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Kinetic Studies of the Complex Formation of Zinc with Glycine Zwitterion by the Ultrasonic Absorption Method

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Received March 17, 1978

The ultrasonic absorption of the aqueous solution of a zinc–glycine complex has been measured in the frequency range 4.5–95 MHz. Both the relaxation frequency and the amplitude of the absorption depend on the ionic concentration and the value of pH. The concentration dependences of the relaxation parameters reveal that the relaxation absorption can be ascribed to the Zn complex formation reaction of glycine zwitterion. The formation and dissociation rate constants are 5.9×10^7 M^{-1} s^{-1} and 5.1×10^7 s^{-1}, respectively, and the volume change of the reaction is 7.5 cm^3 mol^{-1} at 25 °C and $\mu = 2.0$. The ligand dependency of the relaxation absorption has been studied in the solutions of Zn complexes of β-alanine, γ-aminobutyric acid, and ϵ-aminocaproic acid.

Introduction

Among the kinetic studies of the complex formation of labile metal ions, the reactions with amino acid ligands have been extensively studied.[1,2] This research is relevant to our understanding of metal complex formation in inorganic chemistry and also is important in order to understand the role of metal ions in biochemistry. It is well-known that the amino acid takes the form of anion, zwitterion, or cation depending on the pH of the solution. It has been further admitted that only the anion is reactive toward metal ions even though other forms exist in high concentrations. Both the equilibrium[3] and the kinetic studies[1,2,4-6] of the complex formation reactions of amino acids have been limited to those of their anion ligands. Some experimental evidence for complex formation with zwitterions has been given,[7-10] but the kinetic studies have been very limited[11] since (i) the complexes are not very stable, (ii) the rate constants are very large, and (iii) the formation of the complex is restricted to the low-pH region where the detection of the reaction becomes troublesome. The ultrasonic absorption method, which is used in this study, is one of the most appropriate techniques to study these reactions.

In the present investigation, the Zn complex formation reaction with glycine (gly) was studied. Supplementary studies were carried out for the solutions of Zn complexes of β-alanine (β-ala), γ-aminobutyric acid (γABA), and ϵ-aminocaproic acid (ϵACA).

Experimental Section

Samples. All the chemicals used were of a reagent grade and were used without further purification. Deionized and distilled water was used. The sample solutions were prepared from $Zn(NO_3)_2$ and glycine. The pH of the solution was adjusted to the appropriate value by the addition of NaOH or HNO_3 solution. Ionic strength, μ, was kept constant at 2.0 by the addition of $NaNO_3$.

Measurements and Treatments of the Data. Ultrasonic absorptions were measured by the pulse method in the frequency range 4.5–95 MHz. Details of the apparatus have been described elsewhere.[12] The velocity of sound was measured at 1.92 MHz by the sing-around method. The temperature was controlled to 25.0 ± 0.1 °C.

All the relaxation absorption spectra were represented as an excess absorption per wavelength by the single relaxation equation

$$\alpha'\lambda = 2(\alpha'\lambda)_{\max}\frac{f/f_r}{1 + (f/f_r)^2} \tag{1}$$

where α' is the excess absorption coefficient, λ the wavelength, f the frequency, f_r the relaxation frequency, and the subscript max represents the maximum value.

The equilibrium concentrations of all species in the solutions were calculated with use of the equilibrium constants in Table I.

Table I. Equilibrium Constants[a]

reaction	log K^b
$Zn^{2+} + gly^- \rightleftharpoons Zn(gly)^+$	4.898
$Zn(gly)^+ + gly^- \rightleftharpoons Zn(gly)_2$	9.007
$Zn^{2+} + H(gly) \rightleftharpoons Zn(Hgly)^{2+}$	0.12
$H^+ + gly^- \rightleftharpoons H(gly)$	9.382[c]
$H(gly) + H^+ \rightleftharpoons H_2(gly)^+$	2.380[c]

[a] C. Childs and D. Perrin, *J. Chem. Soc. A*, 1039 (1969). [b] At 37 °C and $\mu = 0.15$. [c] E. J. King, *J. Am. Chem. Soc.*, **67**, 2178 (1945); **73**, 155 (1951).

Table II. Experimental Conditions and Ultrasonic Parameters at 25 °C and $\mu = 2.0$

Σ[M], M	Σ[L], M	pH	f_r, MHz	$(\alpha'\lambda)_{\max} \times 10^4$
0.50	1.00	2.0	14.0	63.3
0.50	1.00	2.5	14.8	91.0
0.50	1.00	3.0	15.8	102.5
0.50	1.00	3.5	16.2	108.2
0.50	1.00	4.0	16.3	103.6
0.50	1.00	4.5	17.3	85.2
0.25	0.25	2.0	11.0	11.4
0.25	0.50	2.0	11.5	22.4
0.25	1.00	2.0	12.5	38.6
0.50	0.50	2.0	13.5	43.2
0.50	1.50	2.0	14.8	100.2
0.50	2.00	2.0	15.8	120.9
0.50	0.25	3.0	13.0	31.4
0.50	0.50	3.0	13.8	55.8
0.50	1.50	3.0	17.5	131.2
2.00[a]	1.00	4.4	22.2	77.1

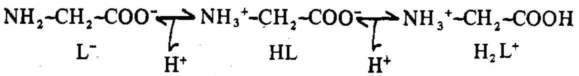
[a] $\mu = 4.1$.

Results and Discussion

The relaxation absorption was observed in all of the solutions containing $Zn(NO_3)_2$ and glycine. Representative absorptions are shown in Figure 1. A blank solution of either $Zn(NO_3)_2$ or glycine, however, showed no discernible relaxation absorption. These facts show that the hydrolysis reaction of Zn^{2+} and the acid dissociation reaction of glycine are not involved in the present relaxation absorption. The observed absorption is accordingly ascribed to the zinc–glycine complex formation reaction.

The measurements were carried out for solutions containing different metal–ligand concentrations and at different pH values. The experimental conditions and the results are summarized in Table II where the following features can be seen. With increase in the value of pH, f_r increases while $(\alpha'\lambda)_{\max}$ increases and reaches a maximum value at pH 3.5. In the variation of ligand concentration at pH 2.0 both f_r and $(\alpha'\lambda)_{\max}$ increase with an increase of the ligand concentration.

It is well-known that glycine takes the following ionized forms in alkaline, neutral, and acidic ranges:



$$\underset{L^-}{NH_2\text{-}CH_2\text{-}COO^-} \underset{H^+}{\rightleftharpoons} \underset{HL}{NH_3^+\text{-}CH_2\text{-}COO^-} \underset{H^+}{\rightleftharpoons} \underset{H_2L^+}{NH_3^+\text{-}CH_2\text{-}COOH} \tag{2}$$

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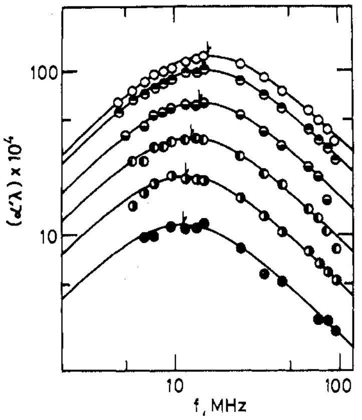

Figure 1. Concentration dependence of the ultrasonic absorption in the Zn–glycine complex solutions at pH 2.0: ●, $\sum$[Zn] = 0.25 M, $\sum$[gly] = 0.25 M; ◑, $\sum$[Zn] = 0.25 M, $\sum$[gly] = 0.50 M; ◐, $\sum$[Zn] = 0.25 M, $\sum$[gly] = 1.00 M; ◒, $\sum$[Zn] = 0.50 M, $\sum$[gly] = 1.00 M; ◓, $\sum$[Zn] = 0.50 M, $\sum$[gly] = 1.50 M; ○, $\sum$[Zn] = 0.50 M, $\sum$[gly] = 2.00 M.

Under the present pH range, the major species are zwitterion, HL, and cation, H_2L^+.

It has been generally believed that the zwitterion of the amino acid is unreactive, even at high concentrations, and only the anion is reactive for the metal complex formation.[1,2,4-6] On the other hand, however, some experimental evidence of complex formation of the zwitterion has been provided.[7] The following mechanisms were accordingly examined in order to explain the observed relaxation absorption data.

Complex Formation Reactions with Glycine Anion. It is well-known that the successive complex formation of Zn with glycine proceeds according to the reactions

$$Zn^{2+} + L^- \rightleftharpoons ZnL^+ \qquad (3)$$

$$ZnL^+ + L^- \rightleftharpoons ZnL_2 \qquad (4)$$

$$ZnL_2 + L^- \rightleftharpoons ZnL_3^- \qquad (5)$$

The concentration dependences of f_r and of the amplitude of absorption, $(\alpha'\lambda)_{max}$, were investigated for each reaction. Several coupled reactions of them were also examined. None of the reactions, however, could give a satisfactory interpretation for the present relaxation absorption. Moreover, the concentration of L^- was less than 10^{-5} M and in an extreme case decreased to 10^{-10} M. The concentrations of ZnL_2, ZnL_3^-, and the higher order complexes were also extremely low. These concentrations are not favorable for study by the ultrasonic absorption technique.

By using a *T*-jump method, Stuehr et al.[5] have found a relaxation phenomenon in a Zn–glycine solution of high concentration which is due to the structural change of the ZnL_2 complex, i.e., tetrahedral–octahedral transition. This reaction is, however, too slow to be assigned for the origin of the present relaxation absorption.

In order to examine the relationship between the relaxation absorption and the chelate-ring closure step, the Zn complexes of the monoaminomonocarboxylic acids of various chain lengths were investigated. The representative relaxation spectra of the Zn complex solutions of gly, β-ala, γABA, and εACA are shown in Figure 2. It is noticeable that a large absorption was also observed in the case of a ligand of long chain. It is well-known that, when chelate-ring size increases beyond five members, the stability of the complex which is formed decreases.[13] Chelate complex formation is not preferred in the case of γABA and εACA ligands since the seven- and the nine-membered chelate complexes, respectively, would be formed. Accordingly the chelate-ring closure step of glycine can be ruled out as the origin of the observed relaxation absorption.

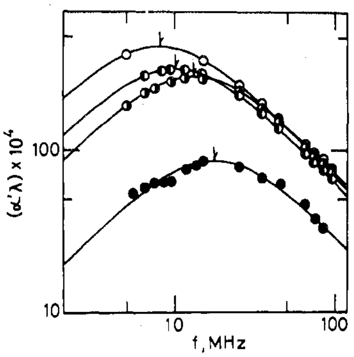

Figure 2. Ultrasonic absorption spectra of the Zn–amino acid solutions at 25 °C. The concentrations are $\sum$[Zn] = 0.50 M and $\sum$[amino acid] = 1.00 M: ●, gly at pH 4.5; ◑, β-ala at pH 5.5; ◐, γABA at pH 5.5; ○, εACA at pH 5.0.

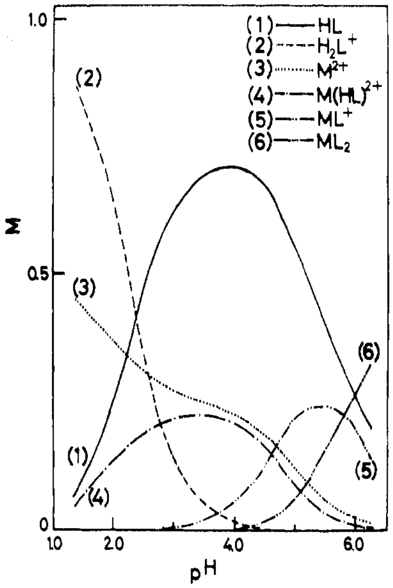

Figure 3. pH dependence of the concentrations of the ionic species in the Zn–glycine complex solution; $\sum$[Zn] = 0.50 M and $\sum$[gly] = 1.00 M.

As shown above, a variety of the complex formation reactions of the glycine anion were examined but could not explain the experimental data.

Complex Formation Reactions with Glycine Zwitterion. Compared with the anion ligand, the zwitterion ligand exists in a relatively high concentration under the present experimental conditions as shown in Figure 3. The complex formation with Zn^{2+} through the carboxyl group is expressed as

$$Zn^{2+} + HL \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Zn(HL)^{2+} \qquad (6)$$

According to the pH titration data by Childs and Perrin,[7] a definite amount of $Zn(HL)^{2+}$ complex is formed at the pH values which were studied. With the use of their data in Table I, the concentrations of the ionic species were calculated and the pH dependences are shown in Figure 3. Under the present experimental conditions, the above reaction is coupled with the acid dissociation equilibrium of glycine.

$$H_2L^+ \underset{k_b'}{\overset{k_f'\ \ H^+}{\rightleftharpoons}} HL \underset{k_b}{\overset{Zn^{2+}\ \ k_f}{\rightleftharpoons}} Zn(HL)^{2+} \qquad (7)$$

Since the protonation reaction is very rapid, the relationship

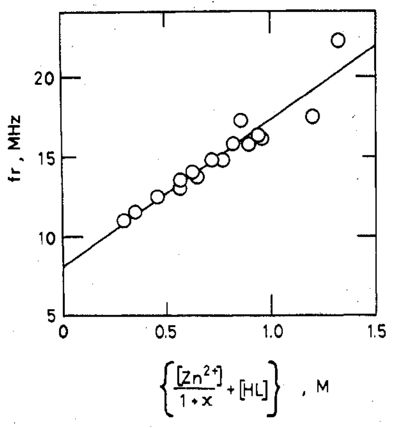

Figure 4. Plot of f_r vs. the concentration term of eq 8.

between the relaxation frequency and the rate constants is given as

$$2\pi f_r = k_f\left(\frac{[Zn^{2+}]}{1+x} + [HL]\right) + k_b \qquad (8)$$

where $x = [H^+]/(K_a + [HL])$ and K_a is the acid dissociation constant of glycine. The plot of f_r vs. the concentration term of eq 8 is shown in Figure 4. The values of k_f and k_b were obtained from the slope and the intercept of the straight line, respectively; $k_f = 5.9\ (\pm 0.4) \times 10^7\ M^{-1}\ s^{-1}$ and $k_b = 5.1\ (\pm 0.5) \times 10^7\ s^{-1}$. The ratio of these values gives the stability constant; $K = k_f/k_b = 1.2\ (\pm 0.2)\ M^{-1}$. This value is in fair agreement with that used for the calculation of the ionic concentrations.[7] This fact, together with the linearity of the plot, implies that reaction 7 is the origin of the relaxation absorption.

Further support of this assignment was given by the ionic concentration dependence of $(\alpha'\lambda)_{max}$. For reaction 7, $(\alpha'\lambda)_{max}$ is given by

$$(\alpha'\lambda)_{max} = \frac{\pi(y\Delta V_1 + \Delta V_2)^2}{2\beta_0 RT}\left\{y^2\left(\frac{1}{[H_2L^-]} + \frac{1}{[H^+]} + \frac{1}{[HL]}\right) - \frac{2y}{[HL]} + \frac{1}{[HL]} + \frac{1}{[Zn^{2+}]} + \frac{1}{[Zn(HL)^{2+}]}\right\}^{-1} \qquad (9)$$

where

$$y = \frac{-k_f'[H^+]}{\{k_f([Zn^{2+}] + [HL]) + k_b\} - \{k_f'([H^+] + [HL]) + k_b'\}}$$

R is the gas constant, β_0 is the adiabatic compressibility, T is the temperature, and ΔV_1 and ΔV_2 are the volume changes of the protonation reaction and reaction 6, respectively. Examination of the kinetic studies of the protonation reaction of carboxylic acids[14] revealed that the value of y is much smaller than unity. Equation 9 can, therefore, be transformed approximately under the present experimental conditions as

$$(\alpha'\lambda)_{max} = \frac{\pi(\Delta V_2)^2}{2\beta_0 RT}\left\{\frac{1}{[HL]} + \frac{1}{[Zn^{2+}]} + \frac{1}{[Zn(HL)^{2+}]}\right\}^{-1} \qquad (10)$$

In Figure 5, the experimental values of $(\alpha'\lambda)_{max}$ are plotted against the concentration term of eq 10. The fact that these points form a straight line indicates the validity of the assignment. From the slope of the plot, the value of ΔV_2 was found to be 7.5 $cm^3\ mol^{-1}$.

Complex formation reactions in solution have usually been interpreted by a stepwise mechanism in which the ion-pair and the inner-sphere complex are included.[15] If the inner-sphere complex formation step is rate determining for the reaction, k_f is expressed as

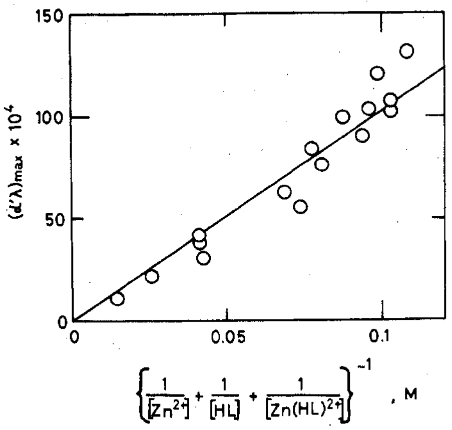

Figure 5. Plot of $(\alpha'\lambda)_{max}$ vs. the concentration term of eq 10.

$$k_f = K_O k_{H_2O} \qquad (11)$$

where K_O is the ion-pair formation constant and k_{H_2O} is the dissociation rate constant of the water molecule coordinated to the metal ion. In the present case, however, the experimental condition of a very high ionic strength and the specificity of the ligand (i.e., zwitterionic form) prevented us from estimating the K_O value, so that the derivation of the k_{H_2O} value was not possible.

The ultrasonic absorption of glycine complex can be compared to those of other homologous amino acid complexes. The ultrasonic absorptions of the solutions of Zn complexes of gly, β-ala, γABA, and ϵACA in Figure 2 indicate definite tendencies for f_r and $(\alpha'\lambda)_{max}$; the longer the chain length of the ligand the smaller f_r and the larger $(\alpha'\lambda)_{max}$. It may generally be observed that the complex becomes more stable[16] and k_f increases while k_b decreases with the increase of the chain length of the zwitterion ligand. At the same metal–ligand concentrations as in the case of Figure 2, the free ion concentrations decrease with the increase of the stability of the complex. According to the above ideas, the behavior of f_r is consistent with that of k_b and of the free ion concentrations. This fact, with eq 8, implies that the variation of f_r in Figure 2 is induced mainly by that of k_b and/or the free ion concentrations ($[Zn^{2+}] + [HL]$). A similar relation between f_r (or k_b) and the pK_a of the ligand has been observed in other analogous reactions.[17,18]

On the assumption that the value of ΔV_2 is constant and independent of the ligands, the predominant factor in the concentration term determining the magnitude of $(\alpha'\lambda)_{max}$ is $[Zn(HL)^{2+}]$. Moreover, in the solutions with the same metal–ligand concentrations, $[Zn(HL)^{2+}]$ becomes larger with an increase of the chain length of the ligand. These facts provide a satisfactory interpretation for the behavior of $(\alpha'\lambda)_{max}$ in Figure 2.

Registry No. Zn, 7440-66-6; gly, 56-40-6; β-ala, 107-95-9; γABA, 56-12-2; ϵACA, 60-32-2.

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(16) The stability constants of the Zn complexes of amino acid zwitterions have not been reported except that of glycine. The following view, however, can be approved. It is generally admitted that the larger the pK_a value of a ligand is, the more stable is the complex. The pK_a values of the carboxyl group of the amino acids used were as follows: gly, 2.45; β-ala, 3.70; γABA, 3.94; ϵACA, 4.34. In the case of a zwitterion ligand, the electrostatic repulsion between the charge of the metal ion and that of the $-NH_3^+$ group interferes with complex formation. This interference effect decreases with the increase of chain length of the ligand. After all, it is concluded that the complex becomes more stable with the increase of chain length of the zwitterion ligand.

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A Kinetic Study of Americium(III) and *trans*-1,2-Diaminocyclohexanetetraacetate

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Received May 4, 1978

The rates of formation and dissociation of $AmDCTA^-$ (DCTA = *trans*-1,2-diaminocyclohexanetetraacetate) have been measured respectively by stopped-flow and conventional spectrophotometric methods. A mechanism is proposed which involves coordination of Am(III) to three acetate groups of $H_n(DCTA)^{n-4}$ to form the relatively long-lived intermediate AmHDCTA* which subsequently loses the proton. The slow step of the formation reaction is postulated to be associated with the formation of an americium–donor nitrogen bond.

Introduction

The kinetics of lanthanide and actinide complexation by aminopolycarboxylates have been studied by several authors.[1-12] The majority of these studies involve exchange of metal cations which was found to occur through two mechanisms. The major path for the exchange at pH 6 involves hydrogen ion catalysis in which the metal complex is protonated in a series of proton additions leading ultimately to the decomposition of the complex and, hence, to metal exchange. A second term in the kinetic equation is acid independent. Some authors have interpreted this as related to a metal ion catalyzed decomposition of the complex in which the ligand serves as a bridge between the incoming and outgoing metal ions. However, alternate explanations are possible for the hydrogen ion independent path which do not involve formation of a binuclear intermediate.

Glentworth[2] found both acid-dependent and -independent terms in the Ce(III) isotopic exchange reaction with $CeDCTA^-$. D'Olieslager and Choppin[3] observed only an acid-dependent term in the equation for the exchange reactions between $LaEDTA^-$ and $^{152-154}Eu(III)$, $^{144}Ce(III)$, and $^{241}Am(III)$. Subsequently, Choppin and Williams[4] showed that the dynamics of the metal-exchange reaction between $EuEDTA^-$ and Am^{3+} has an empirical rate law with acid-dependent and -independent terms. Both DeJonghe,[5] who studied $^{177}Tm(III)$ exchange in $TmEDTA^-$, and D'Olieslager and Oeyen,[6] who studied the $^{152-154}Eu(III)$ exchange of $EuDCTA^-$, found kinetic descriptions with both terms. Studies that use radioactive isotopes of the aquo ions are complicated by the necessity of incorporating the criteria of isotopic equilibria in the treatment of the data. The procedure of Margerum et al.[12,13] used the exchanging metal ion in excess over the bound metal ion. This exchanging metal ion has a higher formation constant with the ligand than the chelated metal ion. In such cases the equilibrium concentration of the original chelated species is small enough to be neglected, greatly simplifying the data treatment.

The formation rates of metal chelates may be measured directly by stopped-flow techniques.[12-14] Nyssen and Margerum[12] used stopped-flow spectrometry to study the direct formation of the La–DCTA complex and conventional spectrometry to measure the decomposition of the DCTA complexes of Y and all (except Pm) of the lanthanide elements. They derived a mechanism for lanthanide complexation by DCTA in whch the complex LnHDCTA* is formed as an intermediate species in a rapid preequilibrium step. In their scheme this intermediate has the metal ion bound to three acetates of the ligand. They postulated slow deprotonation of LnHDCTA* as the rate-determining step in the formation of the stable complex $LnDCTA^-$.

We have investigated the formation and dissociation kinetics of $AmDCTA^-$ by both stopped-flow and conventional spectrophotometry to determine whether this mechanism for lanthanide complexation may be used to explain the kinetics of formation of trivalent actinide ion complexes with DCTA.

Experimental Section

The crystalline monohydrate acid form of DCTA (*trans*-1,2-diaminocyclohexane-*N*,*N*,*N'*,*N'*-tetraacetic acid) was obtained from the Aldrich Chemical Co. It was recrystallized by dissolution in dilute base, followed by lowering the pH to about 5 with $HClO_4$.

The ^{243}Am was obtained from Argonne stocks and was used without any further purification.

The $LiClO_4$ used to adjust the ionic strength and the acetate buffer was made from reagent grade materials. Hydrogen ion concentrations, pcH, were calculated from pH measurements on a Beckman Model G digital pH meter using a combination electrode. The pcH calibration was made by measuring the pH of 0.1, 0.01, and 0.001 M acid solutions (μ = 0.1). The calibration was found to fit the equation

$$\mathrm{pcH} = \mathrm{pH} + 0.10$$

All aqueous solutions were prepared with triply distilled water.

The stopped-flow experiments were performed on a Durrum-Gibson (Model D 110) stopped-flow spectrometer. The flow system was thermostated to ±0.1 °C. The output of the spectrometer was stored in a Biomation 802 transient recorder which was interfaced for on-line operation with a Xerox Sigma V computer. Seven to twelve replicate determinations were carried out for each set of conditions. The absorbance was monitored at 245 nm ($\epsilon \sim 560\ cm^{-1}\ M^{-1}$) where a strong absorption characteristic of $AmDCTA^-$ on the shoulder of an americium–acetate UV absorption was observed. The concentrations of all reagents and of the buffer were varied independently to determine their effect on the rate of reaction.

Though a detailed analysis of the UV absorption spectra of the $AmDCTA^-$ complex (Figure 1) was not made, it is believed that the 245-nm band on the shoulder of the larger band is due to metal-to-ligand charge transfer. A spectrum of Am(III) in acetate solution shows a broad band centered at ca. 200 nm. A similar spectrum of $AmDCTA^-$ in acetate medium exhibits a broadening of the amer-

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0020-1669/78/1317-3374$01.00/0