IBM 1800 computer. As the spectra were taken, they were directly digitized (up to 1024 points/spectrum) and stored on magnetic tape[27] and punched cards. The data were preprocessed on an IBM 360/67 computer for base-line adjustment and conversion to conventional nmr units. The spectra were analyzed on the 360 with a computer program based on the classical modified Bloch equations for uncoupled two-site exchange.[1c] The search routine for this program is based on that described by Gutowsky, *et al.*,[18] and has been modified slightly by Professor T. L. Brown and his students at the University of Illinois and ourselves. The program requires, as input parameters, information about the scan, the populations of the two sites, and the line widths ("effective" T_2's) of the two sites and the chemical shift difference ($\Delta\nu_\infty$) in the absence of exchange. The preexchange lifetime, τ, is varied by the program until a best fit is found. The fitted spectra were edited (the large number of points produced a cluttered plot-out) by the 360 and plotted with a Calcomp 1627 plotter by the 1800.

(27) E. J. Runde, M.S. Dissertation, State University of New York at Stony Brook, 1970; to be published by E. J. Runde and P. C. Lauterbur.

Acknowledgments. The authors wish to thank Professor F. W. Fowler for many stimulating and helpful discussions. M. P. (PRF Postdoctoral Fellow) and C. S. S. wish to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work. S. R. T. wishes to thank the National Science Foundation (Grant No. GP20099) for financial support during the period of this research.

A Probe of Chelated Zinc(II) Environments Using Chlorine-35 Nuclear Magnetic Resonance[1]

James A. Happe

Contribution from the Lawrence Livermore Laboratory, University of California, Livermore, California. *Received March 2, 1973*

Abstract: Nine zinc chelate systems have been studied by the ^{35}Cl nmr probe method. Bidentate, tridentate, and quadridentate ligand molecules have been included in the study. Molar relaxivities are reported for both 1 : 1 and 1 : 2 zinc chelates. This parameter is suitable for characterizing a metal ion environment in terms of the quadrupolar relaxation of ^{35}Cl nuclei that it can produce in 0.5 *M* NaCl. It is shown that bidentate chelation of $Zn^{2+}(aq)$ can be expected to increase its effectiveness in producing ^{35}Cl relaxation. Tridentate chelation can either increase or decrease the amount of relaxation caused by zinc. The least relaxation is produced when ligand atoms have formal negative charges and large acidity constants. In some instances, Zn(II) which is chelated by four ligand atoms is effective at ^{35}Cl relaxation. This, therefore, does not necessarily represent a coordinately saturated environment. Molar relaxivities for 1 : 1 and 1 : 2 chelates usually differed. In these cases it was possible to derive formation constants from the nmr data. These in general are consistent with literature values. In a number of systems hydrolysis-type reactions are readily identified.

There have been a number of reports in which ^{35}Cl nmr has been used to study bound metal ion environments in solution. The method has been called the halide ion probe method and has been described in several places.[2-4] Usually the systems studied have contained bound metal ions in rather poorly defined environments as sensed by halide ions. The experiments were used either to monitor changes in those environments or to derive a more detailed understanding of the actual metal ion binding site. The latter application is difficult because model systems have not been studied. In the work reported here, relatively simple chelate systems are examined whose structures have been previously investigated by other methods. The aim has been to study how Cl^- probe ions interact with these model systems.

It was reported some time ago that the presence of certain metal ions in NaCl solutions increased the observed nmr relaxation rate for ^{35}Cl nuclei. This happens because small concentrations of chloro complexes are formed and these provide centers for quadrupolar relaxation. The relatively few bound ^{35}Cl nuclei have very short relaxation times. The remaining ^{35}Cl are in a rather symmetrical aqueous environment characterized by a relatively long T_2. If chemical exchange is sufficiently rapid, only a single nmr line is observed whose width can be used to infer the properties of the metal ion sites at which Cl^- bind. Nuclear relaxation times and line widths are related by $T_2 = (\pi\Delta\nu)^{-1}$. The full nmr line width at half-maximum, $\Delta\nu$, is given by eq 1 for a particular ^{35}Cl environment[5] and by eq 2 for an exchange averaged line.

$$\Delta\nu_i = (^2/_5\pi)(e^2q_iQ)^2\,\tau_i \quad (1)$$

$$\Delta\nu_{obsd} = (^2/_5\pi)(e^2Q)^2\sum_i(f_iq_i^2\tau_i) \quad (2)$$

In the equations, e is the electronic charge, Q is the quadrupole moment for ^{35}Cl, and f is the probability that a ^{35}Cl nucleus will be found at a site of type i. It is assumed that a bound ^{35}Cl nucleus experiences an axially symmetric electric field gradient, q, whose orientation is time dependent and characterized by a correlation time, τ.

(1) This work was performed under the auspices of the U. S. Atomic Energy Commission.

(2) T. R. Stengle and J. D. Baldeschwieler, *Proc. Nat. Acad. Sci. U. S.*, **55**, 1020 (1966).

(3) T. R. Stengle and J. D. Baldeschwieler, *J. Amer. Chem. Soc.*, **89**, 3045 (1967).

(4) R. L. Ward, *Biochemistry*, **8**, 1879 (1969).

(5) A. Abragam and R. V. Pound, *Phys. Rev.*, **92**, 943 (1953).

In the initial studies, Hg^{2+} binding to macromolecules was studied.[2,3] More recently ^{35}Cl nmr experiments have been extended to include studies of systems containing Zn^{2+} rather than Hg^{2+}.[4,6] In one of these[6] it was shown that metal ion environments could be characterized by molar relaxivity parameters. These contained the information on q, τ, and f describing the relaxation of ^{35}Cl at a particular Zn(II) site. In terms of molar relaxivities, eq 2 was written

$$\Delta\nu_{obsd} - \Delta\nu_{Cl^-} = \sum_i \bar{\nu}_i[Zn(II)]_i \qquad (3)$$

The $\bar{\nu}_i$ refer to experiments at a particular NaCl concentration which is large enough so that it can be considered constant.

A great deal of information, contained in the $\bar{\nu}$ parameters, is usually unavailable because the experiments only give direct information on the $(fq^2\tau)$ product for a given site rather than on the individual components. With reliable values of τ, for example, one might better understand conformational changes at the metal binding sites of protein molecules. In other applications an evaluation of q might suggest chelation by a specific type of ligand atom or group of atoms. Measurements of f for Cl^- binding could quite possibly be useful in assessing coordination numbers for bound metal ions. In spite of the present limitations of the method it becomes increasingly more useful the better one can estimate relative values for the components of $\bar{\nu}$ in specific instances. This report describes experiments in this area where at present little is known.

In the studies reported here $\bar{\nu}$ parameters have been determined for a number of zinc chelates. The systems studied include bidentate, tridentate, and quadridentate ligands and provide a wide variation in metal ion environments. It is found that the $\bar{\nu}$ values measured also vary widely. In spite of the limitations set by the inability to observe separate q, τ, and f values, several conclusions have been reached regarding the relation between metal ion binding site characteristics and ^{35}Cl relaxation produced, *i.e.*, its $\bar{\nu}$. (1) Bidentate chelation of Zn(II) does not seriously reduce Cl^- binding. Instead ^{35}Cl relaxation increases because of more important changes in the $(q^2\tau)$ product. (2) Tridentate chelation of Zn(II), while generally increasing τ, decreases (q^2f). Ligand atom basicities may provide guidelines to the extent of these decreases. (3) Tetracoordination of Zn(II) did not usually prevent ^{35}Cl relaxation and therefore did not in general lead to coordinately saturated tetrahedral species. However, ligand sulfur atoms can produce exceptions. Proton removal from Zn(citrate)$^-$ is discussed as an example of a process differing from normal hydrolysis involving bound H_2O. The nmr line width measurements can be described quantitatively and used to derive reliable formation constants.

Experimental Section

The nmr measurements were made at 5.88 MHz with the spectrometer system described earlier.[4] The probe temperature at which line widths were measured was 31.6°. Line broadenings are presented in the figures. These are defined as $\Delta\nu_{obsd} - \Delta\nu_{Cl^-}$. The line width for Cl^-(aq), $\Delta\nu_{Cl^-}$, was usually found to be about 11-12 Hz.

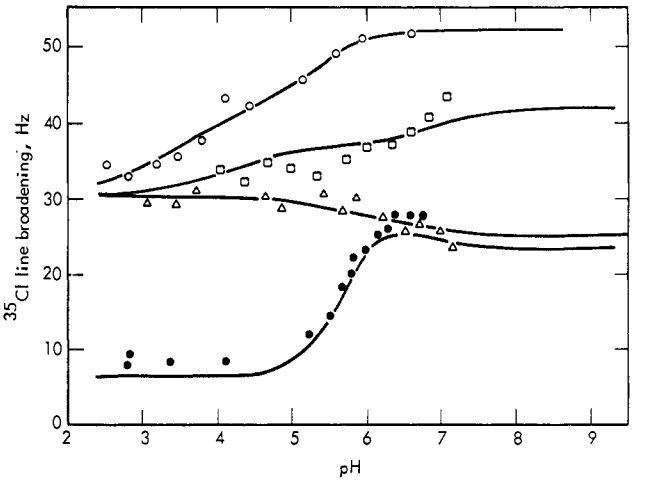

Figure 1. Titrations giving 1:1 chelates with bidentate ligands: ○, 16.0 m*M* Zn + 60.5 m*M* succinic acid; □, 15.6 m*M* Zn + 16.0 m*M* glutamic acid; △, 15.6 m*M* Zn + 16.0 m*M* glycine; ●, 3.4 m*M* Zn + 1.7 m*M* cysteine.

The ionic strength of the samples was 0.5 *M*. Hydrogen ion concentrations were obtained from pH readings using $\gamma_{H^+} = 0.69$. Formation constants for zinc chelates were estimated from the nmr data as described in the text. In these calculations literature values were used for the acidity constants of the ligands. Ionic strength corrections were applied[7,8] to give constants appropriate for $I = 0.5$. Because hydrolysis reactions influenced the nmr line widths, only data below pH 7.0 were used in the calculations. Data used for cysteine calculations were limited to low cysteine concentrations (1.7×10^{-3} *M*) and metal to ligand ratios between 0.8 and 10.0. This was done in order to decrease the importance of polynuclear species.

Results

Data were obtained in the form of titration curves. The ^{35}Cl nmr line width was measured as a function of solution pH. So that the data could be analyzed in terms of association constants and $\bar{\nu}$ parameters, each system was titrated at a number of different total metal to total ligand concentration ratios. Only certain of these are shown in the figures. Their interpretation becomes easier if the following is noted. If there were no chelate formation, line broadening in any of the experiments would be about twice the total millimolar zinc concentration and independent of pH.

Zn(II) Complexes with Bidentate Ligands. Representative titrations are shown in Figure 1 which correspond to the formation of 1:1 chelates. Although chelate formation may be incomplete at the titration end, the curves clearly indicate the line broadening properties of the species formed. At the particular zinc to ligand ratios shown, there should be little 1:2 chelate formation. At sufficiently low pH values ligand chelating atoms are protonated and line broadening corresponds to that by Zn^{2+}(aq). As the pH is raised a region is found over which ligand protons are titrated as the Zn(II) complex forms. The region over which this occurs is of course dependent on the p*k*'s of the ligand donor atoms and on the stability of the complex formed. In three of the four systems

(6) J. A. Happe and R. L. Ward, *J. Amer. Chem. Soc.*, **91**, 4906 (1969).

(7) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958, pp 441-443.

(8) R. G. Bates and G. D. Pinching, *J. Amer. Chem. Soc.*, 71, 1274 (1949).

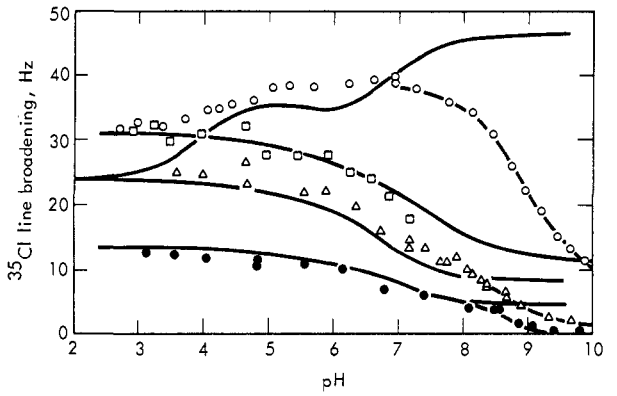

Figure 2. Titrations giving 1:2 chelates with bidentate ligands: ○, 12.2 m*M* Zn + 62.5 m*M* glutamic acid; □, 15.6 m*M* Zn + 32.0 m*M* glycine; △, 12.2 m*M* Zn + 62.5 m*M* glycine; ●, 6.8 m*M* Zn + 40.0 m*M* glycine.

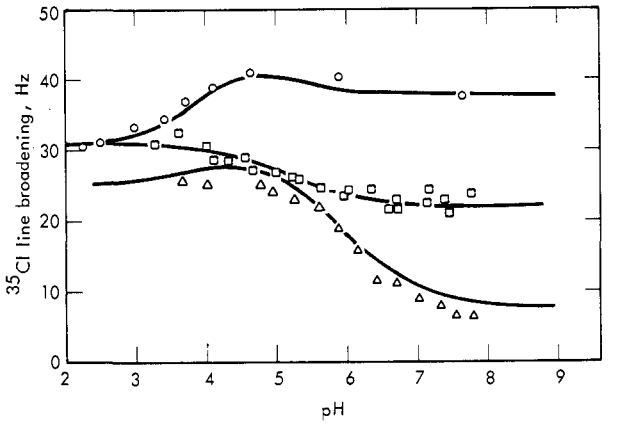

Figure 3. Titrations giving 1:1 chelates with tridentate ligands: ○, 15.6 m*M* Zn + 16.0 m*M* methyliminodiacetic acid; □, 15.6 m*M* Zn + 16.0 m*M* iminodiacetic acid; △, 12.5 m*M* Zn + 12.5 m*M* aspartic acid.

shown a chelate is formed which broadens the nmr line more than does Zn^{2+}(aq). The Zn(glycinate)$^+$ chelate produces slightly less ^{35}Cl relaxation than does Zn^{2+}(aq). In each case the formation of a precipitate terminated the titration near pH 7.0. In the cysteine experiment a precipitate was noted near pH 6.0. For a particular system the titration curves were qualitatively the same for equimolar or excess zinc solutions. The formation constant for Zn(succinate) is low and excess ligand was used in order to produce the complex. It was assumed that the species Zn(succinate)$_2^{2-}$ could be neglected.[9] A formation constant for the later complex has not been reported.

Typical titration curves are shown in Figure 2 for solutions in which the presence of excess ligand leads to the formation of a 1:2 complex. For glycine, three titrations are shown at different ligand to metal ratios. When this ratio was 2.0 the titration could be carried only to pH 7.6 before a precipitate formed. In the other two experiments there was a five- to sixfold ligand excess and the titrations could be carried almost to pH 10.0. In these two titrations the pH region 5.5 to 8.0 represents the chelation of Zn^{2+} leading to the formation of Zn(glycinate)$_2$. Either published formation constants[10] or those derived here could be used to estimate that at pH 8.0 this complex accounted

(9) E. Campi, *Ann. Chim. (Rome)*, **53**, 96 (1963).
(10) C. W. Childs and D. D. Perrin, *J. Chem. Soc. A*, 1039 (1969).

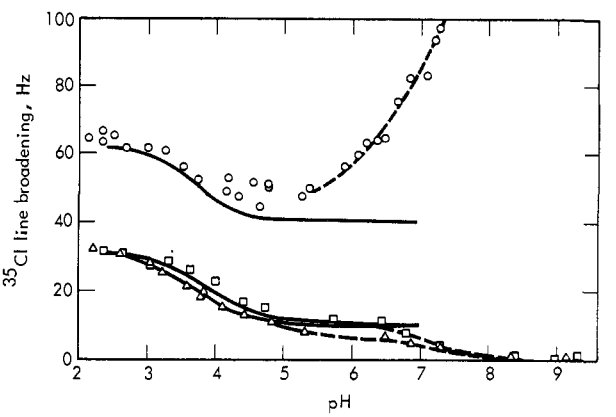

Figure 4. Titrations of some citric acid-zinc solutions: ○, 31.2 m*M* Zn + 16.0 m*M* citric acid; □, 15.6 m*M* Zn + 16.0 m*M* citric acid; △, 15.6 m*M* Zn + 32.0 m*M* citric acid.

for 90 to 95% of the total Zn(II). Similarly, for the glutamic acid solution whose titration is shown, the formation of Zn(glutamate)$_2^{2-}$ is estimated[11] to exceed 90% at pH 8.0. The ^{35}Cl line broadening does not reach zero after the 1:2 complexes are formed. There is an additional change in these complexes in the pH region above 8.0 which is attributed to the formation of a hydroxo species. The dashed curves in Figure 2 show how the data deviate from curves calculated neglecting hydrolysis as described later. It appears that ^{35}Cl nmr line broadening by the hydrolyzed species is zero. Although no analysis of the hydrolysis region was made, the titrations indicate p*k* values near 8.9 and 9.1 for Zn(glycinate)$_2$(OH)$^-$ and Zn(glutamate)$_2$(OH)$^{3-}$ formation, respectively.

Zn(II) Complexes with Tri- or Quadridentate Ligands. The results shown in Figure 3 demonstrate the formation of 1:1 chelates. The Zn(iminodiacetate) and Zn(aspartate) chelate titrations were not carried beyond pH 7.8 because a precipitate formed. The curves show that both complexes produce less ^{35}Cl line broadening than does Zn^{2+}(aq), although for Zn(iminodiacetate) the decrease in broadening power is small. The formation of Zn(methyliminodiacetate) appeared to reach a maximum value between pH 5.0 and 6.0 after which small additions of base caused a sharp rise in pH and the appearance of a precipitate. It is clear, however, that the complex formed produces more ^{35}Cl relaxation than does Zn^{2+}(aq). This result was not obtained with the other two chelates.

Three citric acid–zinc titrations are shown in Figure 4. Two of these, the equimolar and the excess citric acid titrations, are qualitatively the same. This is in agreement with reports[12,13] indicating that Zn(citrate)$_2^{4-}$ is not important when the ligand excess is not large. These two titrations show that formation of zinc–citric acid complexes decreases the metal ion–chloride interaction. With recent formation constants[12] it is possible to calculate the progress from Zn^{2+}(aq) near pH 2.0 to a maximum concentration of Zn(H citrate) somewhat below pH 4.0 and then to practically complete formation of Zn(citrate)$^-$ at higher

(11) L. G. Sillen and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. 17 (1964).
(12) E. Campi, *J. Inorg. Nucl. Chem.*, **26**, 553 (1964).
(13) M. Bobtelsky and J. Jordan, *J. Amer. Chem. Soc.*, **67**, 1824 (1945).

pH. For example, in the equimolar and excess citric acid solutions at pH 6.0, the formation of Zn(citrate)$^-$ is calculated to be about 98% complete. Figure 4 shows that line broadening by this complex is only about one-third as great as by Zn^{2+}(aq). The equimolar and excess citric acid titrations also show that Zn(citrate)$^-$ loses an additional proton over the region pH 6.0 to 8.0. With these relative metal to ligand concentrations this reaction, which has also been observed potentiometrically,[13] gives a species which does not produce ^{35}Cl relaxation.

The citric acid system was the only one studied in which the titration curves for equimolar and excess zinc solutions were qualitatively different. The third titration in Figure 4 shows that, when excess zinc is present, the proton loss in the high pH region leads to a species which is very effective in producing ^{35}Cl relaxation. This is presumably a polynuclear species containing more than one zinc ion for each citrate ligand. The excess zinc titration in Figure 4 shows the dramatic rise in ^{35}Cl relaxation over the pH region above 5.0.

The titrations in Figure 5 are included to demonstrate the formation of several 1:2 chelates. In each case the titrations could be carried beyond pH 9.0 without precipitation. The initial part of the methyliminodiacetic acid titration shows the formation of the 1:1 complex which as already noted is more effective than Zn^{2+}(aq) in ^{35}Cl relaxation. At higher pH values this complex is converted to the 1:2 species which produces little if any ^{35}Cl relaxation. The titrations shown for aspartic acid and iminodiacetic acid also demonstrate the formation of 1:2 chelates which do not significantly broaden the ^{35}Cl nmr line.

Nitrilotriacetic acid forms a tetracoordinated zinc chelate. The titration of an equimolar ligand to metal ion solution is included in Figure 5. The complex, Zn(nitrilotriacetate)$^-$, is very stable (log $K_f \approx 10.6$) and forms below pH 4.0. Both Zn^{2+}(aq) and the complex broaden the ^{35}Cl line to about the same extent. After formation of the complex the pH was found to rise sharply beyond 7.0. Further additions of base remove an additional proton from the complex in a hydrolysis-type reaction. The reaction has a pk near 8.7 and leads to a species which does not appear to interact with Cl^-.

Formation Constants and Molar Relaxivity Parameters. A quantitative analysis of the data has been carried out in order to derive values of formation constants and $\bar{\nu}$ parameters which best reproduce the experimental line width *vs.* pH titrations. An attempt was made to derive the constants from the nmr data because literature values sometimes varied between wide limits and only rarely were they available at the ionic strength used in these studies. The derivation of reasonable formation constants was also considered to be very strong confirmation of the general interpretation of ^{35}Cl relaxation in terms of eq 3. The method used is outlined below.

The following equilibria have been considered in solutions containing a tribasic ligand molecule[14] and Zn^{2+}.

(14) The analysis was formulated in terms of a tribasic ligand molecule so that all systems studied could be treated either directly or as special cases in which the appropriate equilibria were either vanishingly small or very large.

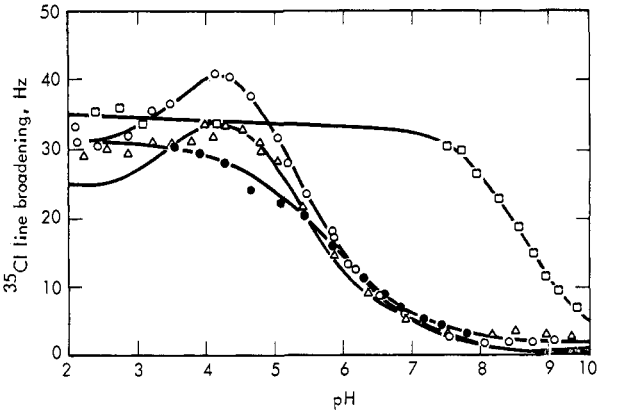

Figure 5. Titrations in which there is a quadridentate ligand or an excess of tridentate ligand: ○, 15.6 mM Zn + 31.8 mM methyliminodiacetic acid; □, 15.6 mM Zn + 16.0 mM nitrilotriacetic acid; △, 12.2 mM Zn + 62.5 mM aspartic acid; ●, 15.6 mM Zn + 31.8 mM iminodiacetic acid.

$$H_3L \overset{k_1}{\rightleftharpoons} H_2L + H$$

$$H_2L \overset{k_2}{\rightleftharpoons} HL + H$$

$$HL \overset{k_3}{\rightleftharpoons} L + H$$

$$Zn + L \overset{K_1}{\rightleftharpoons} ZnL$$

$$ZnL + L \overset{K_2}{\rightleftharpoons} ZnL_2$$

$$Zn + HL \overset{K_3}{\rightleftharpoons} ZnHL$$

From these equilibria and the conservation expressions for total metal, T_M, and total ligand, T_L, it is straightforward to derive the following expression for the fully ionized free ligand concentration, [L].

$$[L]^3 + \{1/K_2 + K_3[H]/K_1K_2k_3 + (2T_M - T_L)/f(k,H)\}[L]^2 + \{(1/K_2 + K_3[H]/K_1K_2k_3)f(k,H) + 1/K_1K_2\}[L] - T_L/K_1K_2f(k,H) = 0 \quad (4)$$

where

$$f(k,H) = \{1 + [H]/k_3 + [H]^2/k_2k_3 + [H]^3/k_1k_2k_3\}$$

Under the assumption that a set of equilibrium constants and initial reactant concentrations have been specified, eq 4 can be solved for [L] at experimental [H] values. The Zn(II) species concentrations can then be evaluated at each experimental point using eq 5–8.

$$[Zn] = T_M/(1 + K_1[L] + K_1K_2[L]^2 + K_3[L]/k_3) \quad (5)$$

$$[ZnL] = K_1[Zn][L] \quad (6)$$

$$[ZnHL] = K_3[Zn][L]/k_3 \quad (7)$$

$$[ZnL_2] = K_2K_1[Zn][L]^2 \quad (8)$$

Equations 4–8 were sufficient to construct a set of experimental "line broadening" *vs.* "concentration set" pairs which were then used in a least squares calculation to obtain the $\bar{\nu}$ coefficients of eq 3. These $\bar{\nu}$ parameters best fit the data for the selected set of equilibrium constants. Equation 3 for this system is

$$(\Delta\nu_{obsd} - \Delta\nu_{Cl^-}) = \bar{\nu}_1[Zn] + \bar{\nu}_2[ZnL] + \bar{\nu}_3[ZnL_2] + \bar{\nu}_4[ZnHL] \quad (9)$$

Table I. Formation Constants and Molar Relaxivity Parameters for Zinc Complexes

Ligand	$\bar{\nu}_{(ZnL)}$, $kH_z\ M^{-1}$	$\bar{\nu}_{(ZnL_2)}$, $kHz\ M^{-1}$	$\bar{\nu}_{(ZnHL)}$, $kH_z\ M^{-1}$	Log K_1	Log K_2	Log K_3	Ref
Aspartic acid	0.54	0	4.26	5.40	3.15	1.19	*a*
				5.84	4.31		*b*
Cysteine	19.06	0	20.35	8.91	8.70	4.80	*a*
				9.04	8.50		*c*
				9.86	8.84		*b*
Methyliminodiacetic acid	3.52	0		7.06	6.19		*a*
				7.66	6.43		*b*
Iminodiacetic acid	1.64	0		6.11	4.92		*a*
				7.03	5.14		*b*
Glycine	1.72	0.69		5.92	4.13		*a*
				4.90	4.11		*d*
				5.19	4.21		*e*
				5.42	4.52		*b*
Citric acid	0.63		1.15			2.42	*a*
				4.98		2.98	*f*
Glutamic acid	2.65	3.82	3.82			1.39	*a*
				5.45	4.01		*b*
Nitrilotriacetic acid	2.21			10.67			*b*
Succinic acid	3.47		2.65	2.33		2.14	*a*
				1.76		0.96	*f*
				2.48			*b*

[a] This investigation. [b] References and conditions tabulated in ref 11. [c] Reported by Lenz and Martell.[15] [d] Reported by Childs and Perrin.[10] [e] Reported by Sharma and Mather.[16] [f] Reported by Campi.[12]

The computations were made on a CDC 6600 computer using a general linear least squares (GLLS) program which fit the data to eq 9 in the form

$$Y = \bar{\nu}_1 f_1(X) + \bar{\nu}_2 f_2(X) + \bar{\nu}_3 f_3(X) + \bar{\nu}_4 f_4(X) \quad (10)$$

The variables Y and X are defined by

$$Y \equiv (\Delta\nu_{obsd} - \Delta\nu_{Cl^-}); \quad X \equiv [H]$$

and the various Zn(II) species concentrations are represented by the appropriate function of [H]. The program GLLS solves the least squares problem with any set of fitting functions which are linear in the coefficients. The degree to which the chosen set of equilibrium constants reproduced the data was assessed by evaluating the statistical variance for the set of observed Y_i and those calculated from eq 9 with the derived $\bar{\nu}$'s and calculated concentrations.

In order to find a set of formation constants best fitting the data, a search was made in which K_1, K_2, and K_3 were systematically varied until that set was found which gave the smallest variance. These constants together with the associated $\bar{\nu}$ parameters are given in Table I. The solid curves in the figures were calculated using eq 9 and the listed parameters. Some typical literature formation constants are included for comparison.

In most of the systems some simplification of the problem could be made either from an examination of the data or from earlier studies of the system by others. The following are examples. For cysteine and the tridentate ligands studied, citric acid excepted, the titrations indicated that $\bar{\nu}_3$ could be set equal to zero. For citric acid, potentiometric studies indicated that K_2 could be made negligibly small. Similarly K_3 was considered to be negligible for glycine.

The formation constants derived here are in general lower than the literature values. This is in the direction expected on the basis of ionic strength and temperature differences. The values of these two variables were considerably higher in the present study. Most of the literature values in Table I were measured at 20° and $I = 0.1$. As indicated in the table, formation constants were not derived for the glutamic acid system. The measured line width *vs.* pH variations were small and indicated that both Zn^{2+}(aq) and the various chelated species have similar $\bar{\nu}$ parameters. For this reason, uncertainty in derived constants was large and literature values for K_1 and K_2 were used to derive the $\bar{\nu}$ parameters. It was found, however, that the fit of the data in the low pH region could be improved by including an equilibrium involving the formation of a protonated species and this formation constant is included in the table.

Nearly the same formation constant for Zn(citrate)$^-$ has been obtained in several reliable studies.[12,17] This value of K_1 was used after being adjusted for ionic strength differences and only K_3 was varied to find a best value. For the nmr analysis, only citric acid data below pH 5.0 were used in order to avoid complications from hydrolysis-type reactions.

Discussion

The characteristic molar relaxivity parameters derived for typical members of several chelate classes are examined below with a view toward understanding how variations in $\bar{\nu}$ arise within a class of chelates. Two such classes would be chelates with bidentate ligands and chelates with tridentate ligands. Because separate values are not obtained for the q, τ, or f parameters that determine $\bar{\nu}$, comparisons between $\bar{\nu}$'s or changes in $\bar{\nu}$ that accompany chelation will be of most interest. The conclusions to be reached were summarized earlier. In brief, they are: (1) the most important effect of bidentate chelation of Zn(II) is to increase ^{35}Cl relaxation *via* the $(q^2\tau)$ product; (2) tridentate chelation of Zn(II), while increasing τ, decreases (q^2f) to an extent determined by the ligand

(15) G. R. Lenz and A. E. Martell, *Biochemistry*, 3, 745 (1964).
(16) V. S. Sharma and H. B. Mather, *Indian J. Chem.*, 3, 475 (1965).
(17) N. C. Li, A. Lindenbaum, and J. M. White, *J. Inorg. Nucl. Chem.*, 12, 122 (1959).

atoms; (3) in aqueous solution tetracoordination of Zn(II) most often leads to octahedral species.

The bidentate zinc chelators which we have examined are glycine, succinic acid, glutamic acid, and cysteine. In all but the glycine complex, the dominant effect of chelation is not to decrease Cl^- binding but to increase $\bar{\nu}$ by increasing the $(q^2\tau)$ product. The glycine chelate was the smallest studied and probably represents the point at which the τ increase from chelation is not quite large enough to compensate for any decrease in Cl^- binding.

Increases in both q and τ have been identified with the bidentate chelates. First of all, the molar relaxivities for glycine and glutamic acid differ by a factor of 1.5 and this is estimated to be a direct measure of τ differences. These two ligands have nearly identical pk_a's for their amino and basic carboxyl groups. Albert[18] reports for glycine $pk_a = 9.86$ and $pk_{a'} = 2.22$ and for glutamic acid $pk_a = 9.92$ and $pk_{a'} = 2.19$. The details of the metal–ligand atom bonding should on this basis be very similar in their zinc chelates. This would lead to nearly equal probabilities of Cl^- binding and values of q at bound ^{35}Cl. This leaves τ as the only variable differentiating ^{35}Cl relaxation by the two chelates. Secondly, the high molar relaxivity for Zn(cysteinate) must be due to a relatively large q at ^{35}Cl nuclei bound to the zinc. The chelate is one of the smallest studied and cannot have a large τ. The unusual chelation increase in q is associated with the ligand S atom and presumably would be a general property of chelates in which S binds the metal ion. Indeed we have also observed correspondingly high ^{35}Cl relaxation in similar Cd(II) chelates.

The conversion of $Zn^{2+}(aq)$ to a tridentate chelate often changes $\bar{\nu}$, but the interpretation of these changes in terms of the $(q^2\tau f)$ components presents many difficulties. Chelation would clearly increase τ for a bound ^{35}Cl but this did not dominate $\bar{\nu}$ changes since in two instances $\bar{\nu}$ decreased from 2.0×10^3, the Zn^{2+}-(aq) value. In these two cases at least, there was clearly a more important decrease in the product (q^2f) on chelation. Neither did the formation of tridentate chelates change $\bar{\nu}$ in a way indicating that a general lowering of f had dominated the change. Thus, neither τ increases nor f decreases alone dominate the $\bar{\nu}$'s and it appears that there must be variations in the (q^2f) product which depend markedly on the properties of the ligand atoms that bind the metal ion. Only in this way could the sevenfold variation in $\bar{\nu}$'s, ranging from 0.53×10^3 for a Zn(aspartate)Cl^- complex to 3.5×10^3 for the Zn(methyliminodiacetate)-Cl^- complex, have occurred. These variations are then superimposed on a general τ increase. The $\bar{\nu}$ for Zn(asparate) demonstrates a particularly sizable chelation decrease in (q^2f). These observations suggest a way in which ^{35}Cl relaxation might be qualitatively related to a ligand atom property, namely its acidity constant.

The tridentate ligand molecules form a series in which two carboxyl groups bind Zn(II) with the third atom being either an uncharged N or O. In each, the second carboxyl ionization leaves a relatively basic oxygen atom and experimentally the chelates with most basic carboxyl groups have low $\bar{\nu}$ parameters. The citrate and aspartate ligands bind Zn(II) with the most basic carboxyl groups and have the lowest $\bar{\nu}$'s while the methyliminodiacetate ligand has the least basic carboxyl group and highest $\bar{\nu}$. The iminodiacetate chelate has intermediate values for both $\bar{\nu}$ and pk_a. This correlation could be interpreted on the basis of a basic carboxyl group significantly lowering the effective metal ion charge and thereby both decreasing Cl^- binding and decreasing the polarizing forces exerted upon the electrons of a bound Cl^-.

The remaining class of chelate environments on which data have been presented is that resulting from tetracoordination of zinc. Potentially these chelates may be octahedral or tetrahedral species. In the former case two water molecules would be included in the first coordination sphere. The ^{35}Cl measurements serve to distinguish between these environments. The tetracoordinated chelates, Zn(glycinate)$_2$, Zn(glutamate)$_2^{2-}$, and Zn(nitrilotriacetate)$^-$, produce significant ^{35}Cl relaxation and therefore Cl^- ions are forming bonds with the Zn(II). The chelates are clearly not coordinately saturated tetrahedral species. It appears rather that Cl^- is readily replacing a water molecule of an octahedral metal ion environment. Only in the case of cysteine did there appear to form a saturated tetrahedral chelate. The $\bar{\nu}$ for Zn(cysteinate)$_2^{2-}$ was zero; presumably because packing considerations favor a tetrahedral chelate when there are two large sulfur atoms in the first coordination sphere.

With zinc chelates hydrolysis reactions involving bound water are normally characterized by a pk near 9.0. The Zn(nitrilotriacetate)$^-$ and Zn(glycinate)$_2$ titrations show this type of proton loss. The ^{35}Cl titrations of Zn(citrate)$^-$ demonstrate a different behavior, however. A proton is lost over the pH region 6.0 to 8.0 and this eliminates ^{35}Cl relaxation by the Zn(II). The ^{35}Cl titrations thus confirm the proton loss reported earlier by Bobtelsky and Jordan in their study of metal citrates.[13] These and other authors have suggested that a proton is lost from the citrate hydroxyl group. It is interesting that the resulting species does not appear to relax ^{35}Cl. The citrate anion is potentialy a quadridentate ligand, but molecular models indicate that its three carboxyl and one hydroxyl oxygen atoms could not attain either octahedral or tetrahedral arrangements about a Zn(II) ion. As a result, in the Zn(citrate)$^{2-}$ species, which does not show a ^{35}Cl interaction, three formally negative oxygen atoms would occupy the first coordination sphere together with one or more water molecules. The metal ion could therefore still potentially bind and relax Cl^-. A reason why no interaction is observed can be based on the relation between $\bar{\nu}$ and pk_a presented earlier. The citrate carboxylate and hydroxylate groups represent very basic ligand sites and would therefore constitute a Zn(II) binding site characterized by a small $\bar{\nu}$. This is in contrast to the not too different situation in Zn(nitrilotriacetate)$^-$ where the ligand contributes three formally negative oxygen atoms and a neutral nitrogen to bind Zn(II). In this case, however, the carboxyl groups are relatively acid ($pk_1 = 1.8$; $pk_2 = 2.5$) and the chelate shows a significant ^{35}Cl interaction.

(18) A. Albert, *Biochem. J.*, **50**, 690 (1952).