, water presumably occupying the sixth site, and a hexacoordinated structure has been found. References to a number of these studies have been summarized in a recent ultrasonic absorption investigation on determination of rate constants for the equilibrium pentacoordinated structure ⇌ hexacoordinated structure in some Ca-, Sr-, Ba-, and Co-(II)–EDTA complexes.¹⁰

Sudmeier and Reilley^{7b} have postulated the existence of hydrated forms of Cd–EDTA in their ¹H NMR study of the rates and mechanisms of exchange reactions involving Cd–EDTA complexes. This postulate by necessity implies dynamic substitution of ligands at the metal center. Cadmium-113 NMR is ideally suited to observe these dynamics. We report here a detailed ¹¹³Cd NMR investigation of the pH dependence of chemical shifts, spin–lattice relaxation, and ¹¹³Cd–{¹H} nuclear Overhauser effects (NOE) for Cd–EDTA at three magnetic field strengths (2.3, 4.7, and 9.4 T corresponding to ¹¹³Cd NMR frequencies of 22.2, 44.4, and 88.8 MHz, respectively). These data, along with parameters from ¹³C NMR, provide new evidence for a pH dependence of the solution structure of the Cd–EDTA complex.

Experimental Section

Materials. For all in-house prepared H₂O solutions usage was made of triple-distilled water which had been extracted twice with a CCl₄ solution of dithizone (diphenylthiocarbazone), washed with CCl₄, and finally flushed with nitrogen for 14 days. The solutions of the Cd–EDTA complexes (ca. 0.4 M) were prepared by addition of a 0.8 M solution of Na₂H₂EDTA (Titriplex III, analytical grade, Merck Darmstadt) in 25% tetramethylammonium hydroxide (H₂O), TMAOH (purum, Fluka AG), to 0.8 M solutions of Cd(NO₃)₂ in D₂O or H₂O. A slight excess (15%)

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of the solution of $\text{Na}_2\text{H}_2\text{EDTA}$ in TMAOH was used in order to dissolve the precipitate of $\text{Cd}(\text{OH})_2$ which forms initially in the mixing of the standard solutions. Complete homogeneous solutions were achieved after stirring for 0.5–2 h. The pH of the solutions was adjusted using either 65% HNO_3 (analytical grade, Merck Darmstadt) or 25% TMAOH and monitored by a Radiometer Model PHM62 digital pH meter before and after the NMR measurements. Solutions prepared in this way ensured that the NMR parameters, especially T_1 's and NOE's, could be reproduced within the experimental errors of these measurements (see below).

Perdeuterated ethylenediaminetetraacetate tetraanion, $\text{EDTA-}d_{12}^{4-}$, or $\text{H}_4\text{EDTA-}d_{12}$, was prepared using ethylene- d_4 glycol and acetic- d_3 acid as starting materials and with slight modifications of synthetic schemes outlined elsewhere.^{8,11} $\text{H}_4\text{EDTA-}d_{12}$ was converted to $\text{Na}_2\text{H}_2\text{EDTA-}d_{12}$ and lyophilized.

Spectra. ^{113}Cd NMR spectra were recorded at three different magnetic field strengths (2.3, 4.7, and 9.4 T) corresponding to ^{113}Cd resonance frequencies of 22.19, 44.42, and 88.76 MHz, respectively. ^{13}C NMR spectra were recorded at 25.16 MHz corresponding to a magnetic field strength of 2.3 T.

Spectra at 2.3 T were obtained on a Varian XL-100-15 spectrometer, extensively modified for multinuclear NMR operation, and at a temperature of 32 °C. A home-made multinuclear cross-coil probe, capable of accommodating up to 18-mm o.d. spinning tubes, was used with this system. All ^{113}Cd T_1 measurements were performed using special thin-walled 18-mm tubes (Wilmad Glass Co.) which required 5 mL of solution. The amplification scheme of the transmitter pulses for this probe consisted of home-made 50–100-W tuned class C amplifiers followed by a broadband 2-kW Heathkit SB-220 tunable amplifier. This scheme resulted in a pulse width of 20 μs for a ^{113}Cd 90° flip angle using 18-mm tubes. Gated ^1H decoupling experiments were performed for the ^{113}Cd – ^1H NOE measurements (with a recycle time of $10T_1$) and for most of the $T_1(^{113}\text{Cd})$ measurements using the Varian spin decoupler with 1 W of decoupling power and 100-Hz square wave modulation. The low decoupling power ensured that no sample heating occurred for any decoupling sequence. Because of (1) the long $T_1(^{113}\text{Cd})$ values at 2.3 T and (2) the negative value for $\gamma_{^{113}\text{Cd}}$ and resulting partial negative NOE observed for several of the samples, most of the $T_1(^{113}\text{Cd})$ measurements were performed using the fast inversion–recovery FT (FIRFT) method¹² (recycle time of 2–3 T_1) and with the decoupler on only during acquisition of data. A set of 13 τ values was used for each T_1 determination and automatically stored on a magnetic tape cassette using a Sykes Compu/Corder 120. All T_1 values were determined from least-squares nonlinear analyses on a CDC 6400 computer system at the University of Aarhus. Based on these fittings of experimental data, the errors in T_1 are estimated to be within 5%.

^{113}Cd NMR spectra at 44.42 MHz (4.7 T) and 88.76 MHz (9.4 T) were obtained on Bruker WP-200 and WH-400 superconducting spectrometers,¹³ respectively, at a temperature of 28 °C and using 10-mm o.d. sample tubes. $T_1(^{113}\text{Cd})$ measurements at these frequencies were performed using the same gated ^1H decoupling sequence as described above, with low decoupling power, and employing the IRFT and/or FIRFT¹² methods (13 τ values). Analysis of the T_1 data followed the above procedure and gave approximately the same errors (5%) in T_1 .

Results and Discussion

^{13}C NMR Spectra. Because of our interest in understanding the origin of ^{113}Cd relaxation in Cd–EDTA (vide infra), we first examined the ^{13}C NMR parameters of this complex. The $T_1(^{13}\text{C})$ data would be utilized as means for estimating the reorientational correlation time for the complex. The ^{13}C chemical shifts, spin-coupling constants (both to ^{111}Cd and ^{113}Cd), spin–lattice relaxation times $T_1(^{13}\text{C})$, and ^{13}C line widths for Cd–EDTA at two selected pH values, pH 6.55 and 13.75, are summarized in Table I.

As shown in Figure 1 the narrow line width for the carbonyl carbon, C3, of the low pH form (pH 6.55) allowed resolution of two sets of cadmium satellites (^{111}Cd and ^{113}Cd ; 12.86 and 12.34% natural abundance, respectively) for this carbon [$^3J_{\text{C3-}^{111}\text{Cd}} = 12.61$ Hz and $^3J_{\text{C3-}^{113}\text{Cd}} = 13.16$ Hz; $^3J_{\text{C3-}^{113}\text{Cd}}(\text{calcd}) = \gamma_{^{113}\text{Cd}}/\gamma_{^{111}\text{Cd}}$

Table I. ^{13}C Chemical Shifts,^a ^{13}C – $^{111}\text{Cd}/^{113}\text{Cd}$ Spin–Spin Coupling Constants,^b ^{13}C Spin–Lattice Relaxation Times,^c Rotational Correlation Times,^d and Line Widths^e for 0.4 M Cd–EDTA Complexes at pH 6.55 and 13.75

| | pH 6.55 | pH 13.75 |
|--|------------------------------|------------------------------|
| δ C1 ethylene | 52.98 (52.98) ^f | 52.72 (53.38) ^f |
| δ C2 acetate | 59.76 (59.38) ^f | 59.65 (60.32) ^f |
| δ C3 carbonyl | 179.13 (173.07) ^f | 178.88 (180.72) ^f |
| $^3J_{^{13}\text{C3-}^{111}\text{Cd}}$ | 12.61 | 10.51 ^g (10.27) |
| $^3J_{^{13}\text{C3-}^{113}\text{Cd}}$ | 13.16 | (10.75) |
| $^2J_{^{13}\text{C1-Cd}}$ | 4.75 ^h (4.64) | ... |
| | (4.86) | |
| $^2J_{^{13}\text{C2-Cd}}$ | 3.27 ^h (3.20) | ... |
| | (3.34) | |
| $T_1(^{13}\text{C1})$ | 0.196 | 0.177 |
| $T_1(^{13}\text{C2})$ | 0.231 | 0.212 |
| $T_1(^{13}\text{C3})$ | 9.7 | 8.9 |
| τ_c | 1.1×10^{-10} | 1.2×10^{-10} |
| $\Delta\nu_{1/2}(^{13}\text{C1})$ | 2.83 | 3.68 |
| $\Delta\nu_{1/2}(^{13}\text{C2})$ | 2.46 | 3.06 |
| $\Delta\nu_{1/2}(^{13}\text{C3})$ | 0.29 | 0.47 |

^a In ppm (± 0.05 ppm) relative to an external 5-mm o.d. sample of neat TMS placed inside the 12-mm tubes. ^b In Hz (± 0.03 Hz). Signs of the coupling constants were not determined. ^c In seconds with errors less than 5%. ^d In seconds ($\pm 10\%$) calculated from the ^{13}C spin–lattice relaxation times for the ethylene carbons (C1) (see text). ^e Line widths observed for the unweighted FID's (no line broadening) and with no corrections for magnetic field inhomogeneity. ^f Values in parentheses are the ^{13}C chemical shifts for a small excess (10–15%) of free EDTA ligand. ^g Observed mean values for the couplings to ^{111}Cd and ^{113}Cd ; values in parentheses are the ^{13}C – ^{111}Cd and ^{13}C – ^{113}Cd coupling constants calculated from these mean values and $\gamma_{^{113}\text{Cd}}/\gamma_{^{111}\text{Cd}} = 1.046$.

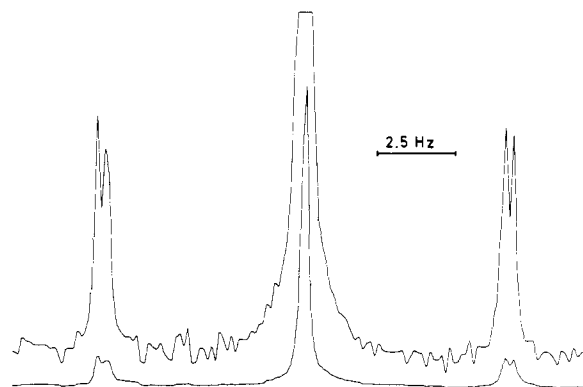


Figure 1. ^{13}C NMR spectrum for the carbonyl carbon of Cd–EDTA at pH 6.55. Satellites are due to ^{111}Cd and ^{113}Cd .

$\times ^3J_{\text{C3-}^{111}\text{Cd}}(\text{obsd}) = 13.19$ Hz]. This is in contrast to the observation made in an earlier ^{13}C NMR study ($^3J_{\text{C3-}^{111}\text{Cd}} = 12.7$ Hz).⁸ Furthermore, at this pH we were also able to observe mean values for the two-bond ^{13}C – $^{111}\text{Cd}/^{113}\text{Cd}$ coupling constants from the cadmium satellites for the two methylene carbon signals (ethylene, C1, and acetate, C2, carbons): $|^2J_{\text{C1-Cd}}| = 4.75$ Hz and $|^2J_{\text{C2-Cd}}| = 3.27$ Hz. The relative magnitudes of these long-range ^{13}C –Cd couplings are in accord with $|^3J_{\text{C-}^{111}\text{Cd}}| > |^2J_{\text{C-}^{113}\text{Cd}}|$ observed for organocadmium compounds.^{26,14} At pH 13.75, the presence of cadmium satellites surrounding the carbonyl carbon resonance indicates that at high pH the complexation between Cd(II) and EDTA ligand is still effective for the lifetime of cadmium in the complex to be long on the NMR timescale. However, at high pH ($\text{pH} > 11$) the satellite signals for ^{111}Cd and ^{113}Cd could not be separately resolved owing to the somewhat larger line width, and no satellites were observed for the methylene carbons for the same reason. Furthermore, a decrease is observed for the magnitude of the average three-bond ^{13}C – ^{111}Cd coupling constant with increasing pH; the average coupling $^3J_{\text{C3-Cd}} = 10.51$ Hz at pH 13.75. For the samples at both pH 6.55 and 13.75, separate ^{13}C

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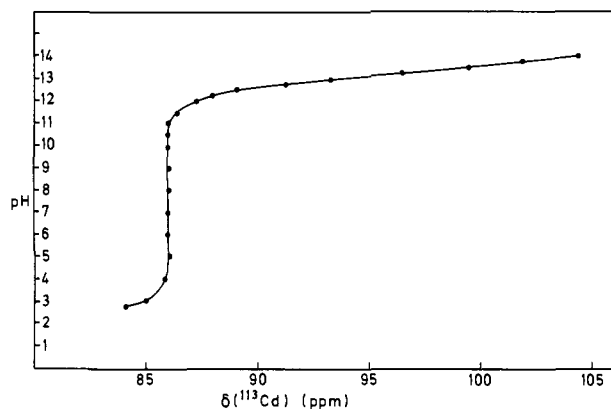


Figure 2. ^{113}Cd chemical shift (relative to external 0.1 M $\text{Cd}(\text{ClO}_4)_2$) as a function of pH for Cd-EDTA.

resonances were observed for the small excess of free EDTA ligand added to these samples (Table I).

The molecular rotational correlation times for the Cd-EDTA complexes at low and high pH values, necessary for the interpretation of the $T_1(^{113}\text{Cd})$ results, were derived from determinations of the ^{13}C spin-lattice relaxation times, $T_1(^{13}\text{C})$, and $^{13}\text{C}\{-^1\text{H}\}$ NOE's at 25.16 MHz. The NOE's for the ethylene and acetate methylene carbons (C1 and C2, respectively) at both pH 6.55 and 13.75 are all equal and maximum within the experimental error (5%), i.e., $1 + \eta = 2.99$. This shows that the $^{13}\text{C}\{-^1\text{H}\}$ dipole-dipole mechanism governs the relaxation for these carbons. However, we will have to use the more general equation for $(T_1^{\text{DD}})^{-1}$ (eq 1) for a calculation of the correlation times. Since isotropic motion is a good approximation for these ball-shaped molecules, a single correlation time has been employed in eq 1

$$\frac{1}{T_1^{\text{DD}}} = \frac{1}{5} \hbar^2 \gamma_X^2 \gamma_H^2 r_{\text{XH}}^{-6} \left[\frac{\tau_c}{1 + (\omega_H - \omega_X)^2 \tau_c^2} + \frac{3\tau_c}{1 + \omega_X^2 \tau_c^2} + \frac{6\tau_c}{1 + (\omega_H + \omega_X)^2 \tau_c^2} \right] \quad (1)$$

($X = ^{13}\text{C}$) where the γ 's and ω 's are expressed in angular units and all terms have their usual definitions. Further, eq 1 assumes that two protons are providing the relaxation path for the ^{13}C nucleus ($X = ^{13}\text{C}$).

The $T_1(^{13}\text{C})$ values (Table I) at low and high pH are almost identical, the values at pH 13.75 being slightly smaller than the corresponding values at pH 6.55. Furthermore, at both pH values the ^{13}C relaxation times for the ethylene carbons (C1) are smaller than for the acetate carbons (C2) by ca. 0.035 s. This most certainly reflects a higher degree of motional freedom for the more labile acetate groups as compared to the ethylene backbone carbons, a conclusion reached from the results of the ^{113}Cd NMR study (vide infra). For these reasons and since the Cd-N bonds in the five-membered ring involving the ethylenediamine bridge are nonlabile, the molecular rotational correlation times τ_c given in Table I for the Cd-EDTA complexes were calculated using $T_1(^{13}\text{C1})$ for the ethylene carbons and eq 1 assuming $r_{\text{CH}} = 1.08 \pm 0.005 \text{ \AA}$. The value of τ_c obtained from eq 1 at pH 6.55 is $(1.1 \pm 0.1) \times 10^{-10} \text{ s}$. This value of τ_c indicates that the Cd-EDTA anion is significantly immobilized by the solvent molecules. Further, this value of τ_c places the ^{13}C and ^{113}Cd NMR spectroscopy of Cd-EDTA near the intermediate correlation time region.

In general the ^{13}C NMR parameters for Cd-EDTA at pH 6.55 and 13.75 show only minor variations. These small changes do not justify any conclusion to be drawn as regards the differences in the solution structure of the complex at low and high pH. On the contrary, it is shown below that such evidences may be obtained from the ^{113}Cd NMR investigations.

^{113}Cd NMR Spectra. The ^{13}C NMR results indicate that changes of the $^{13}\text{C}\{-^{113}\text{Cd}\}$ coupling constants and ^{13}C line widths

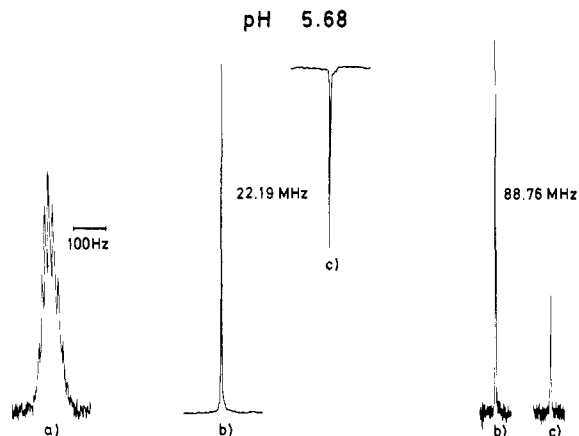


Figure 3. ^{113}Cd NMR spectra of Cd-EDTA at pH 5.68: (a) proton coupled; (b) proton-decoupled NOE suppressed at 22.19 and 88.76 MHz; (c) proton decoupled with NOE at 22.19 and 88.76 MHz.

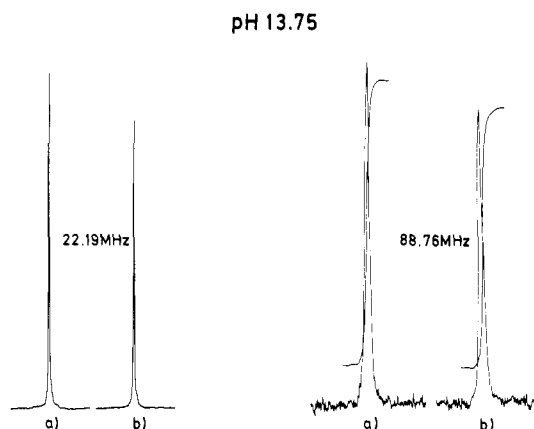


Figure 4. ^{113}Cd NMR spectra of Cd-EDTA at pH 13.75: (a) proton-decoupled NOE suppressed at 22.1 and 88.76 MHz; (b) proton decoupled with NOE at 22.19 and 88.76 MHz.

occur as a function of pH. Thus, in an attempt to understand the origin of this phenomenon and to properly interpret the data of the ^{113}Cd NMR experiments, it became necessary to investigate the ^{113}Cd NMR parameters for Cd-EDTA over a wide range of pH and as a function of magnetic field strength.

The pH dependence of the ^{113}Cd chemical shift for Cd-EDTA is outlined in Figure 2. Just as important, the line width of the ^{113}Cd resonance for these pH values fall in the range ca. 60–90 Hz and furthermore depends on the applied magnetic field strength. However, application of proton decoupling gives rise to a substantial decrease of the observed line width. Thus, for $\text{pH} < 11$ the ^{113}Cd line width in the presence of proton decoupling is 2–3 Hz for all magnetic fields utilized, whereas for pH 13.7 the width of the resonance increases from 5 Hz at 2.3 T to 20 Hz at 9.4 T. Examples of these observations are shown in Figures 3 and 4. In the low pH range the proton-coupled ^{113}Cd NMR spectrum (Figure 3a) appears as a fairly well-resolved first-order multiplet due to three-bond $^{113}\text{Cd}\{-^1\text{H}\}$ spin-spin coupling to the two sets of methylene protons, the average of the two coupling constants being $\frac{1}{2}[^3J_{^{113}\text{Cd}\{-^1\text{H}_1\}} + ^3J_{^{113}\text{Cd}\{-^1\text{H}_2\}}] = 12.1 \text{ Hz}$. The increased ^{113}Cd line width in the high pH range precluded resolution of the multiplet fine structure for the proton-coupled ^{113}Cd spectra at $\text{pH} > 12$. The consequences of proton decoupling on the ^{113}Cd resonance of Cd-EDTA are depicted in Figure 3 (pH 5.68, low pH form) and Figure 4 (pH 13.75, high pH form). Further, these figures also illustrate the effects of magnetic field strength (^{113}Cd resonance frequency) and pH on the $^{113}\text{Cd}\{-^1\text{H}\}$ NOE, numerical values of which are summarized in Table II. The magnetic field and pH dependences of the $^{113}\text{Cd}\{-^1\text{H}\}$ NOE's strongly suggest that the ^{113}Cd spin-lattice relaxation times, $T_1(^{113}\text{Cd})$, and/or relaxation mechanism(s) would also show a similar dependence.

Table II. ^{113}Cd Spin-Lattice Relaxation Times, T_1 , and ^{113}Cd - $\{^1\text{H}\}$ Nuclear Overhauser Effects, η , for Cd-EDTA and Cd-EDTA- d_{12} Complexes at Various Resonance Frequencies and pH Values

| pH | 2.3 T (22.19 MHz) | | 4.7 T (44.42 MHz) | 9.4 T (88.76 MHz) |
|-------|---------------------------|-------------------|----------------------|----------------------|
| | Cd-EDTA | Cd-EDTA- d_{12} | Cd-EDTA | Cd-EDTA |
| 5.68 | T_1 20.4 | | 15.4 | 8.0 |
| | η -1.56 | | -1.0 ^a | -0.63 |
| | η_{max} -2.25 | | -2.22 ± 0.02 | -2.14 ± 0.02 |
| | T_1^{DD} 29.4 | | 34.2 ^a | 27.2 |
| 6.53 | T_1 20.3 | 31.0 | | |
| | η -1.60 | -0.89 | | |
| | η_{max} -2.25 | | | |
| | T_1^{DD} 28.5 | 78.4 | | |
| 13.75 | T_1 1.55 | | 2.6 ^b | 1.66 |
| | η -0.12 | | -0.20 ^b | -0.12 |
| | η_{max} -2.25 | | -2.22 ± 0.02 | -2.12 ± 0.02 |
| | T_1 29.1 | | 28.9 ^b | 29.3 |

^a The observed signal was nearly nulled; hence an accurate determination of η and T_1^{DD} was precluded. ^b pH lower than 13.75 (not measured) because of prolonged standing of the sample (CO_2).

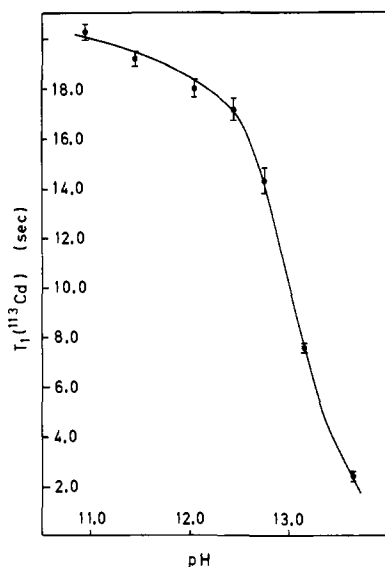
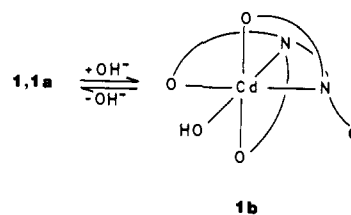


Figure 5. The pH dependence of the ^{113}Cd spin-lattice relaxation time for Cd-EDTA at 22.19 MHz within the range pH 11.0–13.8.

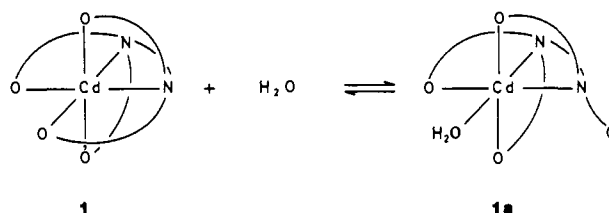
At a field strength of 2.3 T corresponding to a ^{113}Cd resonance frequency of 22.19 MHz, $T_1(^{113}\text{Cd})$ (20.4 s) is independent of pH in the range pH 4–11. However, at pH > 11 there is an abrupt decrease for $T_1(^{113}\text{Cd})$. The results of a detailed investigation of $T_1(^{113}\text{Cd})$ between pH 11 and 13.75 at 22.19 MHz are shown in Figure 5. The field dependences of $T_1(^{113}\text{Cd})$ for two representative pH values, pH 5.68 (low pH form) and pH 13.75 (high pH form), are summarized in Table II.

pH Dependence of the ^{113}Cd Chemical Shift. Figure 2 summarizes the pH dependence of the ^{113}Cd chemical shift of Cd-EDTA. Within the range of pH 4.5 to 11.0 the ^{113}Cd chemical shift is constant with a value of $\delta(^{113}\text{Cd}) = 85.2$ ppm. This value is identical with an earlier reported value obtained at pH 5.^{5b} At pH values in excess of pH 11 there is a dramatic change in the chemical shift toward lower shielding. Between pH 11.5 and 13.5 the ^{113}Cd chemical shift increases by 20 ppm, or $d\delta(^{113}\text{Cd})/d(\text{pH})$ is approximately 10 ppm/pH. The $\delta(^{113}\text{Cd})$ vs. pH dependences depicted in Figure 2 is reminiscent of a titration curve for a weak acid by a strong base. The apparent $\text{p}K_a$ of the weak acid is approximately equal to 12.5 (titration and T_1 experiments). Two potential candidates for weak acid forms of Cd-EDTA, Cd(EDTA)H⁻ and Cd(EDTA)H₂, for which the $\text{p}K_a$'s have previ-

Scheme I



ously^{7h} been determined to be 2.8 and 1.8, respectively, account for the curvature (Figure 2) for pH < 4. In this region only a few measurements could be performed owing to protonation followed by precipitation of the complex. Ionization of water by itself cannot cause the dramatic change observed in the ^{113}Cd chemical shift for pH > 11. Hence, the pH dependence of the ^{113}Cd chemical shift shown in Figure 2 strongly suggests that the solution structure of Cd-EDTA involves a dynamic hydrated form, i.e., **1a**, and that its actual identity depends upon the pH of the solution. The maximum concentration of **1a** occurs at pH ~ 7.



Species **1a** has been postulated earlier by Sudmeier and Reilley^{7h} in their ^1H NMR study of Cd-EDTA. Furthermore, Harada et al.,¹⁰ using ultrasonic absorption measurements, have recently examined the kinetics of reactions that lead to the interconversion of **1** and **1a** for the analogous Ca-, Sr-, Ba-, and Co(II)-EDTA complexes. Thus, the existence of **1a** is reasonably well established. Further, nucleophilic substitution reactions of **1** or **1a** with OH^- and/or ionization of **1a** induced by OH^- could also be envisioned; representative examples of the several possibilities are summarized in Scheme I. Species **1a** can be converted to **1b** by direct reaction with OH^- and displacement of H_2O or by ionization of the bound water molecule. Intramolecular proton transfer from the metal bound water to the "free" carboxylate anion followed by ionization of the carboxylic acid group back to the anion is thought to be improbable because of the stability of the anion. Further substitution reactions of **1b** with H_2O and/or OH^- may also be envisioned.

Such a scheme of rapidly exchanging species could account for the observed pH dependence of the ^{113}Cd chemical shift for Cd-EDTA. It is also consistent with the dynamic solution structure of Cd-EDTA as a function of the solution pH. The above scheme suggests several spin-lattice relaxation experiments which could be employed to test its validity. If species **1a** has a lifetime which is long compared to its reorientational correlation time and further the ^{113}Cd nuclide has a measurable dipolar contribution to the relaxation rate, one should observe a T_1^{DD} contribution arising from the protons of the water bound within the primary solvation sphere of cadmium. If a nonnegligible contribution can be found, it would represent an excellent experimental verification of the existence of species **1a**. The building up of species such as **1b** at higher pH would suggest that $T_1(^{113}\text{Cd})$ should also be pH dependent. Further, if the more common relaxation mechanism (dipole-dipole and chemical shift anisotropy) prevail at high pH values, one would expect the value of $T_1(^{113}\text{Cd})$ to decrease. This arises because of the highly charged nature of, e.g., **1b**, leading to a significant decrease in the mobilization of the anion as a whole.

^{113}Cd Spin-Lattice Relaxation. Within the pH range of pH 4–11, for which the ^{113}Cd chemical shift is independent of pH, the ^{113}Cd spin-lattice relaxation time for Cd-EDTA is constant with a value of 20.4 s at 22.19 MHz (Table II). However, at this same resonance frequency, but in the pH range of pH 11–13.8, $T_1(^{113}\text{Cd})$ abruptly decreases from 20.4 to 1.6 s. The change in

$T_1(^{113}\text{Cd})$ within this pH range is depicted in Figure 5. This dramatic decrease in $T_1(^{113}\text{Cd})$ occurs in exactly the same pH range as the large change observed for the ¹¹³Cd chemical shift (Figure 2).

The behavior of $\eta(^{113}\text{Cd}-\{^1\text{H}\})$ ($\text{NOE} = 1 + \eta$) over the entire pH range, pH 4–13.8, parallels that of $T_1(^{113}\text{Cd})$. That is, within the range pH 4–11 it is essentially constant, $\eta = -1.6$ at 22.19 MHz, whereas it quickly goes to a value of $\eta = -0.1$ between pH 11 and 13.8 (Table II and Figures 3 and 4).

These data are consistent with the dynamic nature of the solution structure of the Cd-EDTA complex. Further, they suggest the existence of different structures for the Cd-EDTA complexes at low and high pH values. Finally, the $T_1(^{113}\text{Cd})$ and $\eta(^{113}\text{Cd}-\{^1\text{H}\})$ data also clearly show that dipolar relaxation processes are important for the low pH form of Cd-EDTA, at least at 22.19 MHz, but apparently unimportant at high pH, e.g., pH 13.75. Hence it is obvious that a determination of the field dependence of $T_1(^{113}\text{Cd})$ at selected values of pH is necessary in order to understand these relaxation behaviors. Since it appears that there are at least two different forms of Cd-EDTA depending on the solution pH, the field dependence studies of these forms will be treated separately.

¹¹³Cd Relaxation of the Low pH Form(s). From the $\delta(^{113}\text{Cd})$ vs. pH dependence in Figure 2 and from Scheme I, it is apparent that species **1** and/or **1a** may be best examined in the low pH range, i.e., at pH 4–11. Hence, in order to obtain indirect evidence for the existence of species **1a**, the ¹¹³Cd spin-lattice relaxation time and associated NOE for the Cd-EDTA complex were determined at the two pH values, pH 5.68 and 6.53, and for the deuterated species Cd-EDTA-*d*₁₂ at pH 6.53. The solution of the isotopically normal Cd-EDTA at pH 5.68 was prepared using commercial Na₂H₂EDTA (Titriplex III) as described in the Experimental Section. However, the isotopically normal Cd-EDTA solution at pH 6.53 was obtained using a lyophilized powder of Na₂H₂EDTA which was prepared in-house from H₄EDTA. The results, summarized in Table II, show that the values of $T_1(^{113}\text{Cd})$ and $\eta(^{113}\text{Cd}-\{^1\text{H}\})$ for both Cd-EDTA solutions are the same, and hence, the two methods of preparation yield the same set of relaxation data. This comparison is important since the Cd-EDTA-*d*₁₂ solutions were prepared by the latter method, whereas all other samples were prepared via the former procedure.

Since the value for η_{obsd} is nonzero, the value of T_1^{DD} can be calculated from eq 2. However, one must be exceptionally careful

$$T_1^{\text{DD}} = (\eta_{\text{max}} / \eta_{\text{obsd}}) T_{1,\text{obsd}} \quad (2)$$

in choosing the value for η_{max} . From the ¹³C relaxation data it is known that the Cd-EDTA τ_c 's of our solutions are near the intermediate correlation time region, and thus the value of η_{max} may not be equal to -2.25 (η_{max} for the extreme narrowing condition) and, further, its value will be field dependent. Therefore, η_{max} has been calculated using eq 3,

$$\eta_{\text{max}} = \frac{\gamma_{\text{H}}}{\gamma_{\text{Cd}}} \frac{W_2' - W_0'}{W_0' + 2W_{1\text{Cd}}' + W_2'} \quad (3)$$

where

$$W_0' = \frac{1}{10} \frac{\tau_c}{1 + (\omega_{\text{H}} - \omega_{\text{Cd}})^2 \tau_c^2} \quad (4)$$

$$W_{1\text{Cd}}' = \frac{3}{20} \frac{\tau_c}{1 + (\omega_{\text{Cd}})^2 \tau_c^2} \quad (5)$$

$$W_2' = \frac{3}{5} \frac{\tau_c}{1 + (\omega_{\text{H}} + \omega_{\text{Cd}})^2 \tau_c^2} \quad (6)$$

In eq 4–6 we have removed the distance factors because of the approximation of a single correlation time. The frequency dependences of η_{max} for the field strengths of interest to the present study are summarized in Figure 6. Utilizing the derived value for τ_c (1.1×10^{-10} s) for the low pH form and eq 3–6, values for

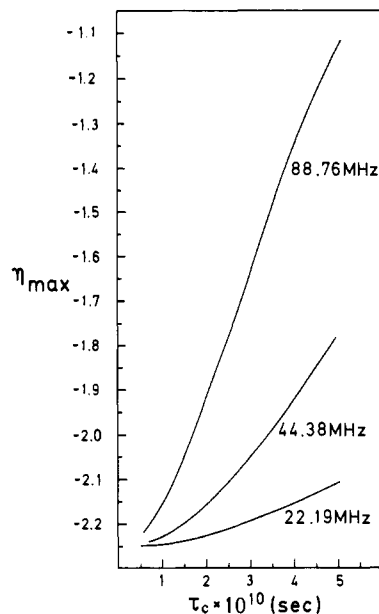


Figure 6. The calculated dependence of $\eta_{\text{max}}(^{113}\text{Cd}-\{^1\text{H}\})$ on the reorientational correlation time τ_c at 22.19, 44.38, and 88.76 MHz and within the range τ_c 0.1–0.5 ns.

η_{max} may be calculated. At the three field strengths of interest η_{max} is -2.25 , -2.22 ± 0.02 , and -2.14 ± 0.02 at 2.3, 4.7, and 9.4 T, respectively. It is essential to note that the values of the frequency-dependent relaxation times and any quantities derived from these parameters can be seriously miscalculated if no corrections are made for the frequency dependence of η_{max} .

Utilizing the values for $T_1^{\text{DD}}(^{113}\text{Cd})$ obtained with deuterated and isotopically normal EDTA, one can separate the importance of EDTA protons vs. H₂O protons in providing a dipolar relaxation pathway for the ¹¹³Cd nuclide. This can be accomplished using the relationships

$$\frac{1}{T_1^{\text{DD}}(\text{obsd})} = \frac{1}{T_1^{\text{DD}}(\text{sol})} + \frac{1}{T_1^{\text{DD}}(\text{L-H})} \quad (7)$$

$$\frac{1}{T_1^{\text{DD}}(\text{obsd})} = \frac{1}{T_1^{\text{DD}}(\text{sol})} + \frac{1}{T_1^{\text{DD}}(\text{L-D})} \quad (8)$$

for the ¹¹³Cd relaxation rates observed in the absence and presence of deuterated ligand, respectively. Here $T_1^{\text{DD}}(\text{sol})$ and $T_1^{\text{DD}}(\text{L-H})$ denote the contributions that the water solvent (H₂O/D₂O, ca. 60–70% protonated) and ligand protons make to $T_1^{\text{DD}}(\text{obsd})$, respectively. $T_1^{\text{DD}}(\text{L-D})$ denotes the dipole-dipole contribution from the ligand deuterons of Cd-EDTA-*d*₁₂ to $T_1^{\text{DD}}(\text{obsd})$. Further, $T_1^{\text{DD}}(\text{L-D})$ is related to $T_1^{\text{DD}}(\text{L-H})$ by eq 9

$$\frac{1}{T_1^{\text{DD}}(\text{L-D})} = \left(\frac{8}{3}\right)(1.004) \left(\frac{\gamma_{\text{D}}}{\gamma_{\text{H}}}\right)^2 \frac{1}{T_1^{\text{DD}}(\text{L-H})} \quad (9)$$

which follows from eq 1. The factor of 8/3 arises from the ratio of $I(I + 1)$ for a spin-1 compared to a spin-1/2 nuclide and the factor 1.004 from the ratio of the correlation time terms at 2.3 T and using $\tau_c = 1.1 \times 10^{-10}$ s. Subtraction of eq 8 from eq 7 and employing eq 9 yields the following expression for $1/T_1^{\text{DD}}(\text{L-H})$:

$$\frac{1}{T_1^{\text{DD}}(\text{L-H})} = \frac{\Delta \left(\frac{1}{T_1^{\text{DD}}(\text{obsd})} \right)}{[1 - (8/3)(1.004)(\gamma_{\text{D}}/\gamma_{\text{H}})^2]} \quad (10)$$

where $\Delta(1/T_1^{\text{DD}}(\text{obsd})) = 1/T_1^{\text{DD}}(\text{obsd}) - 1/T_1^{\text{DD}}(\text{obsd})$. From the values of $T_1^{\text{DD}}(^{113}\text{Cd})$ summarized in Table II, a value of 41.9 s is obtained for $T_1^{\text{DD}}(\text{L-H})$. Utilizing this value in eq 7 yields a value of 89.1 s for $T_1^{\text{DD}}(\text{sol})$. Hence approximately 70% of the ¹¹³Cd dipolar relaxation rate is accounted for by the EDTA ligand

protons, and the remainder (ca. 30%) is provided by the solvent water.

Therefore, from these ^{113}Cd relaxation data it is concluded that a hydrated species of Cd-EDTA such as **1a** exists in solution and its lifetime is long compared to the correlation of the complex ($\tau_c = 1.1 \times 10^{-10}$ s from the ^{13}C relaxation measurements).¹⁰ As a cross-check an estimate of the percentage of the proton content of the mixed $\text{H}_2\text{O}/\text{D}_2\text{O}$ solvent employed here along with an estimate of the Cd-H distance for the coordinated water protons or average number of cadmium bound water molecules has been attempted. These calculations were performed using the relaxation data derived above for the partially deuterated solvent, the value of $T_1(\text{obsd}) = 18.0$ s for the ^{113}Cd spin-lattice relaxation time of Cd-EDTA determined in a fully protonated medium (^{19}F external lock), and the general formula, eq 11, for $T_1(\text{obsd})$. Elimination of $T_1'(\text{other})$ in eq 11 by substituting the values of

$$\frac{1}{T_1(\text{obsd})} = \frac{1}{T_1^{\text{DD}}(\text{L-H})} + \frac{1}{T_1^{\text{DD}}(\text{sol})} + \frac{1}{T_1'(\text{other})} \quad (11)$$

$T_1(\text{obsd})$ for the $\text{H}_2\text{O}/\text{D}_2\text{O}$ and H_2O solutions, respectively, $T_1^{\text{DD}}(\text{L-H}) = 41.9$ s and $T_1^{\text{DD}}(\text{sol}) = T_1^{\text{DD}}(\text{H}_2\text{O}/\text{D}_2\text{O}) = 89.1$ s, gives a value $T_1^{\text{DD}}(\text{H}_2\text{O}) = 57.1$ s for the dipolar contribution from the two protons of H_2O to $T_1(^{113}\text{Cd})$. Using this value and eq 9 gives a value $T_1^{\text{DD}}(\text{D}_2\text{O}) = 900$ s for the analogous contribution from the two deuterons of D_2O . Inserting these values into the expression for the relaxation rate contribution from a mixed $\text{H}_2\text{O}/\text{D}_2\text{O}$ solvent, eq 12, gives a value of $X = 62\%$ for the

$$\frac{1}{T_1^{\text{DD}}(\text{H}_2\text{O}/\text{D}_2\text{O})} = \frac{X}{100} \frac{1}{T_1^{\text{DD}}(\text{H}_2\text{O})} + \frac{(100-X)}{100} \frac{1}{T_1^{\text{DD}}(\text{D}_2\text{O})} \quad (12)$$

proton content of the mixed solvent. This value is in good agreement with the value estimated (60–70%) from the sample preparation. Knowing the contribution $T_1^{\text{DD}}(\text{H}_2\text{O})$ that the solvent protons make to $T_1^{\text{DD}}(^{113}\text{Cd})$ allows the determination of the Cd-H distance, $r_{\text{Cd-H}}$, for the water protons or the average number of water molecules coordinated by the Cd-EDTA complex. Using the appropriate constants in eq 1 and assuming only one water molecule per complex, one obtains a value of 2.65 Å for $r_{\text{Cd-H}}$. Taking a Cd-O distance of 2.15 Å (from the X-ray structure of a "heptacoordinated" Cd-EDTA· H_2O complex)¹⁵ and assuming a HOH bond angle of 104.5° and a H-O distance of 0.96 Å for H_2O , a Cd-H distance $r_{\text{Cd-H}} = 2.84$ Å may be calculated. If these geometrical assumptions are correct, then one would conclude that more than one water molecule (but less than two; $r_{\text{Cd-H}}(\text{calcd}) = 2.98$ Å for two bound water molecules) per complex on the average contributes to the ^{113}Cd relaxation. However, bond distances and/or angles could change such that only one water molecule is bound to Cd-EDTA. Clearly, the separation of these factors is not possible with the present data.

The terms in eq 11 for $T_1(\text{obsd})^{-1}$ may be rewritten in the form

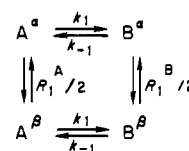
$$\frac{1}{T_1(\text{obsd})} = \frac{1}{T_1^{\text{DD}}} + \frac{1}{T_1^{\text{CSA}}} + \frac{1}{T_1(\text{other})} \quad (13)$$

where T_1^{CSA} and $T_1(\text{other})$ denote the chemical shift anisotropy (CSA) contribution and all other contributing mechanisms to the value of $T_1(\text{obsd})$, respectively. It is well known that T_1^{CSA} varies quadratically with respect to the field strength, i.e.

$$\frac{1}{T_1^{\text{CSA}}} = (2/15)\gamma_{\text{Cd}}^2 H_0^2 (\Delta\sigma)^2 \frac{\tau_c}{1 + \omega_{\text{Cd}}^2 \tau_c^2} \quad (14)$$

where $\Delta\sigma = \sigma_{33} - 1/2(\sigma_{22} + \sigma_{11})$ and $\sigma_{11} \leq \sigma_{22} \leq \sigma_{33}$. The form of eq 14 assumes that the shielding tensor exhibits axial symmetry, i.e., $\sigma_{11} = \sigma_{22} \neq \sigma_{33}$. Further it is assumed that $T_1(\text{other})$ is field independent.¹⁶ Comparison of the relaxation data obtained at

Scheme II



22.19 MHz with those at 88.76 MHz clearly shows that $T_1(\text{obsd})$ at 22.19 MHz is dominated by dipolar processes whereas the mechanism of chemical shift anisotropy dominates at 88.76 MHz. Substituting the values determined for $T_1(\text{obsd})$ and T_1^{DD} at 22.19 and 88.76 MHz (Table II) into eq 13 and employing the relationship 14, describing the frequency dependence for T_1^{CSA} , yields the following values for T_1^{CSA} and $T_1(\text{other})$: $T_1^{\text{CSA}} = 205$ s (22.19 MHz), 51 (44.42), 12.8 (88.76), and $T_1(\text{other}) = 99$ s. In the calculations of the values for T_1^{CSA} and $T_1(\text{other})$, the relaxation data obtained at 44.42 MHz should not be employed because of the experimental uncertainty associated with the NOE determination at this frequency; the value of η is nearly -1; hence an accurate determination of η and T_1^{DD} was precluded. However, for comparison with the experimental value $T_1(\text{obsd}) = 15.4$ s at 44.42 MHz, a value, $T_1(\text{calcd})$, has been calculated using eq 13; $T_1^{\text{CSA}}(44.42 \text{ MHz}) = 51$ s, $T_1(\text{other}) = 99$ s, and $T_1^{\text{DD}} = 28.3$ s (average value) (the average of the T_1^{DD} values at 22.19 and 88.76 MHz was used instead of the more questionable value for T_1^{DD} determined at 44.42 MHz). It is encouraging that the value for $T_1(\text{calcd}) = 15.4$ s exactly equals that determined experimentally. Therefore, the relaxation data for the low pH form(s) demonstrate that although the dipolar contributions represent the dominant relaxation pathway at low magnetic field strengths, the contribution from the CSA mechanism becomes increasingly important at higher fields and is dominating at 9.4 T. The remaining contributions to the observed relaxation rate, $T_1(\text{other})$, are split between spin rotation and/or random field pathways. Using the value calculated for T_1^{CSA} and eq 14 leads to a value for $\Delta\sigma$ of 131 ppm. We hope to confirm this value from a solid-state ^{113}Cd NMR study of the powder spectra of Cd-EDTA solids.

In conclusion, the observed field dependence of $T_1(\text{obsd})$ is simply a manifestation of an increasing importance of the CSA mechanism at higher fields and a gradual increase in the importance of the frequency-dependent terms in the expressions for $1/T_1^{\text{DD}}$ (eq 1 and 13). Moreover, τ_c for the small Cd-EDTA complex in our solutions approaches the intermediate correlation time region, and thus a gradual increase of the importance of the frequency-dependent terms in the expression for $1/T_1^{\text{DD}}$ (eq 1) is apparent at high frequency. Clearly one has to be exceptionally careful in the interpretation of the field dependence of metal T_1 's for complexes within this correlation time region.

^{113}Cd Relaxation of the High pH Forms(s). When the pH of a solution of Cd-EDTA is increased from pH 11.0 to 13.8 there is a dramatic decrease in $T_1(^{113}\text{Cd})$ (Figure 5 and Table II) with only a slight increase in the correlation time (1.1×10^{-10} to 1.2×10^{-10} s). A close examination of the low-field data leads to an interesting paradox. Changing the pH from 5.7 to 13.8 results in no observable change for $T_1^{\text{DD}}(^{113}\text{Cd})$ in accord with the almost constant correlation time. However, the observed T_1 has decreased by a factor of 13.2. Therefore, a new mechanism has to be introduced to account for the increased efficiency of relaxation. Spin rotation, scalar coupling, and random fields can be discounted owing to their relative unimportance at lower pH and because of the increased correlation time. It appears that only chemical shift anisotropy is left to account for the difference. However, the relaxation data at 9.4 T (Table II) quickly leads one to discard that hypothesis. Taking the increased sensitivity of $T_1(^{113}\text{Cd})$ to small fluctuations in pH into account, these data can only be

(15) A. I. Pozhidaev, T. N. Polinova, and M. A. Porai-Koshits, *Acta Crystallogr., Sect. A, Suppl.*, **28**, S76 (1972).

(16) The only other field-dependent relaxation mechanism would arise from scalar coupling to ^{14}N and/or the protons of water. Simple calculations have demonstrated that for these spins this mechanism should not affect T_1 but potentially T_2 .

interpreted in terms of the presumed field-independent new mechanism and a small contribution from dipolar processes.

From the preceding discussion it is apparent that the ¹¹³Cd spin-lattice relaxation time for the Cd-EDTA complex at pH 13.75 cannot be rationalized in terms of the "normal" relaxation mechanisms. However, with the aid of an idealized kinetic scheme, we will demonstrate how chemical exchange processes can, under certain conditions, dominate normal spin-lattice relaxation. For simplicity consider two separate species A and B which can undergo chemical exchange (Scheme II). Here A^α and A^β denote the species A with α spin and β spin, respectively, and likewise for B. Further, we have introduced the kinetic parameters *k*₁ and *k*₋₁ which represent the rate constants for the forward and reverse chemical reactions, and *R*₁^A and *R*₁^B which denote the spin-lattice relaxation rates for species A and B in the absence of chemical exchange. We will defer any further comments about the relative values of *k*₁, *k*₋₁, *R*₁^A, *R*₁^B, and the populations of species A and B until we have obtained an expression for the rate of change of A magnetization.

The equation of motion for the line intensities of the various NMR resonances in the preceding kinetic scheme may be obtained from the so-called master equation for populations¹⁷

$$\frac{dP_i}{dt} = \sum_j W_{ij}[(P_j - P_j^0) - (P_i - P_i^0)] \quad (15)$$

Here the *P*_{*i*}'s denote the populations of species *i* and the *W*_{*ij*} represent the rate constants connecting *i* with *j*; *i* and *j* are indexes which run over species A and B. Using eq 15 and Scheme II, we write

$$\frac{d(P_{A\alpha} - P_{A\beta})}{dt} = -(k_1 + R_1^A)[(P_{A\alpha} - P_{A\alpha}^0) - (P_{A\beta} - P_{A\beta}^0)] + k_{-1}[(P_{B\alpha} - P_{B\alpha}^0) - (P_{B\beta} - P_{B\beta}^0)] \quad (16)$$

$$\frac{d(P_{B\alpha} - P_{B\beta})}{dt} = -(k_{-1} + R_1^B)[(P_{B\alpha} - P_{B\alpha}^0) - (P_{B\beta} - P_{B\beta}^0)] + k_1[(P_{A\alpha} - P_{A\alpha}^0) - (P_{A\beta} - P_{A\beta}^0)] \quad (17)$$

Equations 16 and 17 can be written in terms of the expectation values of the Z components for the A and B magnetizations, i.e.

$$\frac{d\langle I_{ZA}(t) \rangle}{dt} = -(k_1 + R_1^A)[\langle I_{ZA}(t) \rangle - I_{ZA}^0] + k_{-1}[\langle I_{ZB}(t) \rangle - I_{ZB}^0] \quad (18)$$

$$\frac{d\langle I_{ZB}(t) \rangle}{dt} = -(k_{-1} + R_1^B)[\langle I_{ZB}(t) \rangle - I_{ZB}^0] + k_1[\langle I_{ZA}(t) \rangle - I_{ZA}^0] \quad (19)$$

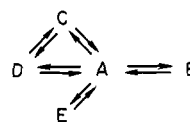
In arriving at eq 18 and 19 we have used the following relations: $\langle I_{ZA}(t) \rangle = P_{A\alpha} - P_{A\beta}$, $I_{ZA}^0 = P_{A\alpha}^0 - P_{A\beta}^0$, etc.

From eq 18 it can be seen that the time dependence of the A magnetization is coupled to that of B by the chemical exchange. In the absence of chemical exchange, the time dependence of the A magnetization will recover exponentially with an apparent relaxation time of *R*₁^A. However, in the presence of chemical exchange, the apparent relaxation time will be a mixture of kinetic and spin-lattice relaxation parameters.

An interesting situation arises when *R*₁^A is small compared to *k*₁ and the exchange rates and/or the population of B are such that only one resonance is observed (A or a weighted average of A and B). Under these circumstances the apparent relaxation time for the observed resonance will be independent of the normal relaxation parameters for A. The A magnetization will relax via the chemical exchange with B. This process will be efficient when the relaxation rate for B is large compared to *R*₁^A, and further *k*₁ and *k*₋₁ are consistent with fast kinetics. Therefore, the relaxation time for A is determined by the existence of a short relaxation time of species B which is coupled to A by chemical exchange.

(17) J. H. Noggle and R. E. Shimer, "The Nuclear Overhauser Effect", Academic Press, New York, 1971.

Scheme III

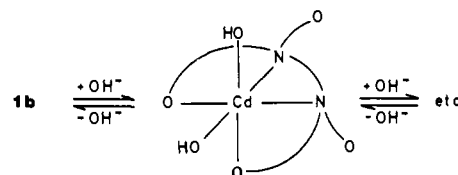


The coupling of chemical exchange and spin-lattice processes was first recognized by Hoffmann and Forsén¹⁸ with their classic saturation transfer experiments. Subsequently, the utilization of spin-lattice relaxation data to measure rate constants for chemical exchange reactions has received moderate attention.¹⁹ There are many examples, however, in organic, inorganic, organometallic, and bioinorganic systems where a spin-1/2 nuclide is involved in chemical exchange processes, e.g., intra- and intermolecular ligand exchange or a metal exchange between different binding sites in a biological system. Two examples involving cadmium, where this exchange is slow compared to the chemical shift differences between sites, are the ligand exchange between dimethylcadmium and diethylcadmium²⁰ and the metal site exchange observed in cadmium-substituted Concanavalin A.^{3d} In these cases all of the relevant exchange rate constants can potentially be determined.

An example of a case where the exchange rates and/or the population of species B are such that only a single resonance is observed, is the Cd-EDTA complex at high pH. However, it is probable that this system is far more complicated than the two-site model proposed above, i.e., a more realistic system can be schematically represented by Scheme III.

In Scheme III the actual identity of species A, B, C, D, . . . , etc., are undetermined, since the observed resonance represents a weighted average of all the species which are present. For the present discussion we will assume that A is the principal species in solution. For chemical-exchange processes to become a dominant factor in spin-lattice relaxation of A, we must have *at least one* species which has a short relaxation time and which undergoes chemical exchange with A. For notational convenience we will denote that species as B.

From the previous discussion, the high pH form(s) of the Cd-EDTA complex evolves at a pH > 11. Hence, the possible candidates for B, C, D, E, . . . are hydroxyl substituted forms of Cd-EDTA, i.e.



These species have been postulated by Schwarzenbach²⁰ and by Matyska and Kössler.²¹ From titration experiments of Mg-EDTA and Ca-EDTA with KOH (*pK*_a = 11.24 and 11.39, respectively), Schwarzenbach et al. postulated the formation of hydroxyl forms of metal-EDTA complexes. The polarographic work of Matyska and Kössler²¹ has shown the existence of the [Hg(EDTA)(OH)]³⁻ species having a formation constant of 10^{5.1}.

Another candidate for the rapid relaxing species B is [Cd(EDTA)₂(OH)_{*n*}]⁽⁶⁺ⁿ⁾⁻. The existence of such species has been postulated for metal-EDTA complexes at higher pH in the early work by Schwarzenbach et al.²⁰ Furthermore, Sudmeier and

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Reilly^{7h} have postulated the existence of disubstituted EDTA-cadmium complexes in the presence of excess EDTA, i.e.



This species should be highly immobilized in aqueous solution because of the presence of the number of negative charges. This degree of immobilization should lead to a dramatic shortening of the spin-lattice relaxation time for the cadmium nucleus. The preceding statement assumes that the ¹¹³Cd spin-lattice relaxation mechanism(s) would be dominated either by dipole-dipole or chemical shift anisotropy processes.

Further evidence for the existence of chemical exchange between different species of Cd-EDTA at high pH values comes from the field dependence of the ¹¹³Cd line width at pH 13.75; $\Delta\nu_{1/2} = 5$ Hz at 2.3 T and 20 Hz at 9.4 T. That is, the kinetic processes have become slower by a factor of 4 at 9.4 T with respect to the ¹¹³Cd NMR chemical shift time scale in hertz. This contrasts with the ¹¹³Cd line width for the low pH form(s) which is independent of the applied field strength ($\Delta\nu_{1/2} = 2-3$ Hz). Currently the actual identities of the solution species of Cd-EDTA at high pH are unknown. However, low-temperature, supercooled aqueous solution studies²² and solid-state NMR experiments are being designed to further investigate the nature of these species.

Summary and Conclusions

The dependence of the ¹¹³Cd chemical shift for Cd-EDTA as a function of pH in the range pH 3.0-13.8 has been determined. This striking pH dependence of the ¹¹³Cd chemical shift clearly points out how informative NMR spectroscopy of metal nuclei can be. In an effort to understand the origins of this pH dependence, the ¹¹³Cd spin-lattice relaxation time was determined at three field strengths (2.3, 4.7, and 9.4 T) and at selected values of pH. However, these data could not be properly analyzed without a corresponding determination of the ¹³C spin-lattice relaxation times for the metal-bound ligand. These data afforded a value of $\tau_c = 1.1 \times 10^{-10}$ s for the low pH form. Thus, with this relatively "simple" anion it was found that the complex is near the region of intermediate correlation time. Within this

region the value of η_{max} for ¹¹³Cd changes rapidly with frequency, and frequency-dependent terms within the expressions for T_1^{-1} become increasingly important. Hence, the frequency dependence of these parameters must be included to properly interpret the experimental data. With a knowledge of $T_1^{\text{DD}}(^{113}\text{Cd})$ for Cd-EDTA in the presence and absence of deuterium in the ligand, it was determined that protons from water in the primary solvation sphere are providing a dipolar relaxation pathway for the ¹¹³Cd nucleus. Hence, part of the observed pH dependence (pH 4-11) of the ¹¹³Cd chemical shift, $T_1(^{113}\text{Cd})$, and $\eta(^{113}\text{Cd}-\{^1\text{H}\})$ is due to the presence of a hydrated form of Cd-EDTA. It is essential to point out that the proper interpretation of the relaxation data was only afforded after a knowledge of the correlation time. Without this, the experimentally determined frequency dependence of T_1 may have been seriously misinterpreted. Therefore, the analysis of relaxation data of any highly charged metal nuclide must be preceded by a determination of τ_c for the complex.

Analysis of the ¹¹³Cd relaxation data of Cd-EDTA at pH 13.8 illustrates how chemical kinetics can, under certain circumstances, dominate the measured value of T_1 . These data argue the importance of hydroxylated forms of Cd-EDTA at this pH. Further, they also demonstrate the importance of examining relaxation parameters at widely separated field strengths.

Finally, the observed behavior of $T_1(^{113}\text{Cd})$ and $\eta(^{113}\text{Cd})$ for Cd-EDTA are in all probability a function of the nature of the counterion. The present work utilized the tetramethylammonium ion. However, other counterions may yield different results. These phenomena could arise because of the differences in the nature of the solvation of the Cd-EDTA anion/counterion complex. Further work on these systems is planned.

Acknowledgment. The authors acknowledge the NATO Science Affairs Division, Brussels (Research Grant No. 1831), the Danish Natural Science Research Council (J. No. 511-15401), the National Science Foundation Regional Nuclear Magnetic Resonance Facility at the University of South Carolina (CH78-18723), the National Institutes of Health (GM 26295), and the National Science Foundation (CH78-03115) for partial support of this research. Further, S.D. thanks DANIDA for the research fellowship. Finally, the authors thank Mr. K. E. Holst for synthesizing the EDTA-*d*₁₂ ligand and the continuing support of Mr. P. Daugaard with the XL-100 spectrometer (Aarhus).

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NMR and Conductometric Studies of 2-Pyridyl-Substituted Carbanions. 2. Effects of Cation Size and Coordination

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Received October 22, 1980

Abstract: ¹H and ¹³C NMR spectra of the lithium, sodium, and potassium salts of 2-ethylpyridine without and in the presence of crown ethers and cryptands indicate that the uncomplexed salts are present as contact ion pairs. Conductometric measurements carried out on the sodium salt confirm this conclusion and also indicate that the dibenzo-18-crown-6 and [2.2.2]cryptand complex of the sodium salt exist as a 1:1 crown complexed contact ion pair and a partially separated cryptate, respectively. The partial double-bond character of the C_α-C₂ bond leads to the occurrence of *E* and *Z* torsional isomers that are distinguishable by H_α-H₄ coupling. Coalescence of the CH₃ group for the Na, K, (Li, tetraglyme), and (Na, tetraglyme) salts in THF is observed at 105, >120, 65, and 95 °C, respectively. The *E/Z* isomer ratio, at least at higher temperatures, appears therefore to be thermodynamically controlled and decreases with increasing cation size and coordination. The ΔH of the equilibrium appears to be close to zero. This seems to indicate that differences in *E/Z* isomer ratios are entropy rather than enthalpy determined and may be due to restrictions on the rotation of the CH₃ group and the coordination sphere of the cation that is bonded in an allylic type fashion to C_α and N. Charge distribution within the anions was determined from ¹H and ¹³C chemical shifts by using Me₄Si as internal standard and was found to be in qualitative agreement with CNDO/2 calculations.

Although the structure of ion pairs of delocalized hydrocarbon anions has been extensively studied in recent years,¹⁻³ relatively

little detailed information is available about ion pairing in heteroatoms containing analogue anions in which the heteroatom