



Kinetics and mechanism of the reactions of Cu(II)-N,N-Diglycylethylenediamine with triethylenetetramine and Ethylenediaminetetraacetate
by Presley Kirkland Mitchell

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University
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Abstract:

The substitution kinetics and mechanisms of copper(II)-N,N'-diglycylethylenediamine (CuH-2DGEN) with triethylenetetramine (TRIEH) and ethylenediaminetetraacetate (EDTA) ion were investigated. The overall rate expression for each reactant was resolved, and the rate constants for the reaction pathways were determined.

The reaction between Copper(II)-N,N'-diglycylethylenediamine and triethylenetetramine proceeded via a nucleophilic and general acid catalysis pathway. The rate constants are $k_T = 650 \text{ M}^{-1} \text{ s}^{-1}$, $k_{HT} = 210 \text{ M}^{-1} \text{ s}^{-1}$, $k_{H_2T} = 87 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_3T} = 2100 \text{ M}^{-1} \text{ s}^{-1}$.

The reaction between copper(II)-N,N'-diglycylethyethylenediamine and ethylenediaminetetraacetate ion proceed via a general acid catalysis pathway. The rate constants for the mono- and diprotonated species are $k_{HEDTA} = 56 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_2EDTA} = 1.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

Other replacing ligand independent rate constants that are applicable to both systems are $k_d = 0.043 \text{ s}^{-1}$, $k_{H_3BO_3} = 116 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_3O} = 1.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$.

KINETICS AND MECHANISM OF THE REACTIONS OF Cu(II)-N,N' -
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A thesis submitted in partial fulfillment
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of

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in

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The substitution kinetics and mechanisms of copper(II)-N,N'-diglycylethylenediamine (CuH_2DGEN) with triethylenetetramine (TRIE) and ethylenediaminetetraacetate (EDTA) ion were investigated. The overall rate expression for each reactant was resolved, and the rate constants for the reaction pathways were determined.

The reaction between copper(II)-N,N'-diglycylethylenediamine and triethylenetetramine proceeded via a nucleophilic and general acid catalysis pathway. The rate constants are $k_T = 650 \text{ M}^{-1} \text{ s}^{-1}$, $k_{HT} = 210 \text{ M}^{-1} \text{ s}^{-1}$, $k_{H_2T} = 87 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_3T} = 2100 \text{ M}^{-1} \text{ s}^{-1}$.

The reaction between copper(II)-N,N'-diglycylethylenediamine and ethylenediaminetetraacetate ion proceed via a general acid catalysis pathway. The rate constants for the mono- and diprotonated species are $k_{HEDTA} = 56 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_2EDTA} = 1.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

Other replacing ligand independent rate constants that are applicable to both systems are $k_d = 0.043 \text{ s}^{-1}$, $k_{H_3BO_3} = 116 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{H_2O} = 1.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$.

CHAPTER 1

INTRODUCTION

The kinetics and mechanisms of the transfer of copper(II) between peptide and amino acid complexes are important to the understanding of the biological transport of this metal ion.

The imbalance of copper within the biological system has been associated with an assortment of diseases. For example, the rare inherited Wilson's disease involves the inability to excrete copper and is characterized by high free copper levels (1). The treatment of this disease involves the removal of copper via copper chelating agents such as ethylenediaminetetraacetate ion and D-penicillamine (1). Triethylenetetramine (TRIEEN) was also found to be an effective chelating agent (2).

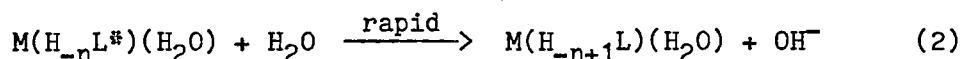
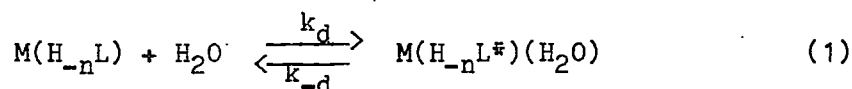
Metal peptide or oligopeptide complexes have the general formula $M(H_nL)$, where there are n peptide or amide groups bonded to the metal ion. Evidence for metal-N(peptide) bonding in diglycine, triglycine and tetraglycine have been verified by infrared and visible spectroscopy, as well as potentiometric measurements (3). The results of X-ray crystallographic studies (1) have shown, unequivocally, the peptide proton displacement in the solid and the coordination of the resulting negative peptide groups to metal ions.

The chemical kinetic behavior of the metal-N(peptide) bonds have some interesting properties. Although the N(peptide) group is a strong donor that is tightly bound to metal ions, it tends to be the group displaced in protonation, dissociation and nucleophilic

reactions. The configurations of copper-triglycine, copper-glycylglycyl-L-histidine and copper-tetraglycine are shown in Figure 1. These examples will aid in understanding kinetic variations that occur in metal-N(peptide) complexes. The above complexes are unique because they contain different terminally bonded groups. These structural differences result in different reaction kinetics.

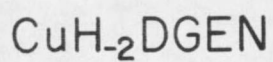
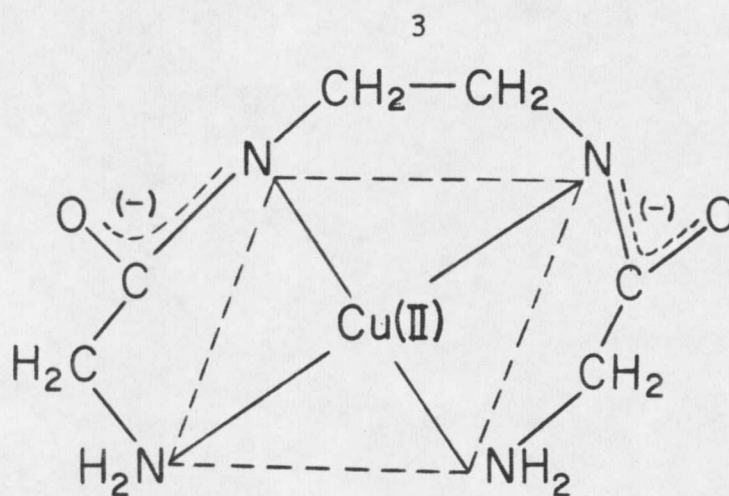
The various reaction pathways that occur with metal-N(peptide) complexes include the solvent dissociation, nucleophilic and protonation pathways (4).

The solvent dissociative pathway is independent of the replacing ligand concentration. The solvent, water in this discussion and study, can act as an acid or nucleophile, leading to the cleavage of the metal-N(peptide) bond. Once this bond breaks, other water molecules will rapidly transfer a proton to the very basic free deprotonated-peptide nitrogen. Equations 1 and 2 illustrate the reactions in which solvent dissociation is believed to occur (5),

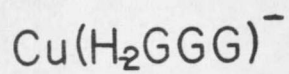
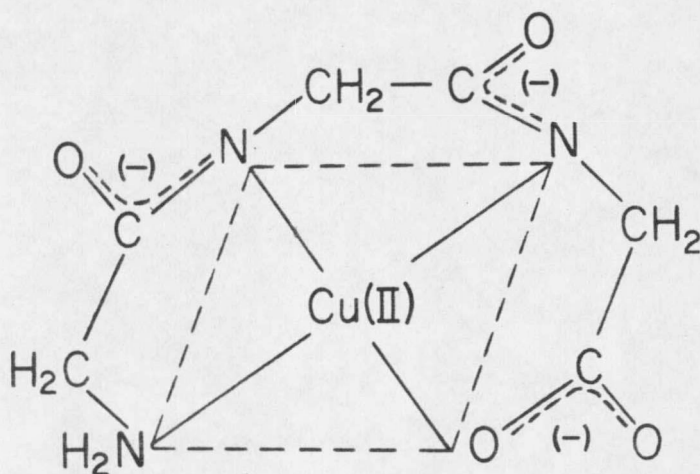


where $(H_{-n}L^*)$ refers to the presence of a free deprotonated-peptide nitrogen.

The dissociative rate constant for copper triglycine was experimentally found to be 0.12 s^{-1} (6). This k_d term is associated

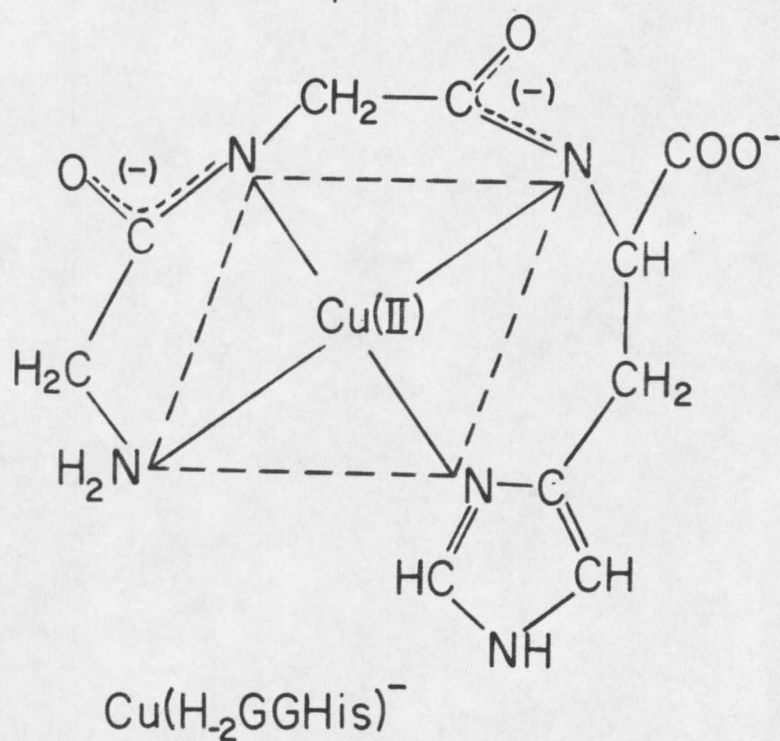


Copper(II)-N,N'-Diglycylethylenediamine

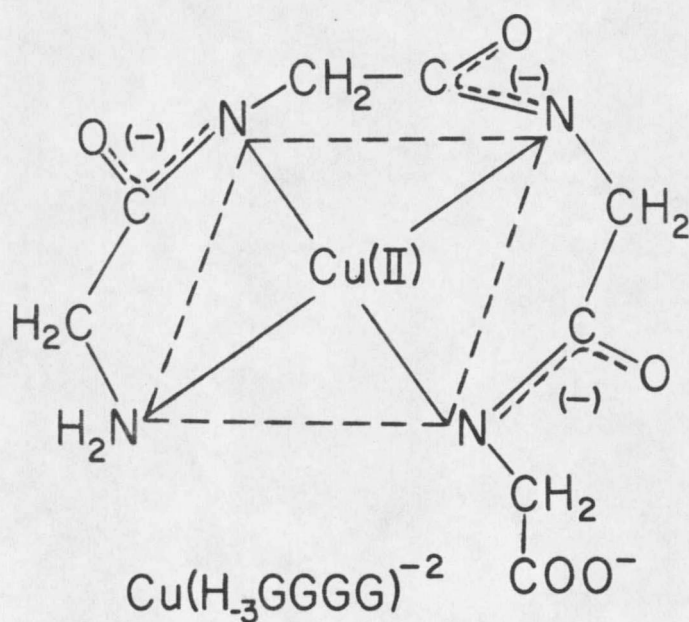


Copper(II)-Triglycine

Figure 1. Structures of several copper(II) complexes.



Copper(II)-Glycylglycyl-L-histidine



Copper(II)-Tetraglycine

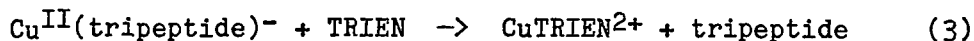
Figure 1 (continued). Structures of several copper(II) complexes.

with the molecular rearrangement of the complex to form a species that can react directly with the replacing ligand. On the other hand, if the terminal carboxylate group of the copper triglycine complex is replaced with a terminal histidine residue, as in the case of copper glycylglycyl-L-histidine, the dissociative rate constant is reduced to $7.5 \times 10^{-4} \text{ s}^{-1}$ (7). This sluggish dissociative rate is due to increased stabilization that is imparted by the presence of the imidazole group.

The second major pathway, the nucleophilic pathway, involves the coordination of the replacing ligand to the metal, replacing one or more of the metal-N(peptide) bonds. These nucleophilic reactions result in the formation of mixed ligand complexes or, if a multidentate ligand is used, complete displacement of the peptide from the metal ion (5).

The most effective nucleophiles for displacing metal-N(peptide) complexes are multidentate ligands which fulfill two conditions (5): a) the ligand must contain an amine group which can easily coordinate in a planar position of the metal-N(peptide) complex; and b) the ligand must have at least one additional amine group available as the entering group to allow an associative type mechanism to operate. Other important factors include the coordinating strength of the donor groups, the chelating ability of the nucleophile and its absence of steric hindrance to coordination in a kinetically accessible planar position of the metal.

An important aspect of metal-N(peptide) reactions are steric effects that occur in nucleophilic reactions in which TRIEN displaces a double-deprotonated tripeptide. Equation 3 illustrates the reaction of such a copper tripeptide (8).



The rate is first-order in each reactant, as shown in Equation 4.

$$\text{rate} = \frac{d[\text{CuTRIEN}^{2+}]}{dt} = k[\text{Cu}^{\text{II}}(\text{tripeptide})^-][\text{TRIEN}] \quad (4)$$

The observed rate in which the tripeptide complex is displaced is greatly affected by amino acid residues in the terminal position. If the terminal residue is a carboxylate group, as in the case of copper triglycine, the nucleophilic rate constant for the unprotonated TRIEN is $1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (9), while the rate constants for the mono- and diprotonated TRIEN species are 5.1×10^6 and $1.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively (9). Since these rates decrease with decreasing nucleophilic potential ($\text{TRIEN} > \text{HTRIEN} > \text{H}_2\text{TRIEN}$), this is conclusive evidence for the nucleophilic pathway.

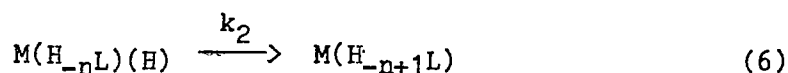
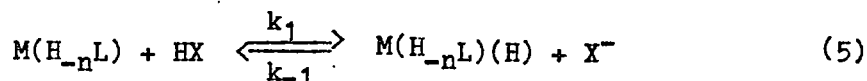
If the carboxylate group of copper triglycine is replaced with a terminal peptide group, as is the case for copper tetraglycine, the rate constant for the unprotonated TRIEN species is $490 \text{ M}^{-1} \text{ s}^{-1}$ (10). This reduction by more than four orders of magnitude is due to the much stronger ligand field donor of the peptide group over the carboxylate group.

In general, the effect of increasing the number of copper-N(peptide) bonds is to decrease the chances for nucleophilic attack (5). Other nitrogen donors in the planar position have similar effects.

Structural considerations within the complexes have tremendous effects on the rates of nucleophilic attack, as was evidenced in a study of peptide steric effects (11). This study showed variations of residues of glycyl to L-alanyl or L-leucyl in the copper tripeptide complexes resulted in steric problems with TRIEN displacement. Methyl groups substituted for hydrogens in the first chelate ring resulted in small variations of TRIEN rate constants. However, if methyl groups were substituted in either the second or third chelated rings, then the rates of nucleophilic substitution were considerably retarded. It was concluded that the methyl groups sterically hinder the TRIEN displacement reactions.

The third pathway of importance in the reaction of metal oligopeptide complexes is represented by the proton-transfer mechanism. Most proton-transfer reactions of base with H_3O^+ and of acids with OH^- are extremely fast and undergo diffusion-controlled rates of approximately 10^{10} to $10^{11} \text{ M}^{-1} \text{ s}^{-1}$ (12).

The rate constants for some of the protonation reactions illustrated in Equations 5 and 6 with Ni(II) and Cu(II) peptide complexes are on the order of 10^4 to $10^8 \text{ M}^{-1} \text{ s}^{-1}$ (6,10,13,14).



As can be seen, the metal-N(peptide) complex reactions with H_3O^+ are many orders of magnitude slower than the diffusion-controlled rates.

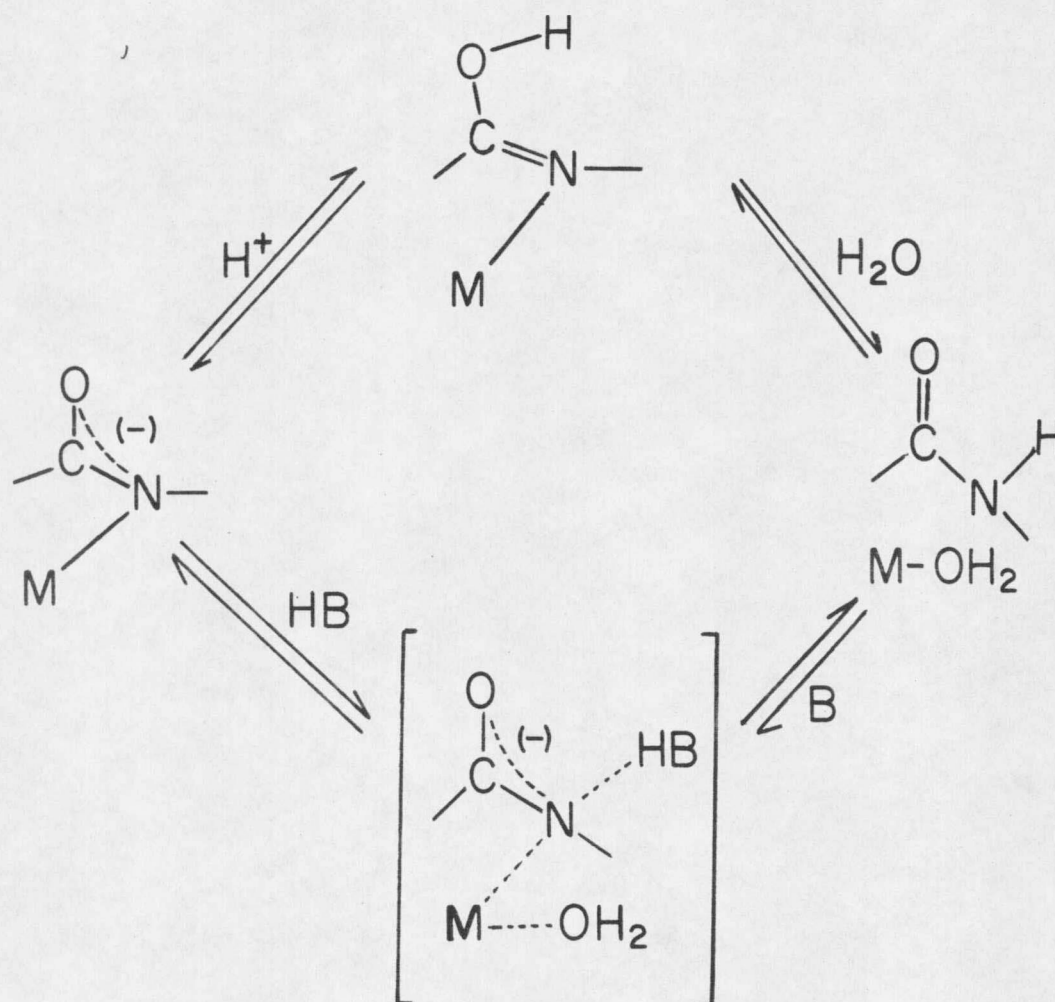
The proton-transfer reactions of metal-N(peptide) complexes occur by two distinct mechanisms (15,16), as depicted in Figure 2.

In the "outside protonation" pathway, rapid protonation of the peptide oxygen is followed by metal-N(peptide) bond cleavage and solvation of the metal ion. The outside protonated species is a true intermediate, and this pathway is not subjected to general acid catalysis because the rate determining step (k_2) occurs after protonation.

The other mechanism is known as the "inside protonation" pathway. This mechanism is characterized by the transfer of a proton directly to the coordinated peptide nitrogen. The proton transfer and metal-N(peptide) bond breaking occur simultaneously. The rate determining step is shifted to k_1 of Equation 5. This pathway is subjected to general acid catalysis.

One of the unique characteristics of general acid catalysis is shown in the Brønsted plot of Figure 3 (5). The plot has the slope of an Eigen-type Brønsted plot (17) in which the slope changes from one to zero as the diffusion-controlled limit is reached. However, in the case of Figure 3, the slope of zero is obtained at values as

OUTSIDE PROTONATION PATHWAY



INSIDE PROTONATION PATHWAY

Figure 2. Proposed mechanism for the proton-transfer reactions of metal-N(peptide) complexes.

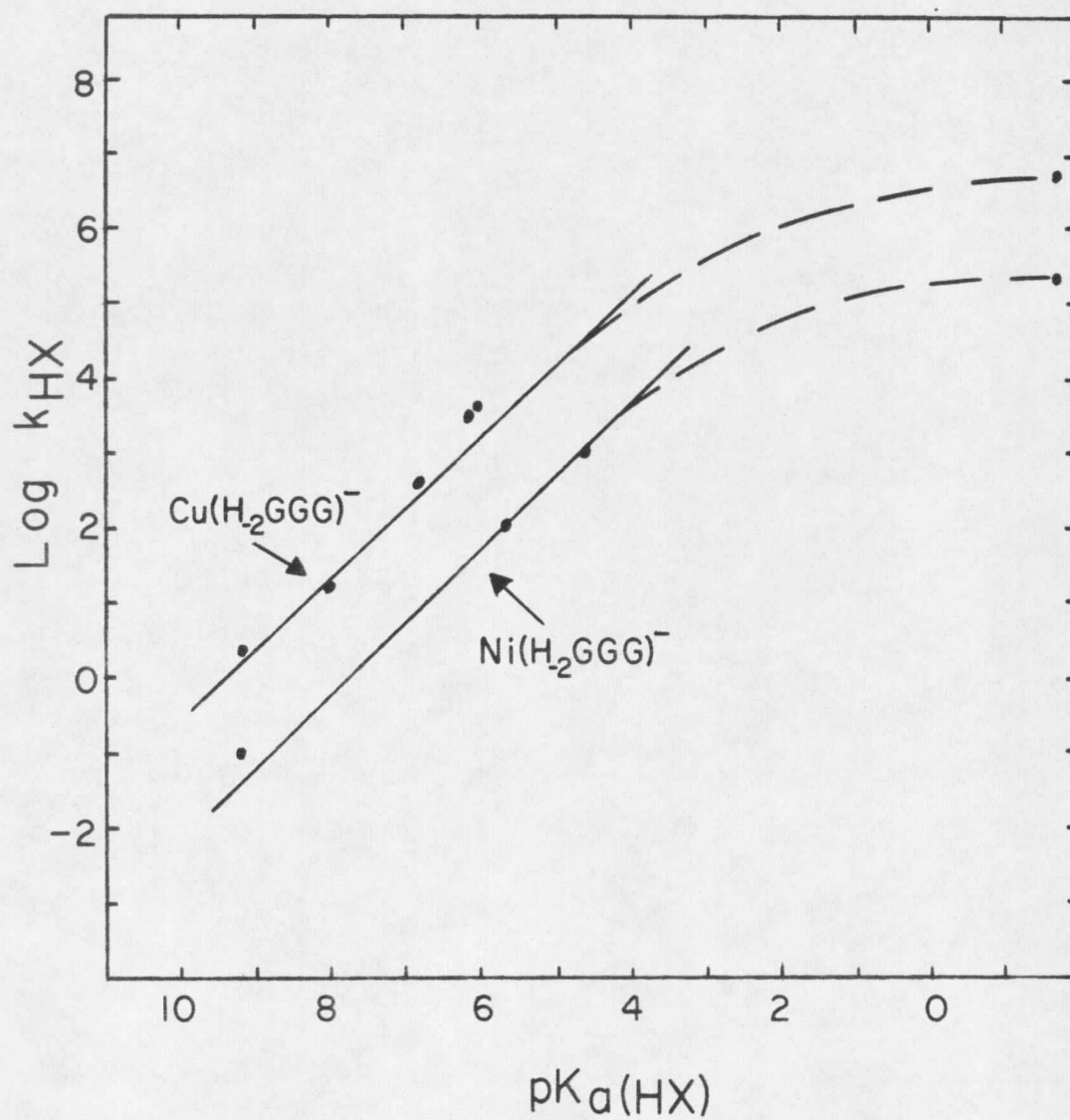


Figure 3. Rate constants for the reactions of $\text{M}(\text{H}_2\text{GGG})^-$ complexes with HX as a function of the acidity of HX . The solid line corresponds to the Brønsted α value of unity.

small as $10^5 \text{ M}^{-1} \text{ s}^{-1}$ rather than the expected $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for diffusion-controlled reactions.

Many protonation reactions have been investigated for different Cu(II) and Ni(II)-N(peptide) complexes. The reaction of both copper(II) and nickel(II) triglycine were experimentally found to be general acid catalyzed (9,18) with protonation rate constants for H_3O^+ of 4.9×10^6 and $2.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. On the other hand, the H_3O^+ protonation constant for copper tetraglycine was $1.8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (10). Therefore, it has been postulated that the presence of a third nitrogen donor in the copper-N(peptide) complexes is more susceptible to protonation reactions (5).

In comparing reaction rates between copper and nickel peptide complexes, one will notice that nickel reacts at a much slower rate than copper for a similar complex. This reduced reactivity pattern is due to the different crystal field stabilization energy of the two metal ions. The nickel ion, with its d^8 system has twice the crystal field stabilization energy of the d^9 system of copper. This factor results in the stronger binding in nickel complexes, and is reflected in reduced reactivity.

The preceding discussions have illustrated the various pathways in which metal-N(peptide) complexes react. In general, those complexes with terminal carboxylate groups are more readily displaced in nucleophilic reactions. The presence of an imidazole group in the terminal position reduces the chances for direct nucleophilic attack. For proton transfer reactions, hydrogen bonding, steric factors and

electronic rearrangements during proton transfer are important in determining the rate of general acid catalysis. These factors, along with many others, are important in determining protonation reaction rates.

The reaction of TRIEN and EDTA with CuH_2DGEN is the focus of this study. The copper(II) complex of DGEN has a configuration different from other oligopeptide complexes. These differences include: a) the presence of an amine group at both terminal binding positions, b) the symmetry of the CuH_2DGEN complex, other oligopeptide complexes being asymmetrical, and c) the location of the peptide oxygen in the third chelate ring. How these differences affect the reactivity patterns will be investigated in the following study.

CHAPTER 2

STATEMENT OF THE PROBLEM

The reactivity patterns of many Cu(II) oligopeptide complexes have been mentioned in the previous discussion. It was found that these complexes are displaced via solvent dissociation, nucleophilic and proton transfer mechanisms and that different terminally bonded groups result in different reaction rates.

N,N'-Diglycylethylenediamine (DGEN) forms a violet, tetragonally distorted octahedral complex with Cu(II) in which both DGEN amide protons have been ionized. Coordination of the ligand to copper is facilitated by two terminal amine groups and two deprotonated amide nitrogens. The deprotonated complex is designated by CuH_2DGEN (Figure 1). This complex is different from other oligopeptide complexes because it has a plane of symmetry through the metal center and the second chelate ring. Also, in the CuH_2DGEN complex, terminal binding is accomplished by amine groups, rather than the usual carboxylate or peptide groups. How these structural differences affect reactivity patterns is unknown.

To investigate these reactivity patterns, the reactions of TRIEN and EDTA with CuH_2DGEN have been studied.

CHAPTER 3
EXPERIMENTAL

Apparatus

A Durrum Model D-103 stopped-flow instrument was used to determine all kinetic measurements. The instrument contains a constant temperature bath housing for reactant syringes and a thermostated observation cell compartment. The ultraviolet light source was powered by a Beckman hydrogen lamp power supply. Interfaced to the instrument was a Tektronic type 564 storage oscilloscope equipped with a type 3A3 dual trace differential amplifier and a type 2B67 time base. An offset voltage was supplied by a 1.4-volt mercury battery. The applied offset voltage was regulated using a series of potentiometers. An IP-128 photomultiplier tube was powered by an Ortec high voltage power supply. The spectral data was stored and manually extracted from the storage oscilloscope.

A Corning 130 pH meter with an Orion 91-05 combination pH electrode was utilized in all pH measurements. The instrument was calibrated using VWR Scientific standard pH buffer solutions.

Reagents

$\text{Cu}(\text{ClO}_4)_2$ was recrystallized twice from double distilled water. A 9.75×10^{-2} M solution was prepared by dissolving the recrystallized salt in double distilled water and standardized by EDTA titration (19).

Anhydrous sodium perchlorate was prepared by dissolving a known weight of the hydrated salt in boiling double distilled water. The solution was filtered through a 0.45 micrometer millipore filter and evaporated to a predetermined volume. The recrystallized salt was oven dried at 70°C for at least 72 hours to ensure dryness. A 1.0 M solution was prepared by dissolving one mole of the dried salt in one liter of double distilled water.

A 0.10 M sodium borate solution was prepared by dissolving a required amount of dried recrystallized salt in the appropriate amount of double distilled water.

Technical grade triethylenetetramine was purified by vacuum distillation at 0.5 mm mercury. The purified compound was converted into the disulfate salt by adding 2.0 moles of concentrated sulfuric acid dropwise to 1.0 moles of triethylenetetramine dissolved in three volumes of toluene. The temperature was maintained at approximately 0°C with an ice bath. Upon complete reaction, the mixture was stirred for an additional hour, and the resultant precipitate was filtered and dried. The disulfate salt was then twice recrystallized in double distilled water.

A TRIEN stock solution was prepared by dissolving a predetermined weight of the recrystallized salt in the required volume of double distilled water. This solution was standardized by the mole ratio method (20) at 575 nm using 9.75×10^{-2} M $\text{Cu}(\text{ClO}_4)_2$. A pH of 4.8 was maintained using 0.05 M acetate buffer, while the ionic strength was held constant ($\mu = 0.10$ M) with NaClO_4 .

The disodium salt of ethylenediaminetetraacetic acid (EDTA) was purified and recrystallized by the method of Blaedel and Knight (21). A stock solution of the disodium salt was prepared by dissolving a required weight of the purified salt in the appropriate amount of double distilled water. The stock solution of EDTA was standardized by the CaCO_3 method (19) using Erio Chrome Black T as the indicator. The standard solution was stored in a polypropylene container to ensure a constant titer.

The ligand N,N'-diglycylethylenediamine (DGEN) was synthesized by the method of Cottrell and Gill (22). The ligand was characterized by carbon-13 NMR and by elemental analysis (23).

The names and abbreviations of all the chemicals used are found in Table 1, along with the manufacturer and the compound purity.

The complex was formed by the addition of 50% excess DGEN to a solution containing the desired amount of copper. The solution was ionically adjusted to $\mu = 0.10 \text{ M}$ with 1.0 M NaClO_4 . The pH of the solution was raised to the desired value using dilute NaOH. Once within range, the solution was buffered with sodium borate such that $\text{borate}_t = 5.0 \times 10^{-3} \text{ M}$. Upon final pH adjustment, the solution was brought to volume with double distilled water and the vessel was placed in a thermostatic bath at 25°C .

Both TRIEN and EDTA solutions were prepared in a similar manner. The desired aliquot of either stock solution was added to an appropriate vessel, and all further procedures were similar to those described in the preceding paragraph.

Table 1. Names of compounds used and their abbreviations

Name	Abbreviation
N,N'-Diglycylethylenediamine	DGEN ^a
Copper(II) perchlorate	$\text{Cu}(\text{ClO}_4)_2$ ^{b,g}
Sodium perchlorate	NaClO_4 ^{c,g}
Sodium borate	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ^{d,g}
Triethylenetetrammine	TRIEN ^{e,g}
Ethylenediaminetetraacetate	EDTA ^{f,g}

^a Elemental analysis:	<u>element</u>	<u>expected value</u>	<u>measured value</u>
	carbon	29.16%	30.16%
	hydrogen	6.53%	6.70%
	nitrogen	22.67%	21.05%

^bG. Frederick Smith Chemical Company

^cFluka AG, Chemische Fabrik

^dJ. T. Baker Chemical Company

^eKodak Chemical Company

^fMatheson, Coleman and Bell Chemical Company

^greagent grade

Kinetic Measurements

CuH_2DGEN absorbs weakly in the visible region of the electromagnetic spectrum of 519 nm with an absorptivity coefficient, $\epsilon = 144 \text{ M}^{-1} \text{ cm}^{-1}$. Unfortunately, the kinetics could not be evaluated at this wavelength because the final product, CuTRIEN^{2+} , also absorbed at 519 nm with an $\epsilon = 108 \text{ M}^{-1} \text{ cm}^{-1}$. The $\Delta\epsilon(\epsilon_{\text{CuH}_2\text{DGEN}} - \epsilon_{\text{CuTRIEN}^{2+}})$ was not large enough to offer reproducible results. To overcome this problem, the kinetics were evaluated in the ultraviolet region where they absorb strongly. It was experimentally determined that the reaction between CuH_2DGEN and TRIEN could be monitored at 255 nm.

At this wavelength the molar absorptivity values are $3920 \text{ M}^{-1} \text{ cm}^{-1}$ and $4890 \text{ M}^{-1} \text{ cm}^{-1}$ for CuH_2DGEN and CuTRIEN^{2+} , respectively. The large $\Delta\epsilon$ value at this wavelength offered kinetic data with reproducible results.

The reaction between CuH_2DGEN and EDTA was evaluated at 286 nm. At this wavelength CuH_2DGEN has an $\epsilon = 966 \text{ M}^{-1} \text{ cm}^{-1}$, while the product CuEDTA has a molar absorptivity value of $2220 \text{ M}^{-1} \text{ cm}^{-1}$. This large $\Delta\epsilon$ value offered excellent conditions for studying the kinetic system.

The stopped-flow instrument was equipped with two reactant syringes. These syringes were filled with the desired reactants and maintained at 25°C by the bath housing. All 100% transmittance conditions were established with either non-absorbing species, TRIEN or EDTA. The desired offset voltage was applied so that the oscilloscope displayed the greatest sensitivity for that transmittance change. Once the conditions were optimized, the reaction was initiated by pressing the actuate button which pneumatically plunged the reactants through a mixing chamber and then into the observation cell. The acquisition of data was initiated by the stopping syringe, which completed the circuit and triggered the oscilloscope.

The reaction trace on the oscilloscope display was manually extracted in terms of percent transmittance versus time. All transmittance values were converted to absorbance.

A_t is defined as absorbance at time t , and A_∞ is the absorbance at infinite time, or when the reaction was complete.

For an irreversible pseudo first-order reaction, Equation 7 is valid:

$$\frac{A_{\infty} - A_t}{A_{\infty} - A_0} = \exp(-kt) \quad (7)$$

provided A increases with time.

Plots of $-\ln(A_{\infty} - A_t)$ versus time were linear, indicating pseudo first-order conditions. The slopes of their plots are the observed pseudo first-order rate constants, k_{obs} .

The observed pseudo first-order rate constants were plotted versus both $[\text{TRIEN}]_t$ and $[\text{EDTA}]_t$. The following plots yielded a slope equal to the second-order rate constants, k_{slope} . Intercepts of such plots are designated as k_i . The second-order rate constants were further resolved using the equilibria and equilibrium constants shown in Table 2.

Table 2. Equilibria and equilibrium constants

Equilibrium	Equilibrium Constants
$\text{CuDGEN}^{2+} \rightleftharpoons \text{CuH}_{-2}\text{DGEN} + 2\text{H}^+$	$K_{1ab} = 10^{-13.8} \quad (25)$
$\text{HTRIEN}^+ \rightleftharpoons \text{H}^+ + \text{TRIEN}$	$K_{a1} = 10^{-9.74} \quad (26)$
$\text{H}_2\text{TRIEN}^{2+} \rightleftharpoons \text{H}^+ + \text{HTRIEN}^+$	$K_{a2} = 10^{-9.08} \quad (26)$
$\text{H}_3\text{TRIEN}^{3+} \rightleftharpoons \text{H}^+ + \text{H}_2\text{TRIEN}^{2+}$	$K_{a3} = 10^{-6.56} \quad (26)$
$\text{HEDTA}^{-3} \rightleftharpoons \text{H}^+ + \text{EDTA}^{-4}$	$K_{a4} = 10^{-10.26} \quad (27)$
$\text{H}_2\text{EDTA}^{-2} \rightleftharpoons \text{H}^+ + \text{HEDTA}^{-3}$	$K_{a3} = 10^{-6.16} \quad (27)$

All experimentally determined pH values were converted to $[H^+]$ values using activity coefficients calculated from the extended Debye-Hückel equation (24).

The experimentally determined k_{slope} values were fitted to the rate expression using the simultaneous equation program on the Texas Instrument TI Programmable 58C calculator, along with a non-linear regression analysis program.

CHAPTER 4

RESULTS

General Rate Expression for the Reaction of CuH₂DGEN with TRIEN

Determination of the general rate expression for the displacement of DGEN from its Cu(II) complex was accomplished by measuring the rate of reaction as a function of total TRIEN concentration. The reactions were evaluated from pH 6.64 to 10.45. This large pH range was necessary to determine which TRIEN species were reactive. All kinetic measurements were run under pseudo first-order conditions with $[\text{TRIEN}]_t \gg [\text{CuH}_2\text{DGEN}]$. The plots of $-\ln(A_\infty - A_t)$ versus time were linear. Equation 8 is valid, assuming the reaction is irreversible and fully complete.

$$\text{rate} = \frac{-d[\text{CuH}_2\text{DGEN}]}{dt} = k_{\text{obs}}[\text{CuH}_2\text{DGEN}] \quad (8)$$

The pseudo first-order rate constants, k_{obs} , were determined from an average of at least three kinetic runs. Table 3 lists these rate constants and their corresponding pH and $[\text{TRIEN}]_t$ values.

Plots of k_{obs} versus $[\text{TRIEN}]_t$ shown in Figure 4 were linear, corresponding to Equation 9,

$$k_{\text{obs}} = k_i + k_{\text{TT}}[\text{TRIEN}]_t \quad (9)$$

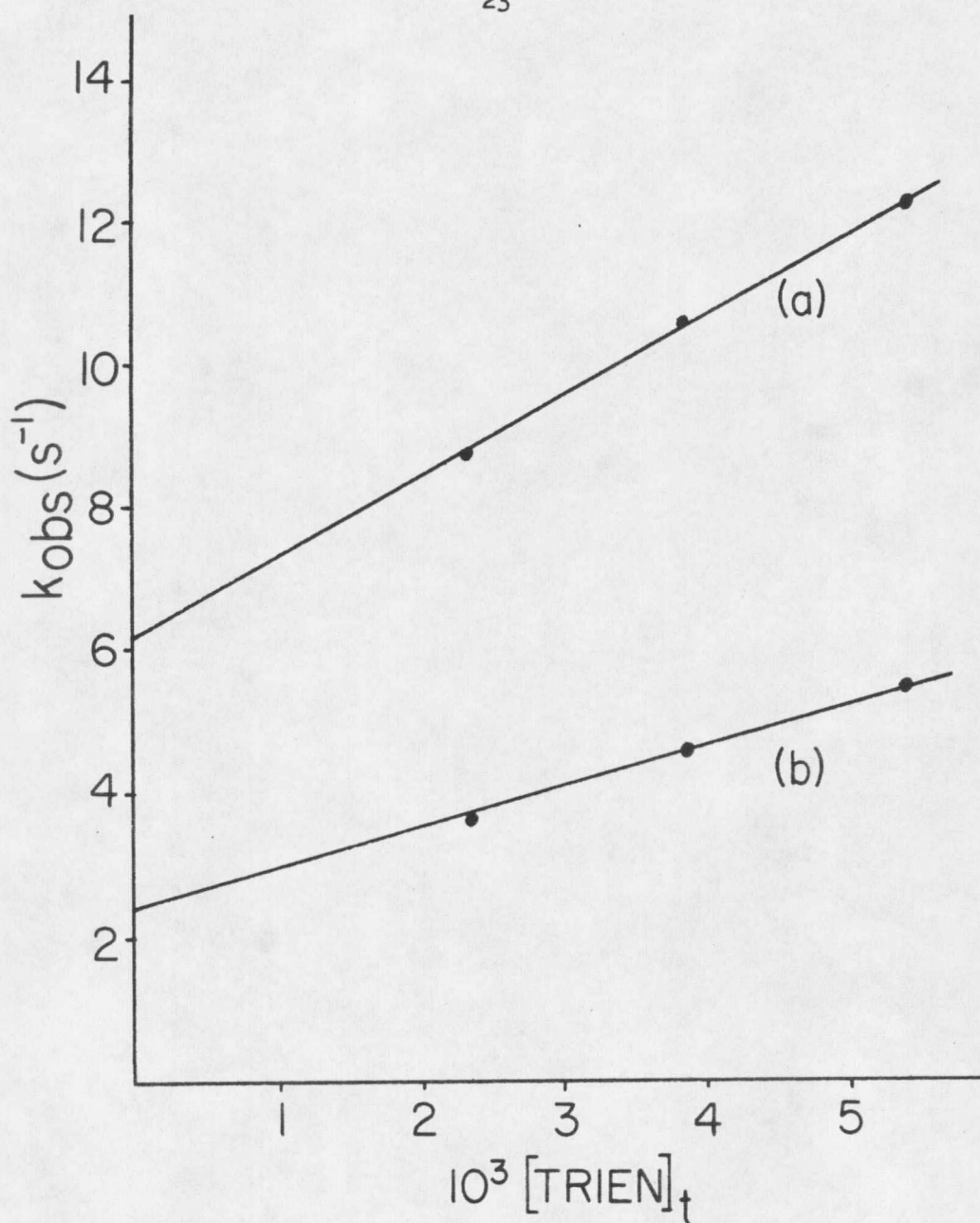
where k_i represents the intercept of the plot, while k_{TT} is the slope. The above k_{obs} relationship can be substituted into Equation 8 to give Equation 10.

Table 3. Concentrations and k_{obs} for the reaction of TRIEN with $\text{CuH}_{-2}\text{DGEN}$

Run number	pH	$10^3[\text{TRIEN}]_t$	$k_{\text{obs}} (\text{s}^{-1})$
2-67-1a	6.64	2.31	8.82
2-68-2a	6.64	3.85	16.61
2-68-3a	6.64	5.40	12.27
2-69-1a	7.15	2.31	3.74
2-69-2a	7.15	3.85	4.58
2-70-3a	7.15	5.40	5.51
1-81-1b	7.55	1.44	1.85
1-81-2b	7.55	4.32	2.46
1-82-3b	7.55	7.20	3.30
1-82-4b	7.55	10.1	4.27
1-76-1b	7.88	1.44	0.89
1-76-2b	7.88	4.32	1.37
1-77-3b	7.88	7.20	1.82
1-77-4b	7.88	10.1	2.62
2-64-1a	8.40	0.77	0.56
2-65-2a	8.40	1.54	0.72
2-65-3a	8.40	3.08	0.90
2-66-4a	8.40	4.62	1.12
1-69-1b	8.66	1.08	0.51
1-61-2b	8.66	3.24	0.90
1-61-3b	8.66	6.50	1.32
1-62-4b	8.66	8.65	1.54
1-57-1b	9.28	1.44	0.68
1-58-2b	9.28	2.16	0.84
1-58-3b	9.28	2.89	1.02
1-59-4b	9.28	3.66	1.13
1-67-1b	9.94	1.44	6.92
1-68-2b	9.94	4.32	2.24
1-68-3b	9.94	7.20	3.67
1-69-4b	9.94	11.5	5.61
1-69-5b	9.94	14.4	6.74
1-70-5b	9.94	18.0	9.37
1-71-1b	10.45	1.44	0.99
1-72-2b	10.45	4.32	2.56
1-72-3b	10.45	10.1	5.57
1-73-4b	10.45	12.9	7.28
1-73-5b	10.45	15.8	8.62

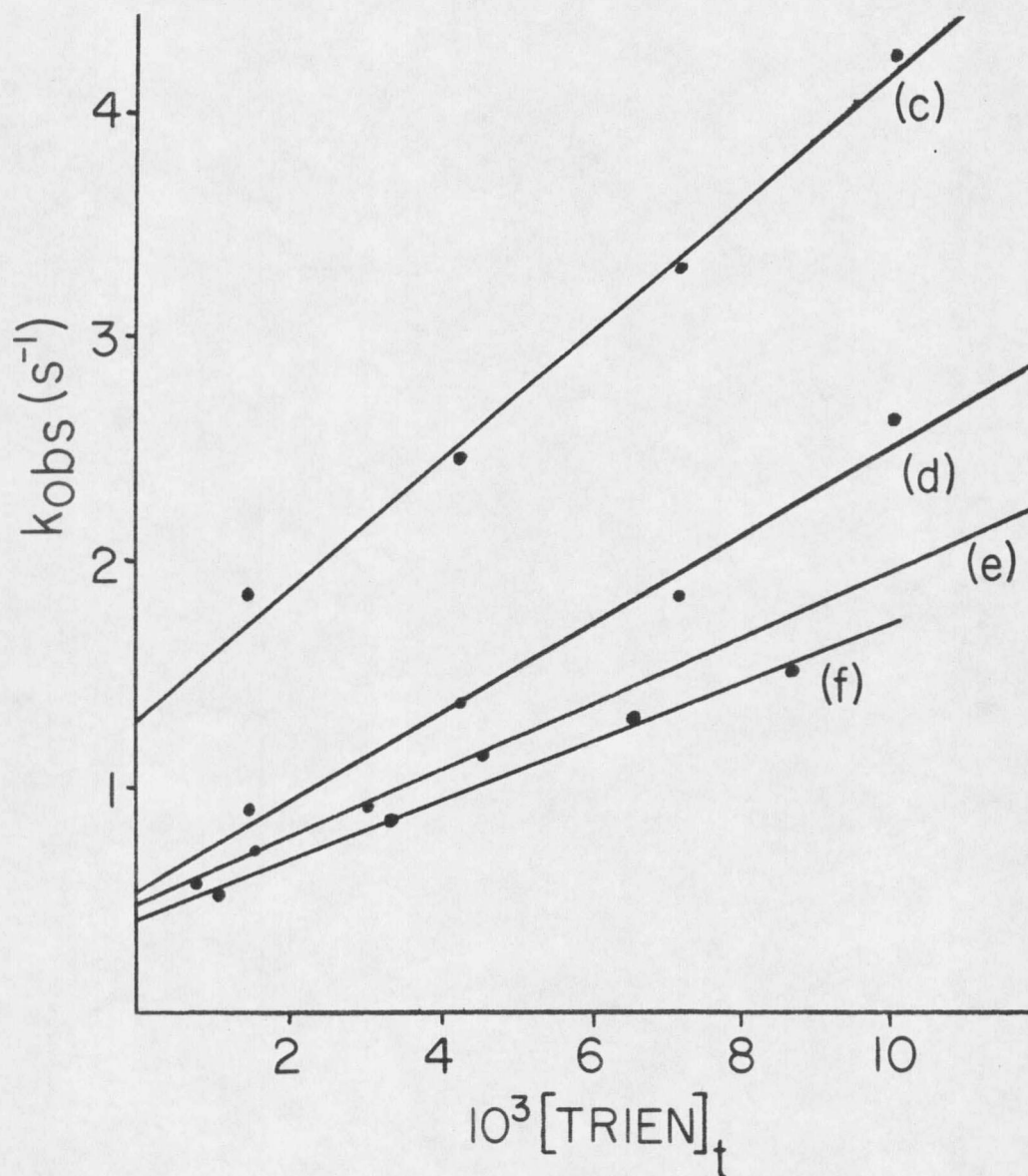
$$^a[\text{CuH}_{-2}\text{DGEN}]_t = 2.92 \times 10^{-5}$$

$$^b[\text{CuH}_{-2}\text{DGEN}]_t = 8.15 \times 10^{-5}$$



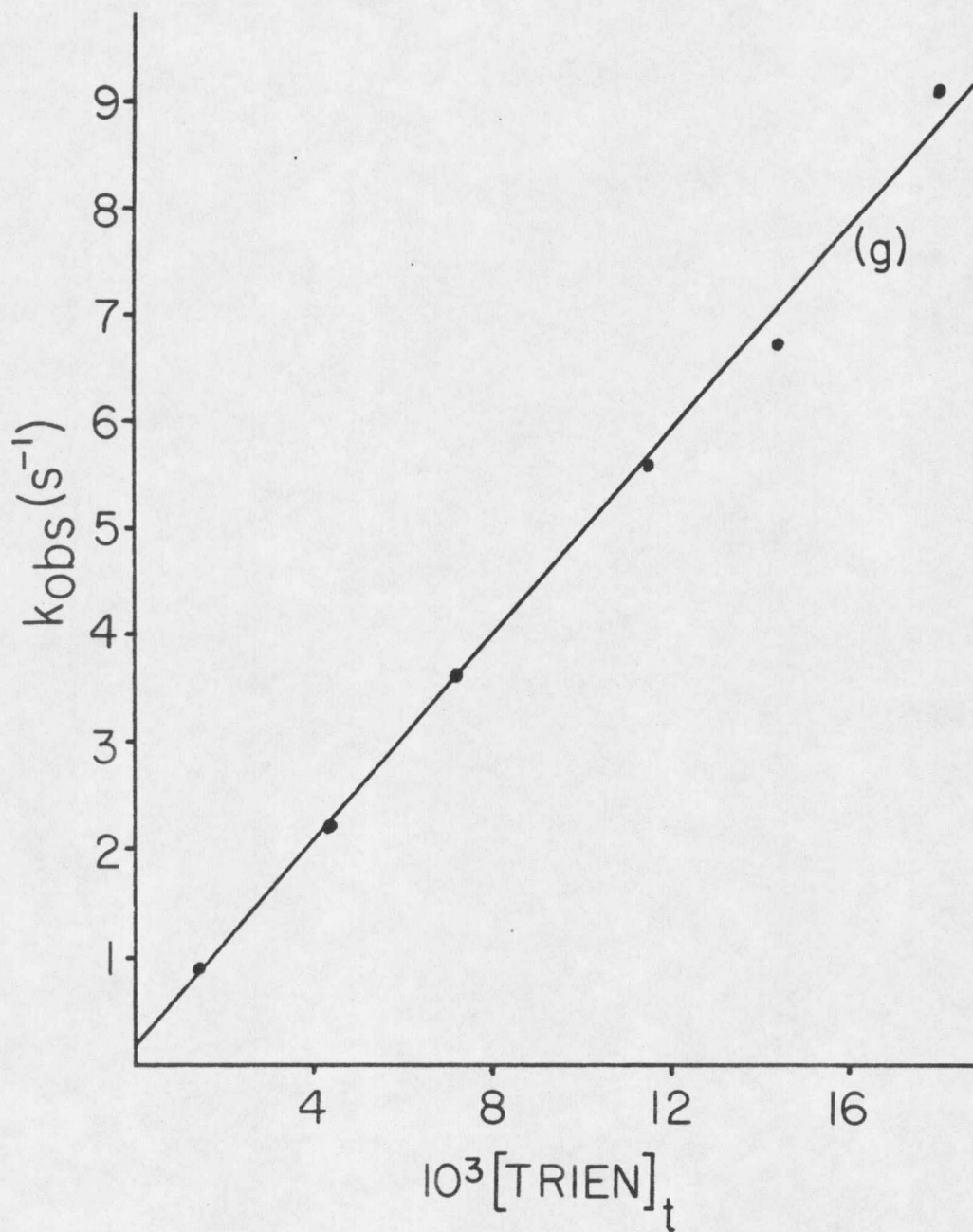
	<u>pH</u>	<u>$k_{\text{TT}} (\text{M}^{-1} \text{s}^{-1})$</u>	<u>$k_1 (\text{s}^{-1})$</u>
(a)	6.64	1130	6.2
(b)	7.15	570	2.4

Figure 4. Dependence of k_{obs} on $[\text{TRIEN}]_t$ for the reaction of TRIEN with CuH_2DGEN .



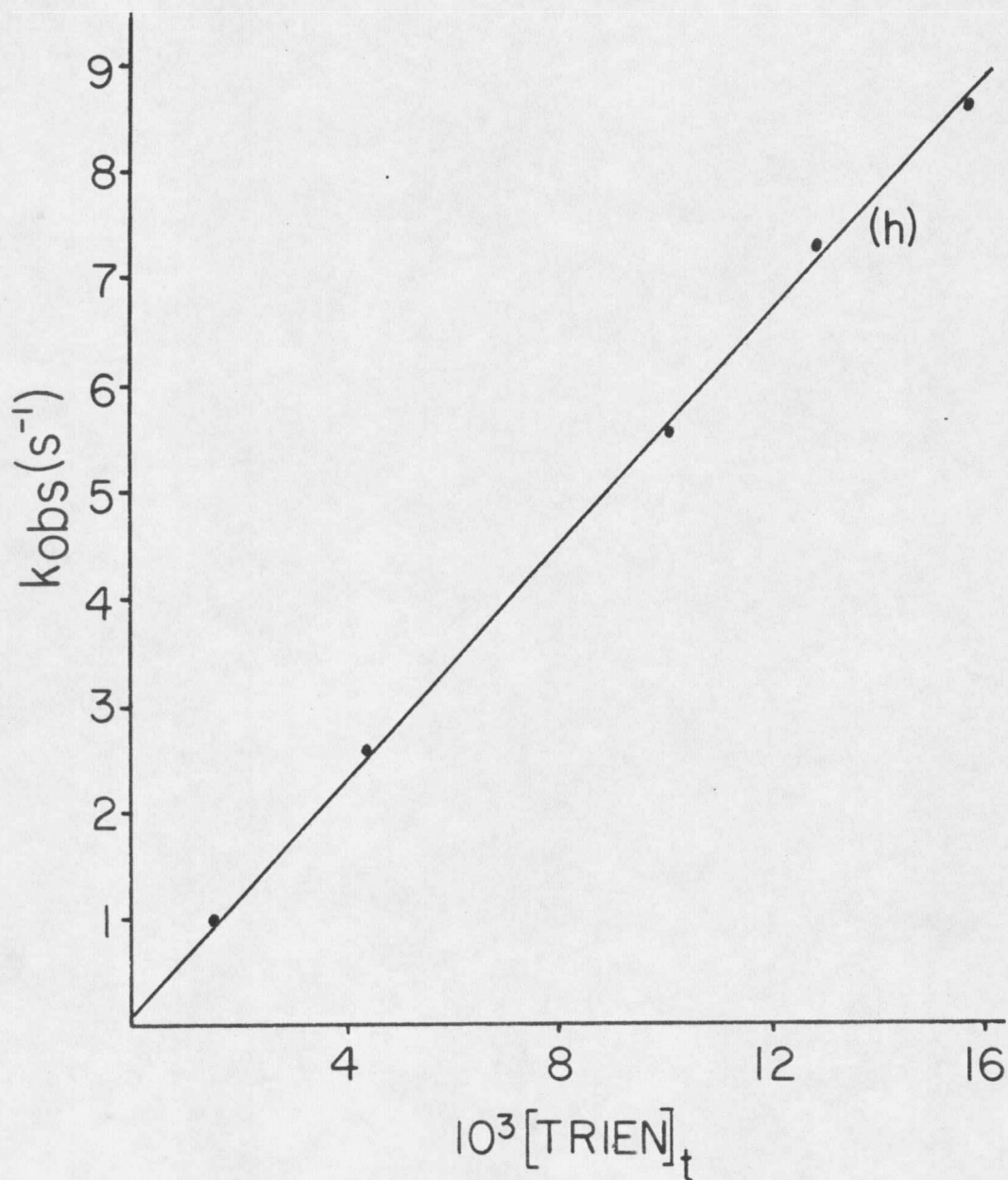
	pH	$k_{TT}(M^{-1}s^{-1})$	$k_i(s^{-1})$
(c)	7.55	270	1.3
(d)	7.88	200	0.53
(e)	8.40	140	0.47
(f)	8.66	135	0.41

Figure 4 (continued). Dependence of k_{obs} on $[TRIEN]_t$ for the reaction of TRIEN with CuH_2DGEN .



pH	$k_{\text{TT}} (\text{M}^{-1} \text{s}^{-1})$	$k_i (\text{s}^{-1})$
(g) 9.94	490	0.16

Figure 4 (continued). Dependence of k_{obs} on $[\text{TRIEN}]_t$ for the reaction of TRIEN with CuH_2DGEN .



<u>pH</u>	<u>$k_{\text{TT}} (\text{M}^{-1} \text{s}^{-1})$</u>	<u>$k_1 (\text{s}^{-1})$</u>
(h) 10.45	580	0.080

Figure 4 (continued). Dependence of k_{obs} on $[\text{TRIEN}]_t$ for the reaction of TRIEN with CuH_2DGEN .

$$\frac{-d[\text{CuH}_2\text{DGEN}]}{dt} = \{k_i = k_{\text{TT}}[\text{TRIEN}]_{\text{TT}}\}[\text{CuH}_2\text{DGEN}] \quad (10)$$

k_i is the first-order ligand independent term and k_{TT} is the second-order rate constant.

From pH 6.6 to 10.5 TRIEN takes on several forms. At low pH, $\text{H}_3\text{TRIEN}^{3+}$ and $\text{H}_2\text{TRIEN}^{2+}$ are the predominant species, while at high pH, HTRIEN^+ and TRIEN become the major species. The relationship between $[\text{TRIEN}]_t$ and its various contributing species is given in Equation 11.

$$[\text{TRIEN}]_{\text{tt}} = [\text{TRIEN}] + [\text{HTRIEN}^+] + [\text{H}_2\text{TRIEN}^{2+}] + [\text{H}_3\text{TRIEN}^{3+}] \quad (11)$$

To determine the contribution of each TRIEN species on the experimental second-order rate constant (k_{TT}), Equation 12 is given,

$$k_{\text{TT}} = \frac{\{k_{\text{T}}K_{\text{HT}}K_{\text{H}_2\text{T}}K_{\text{H}_3\text{T}} + k_{\text{HT}}K_{\text{H}_2\text{T}}K_{\text{H}_3\text{T}}[\text{H}^+] + k_{\text{H}_2\text{T}}K_{\text{H}_3\text{T}}[\text{H}^+]^2 + k_{\text{H}_3\text{T}}[\text{H}^+]^3\}}{K_{\text{HT}}K_{\text{H}_2\text{T}}K_{\text{H}_3\text{T}} + K_{\text{H}_3\text{T}}K_{\text{H}_2\text{T}}[\text{H}^+] + K_{\text{H}_3\text{T}}[\text{H}^+]^2 + [\text{H}^+]^3} \quad (12)$$

where K_{HT} ($10^{-9.74}$), $K_{\text{H}_2\text{T}}$ ($10^{-9.08}$) and $K_{\text{H}_3\text{T}}$ ($10^{-6.56}$) are the acid dissociation constants for HTRIEN^+ , $\text{H}_2\text{TRIEN}^{2+}$ and $\text{H}_3\text{TRIEN}^{3+}$, respectively (26), and k_{T} , k_{HT} , $k_{\text{H}_2\text{T}}$ and $k_{\text{H}_3\text{T}}$ are the resolved second-order rate constants for the reaction of TRIEN with CuH_2DGEN .

The complexity of Equation 12 can be simplified further to give Equation 13.

$$k_{TT} = \frac{k_{H_2T}K_{H_3T} + k_{H_3T}[H^+]}{k_{H_3T} + [H^+]} \quad (13)$$

At the lower pH values of 6.64, 7.15 and 7.55, the contribution from $HTRIEN^+$ and $TRIEN$ becomes negligible, and Equation 13 is valid. This Equation was resolved, and k_{H_3T} was found to be $2.1 \times 10^3 M^{-1} s^{-1}$, while an approximation for k_{H_2T} was extracted. At pH values greater than 7.7, where $HTRIEN^+$ and $TRIEN$ participate, Equation 12 was solved using the already-found k_{H_3T} value and the approximation for k_{H_2T} . With this information and use of a non-linear regression analysis program, the rate constants for k_T , k_{HT} and k_{H_2T} were found to be 6.5×10^2 , 2.1×10^2 and $87 M^{-1} s^{-1}$, respectively. Table 4 gives a comparison of the calculated and experimental k_{TT} values.

Table 4. Calculated and experimental k_{TT} values for the reaction of $TRIEN$ with $CuH_{-2}DGEN$.

pH	$k_{TT}(CALC)$ $M^{-1}s^{-1}$	$k_{TT}(EXP)$ $M^{-1}s^{-1}$	% deviation	$k_i(s^{-1})$
6.64	1110	1130	2	6.2
7.15	577	570	1	2.4
7.55	305	270	13	1.3
7.88	196	200	2	0.53
8.40	137	140	2	0.47
8.66	144	135	7	0.41
9.28	228	200	14	0.32
9.94	441	490	10	0.16
10.45	584	580	1	0.08

The value of k_i in Equation 9 was experimentally found to decrease with increasing pH. This general trend indicated the

existence of buffer participation in the TRIEN independent pathway. From pH 7.88 to 10.45, boric acid and dihydrogen borate are in equilibrium. This buffer contribution can be determined through use of Equation 14.

$$k_i = k_d + k_{H_3BO_3} [H_3BO_3] \quad (14)$$

Between pH 7.88 and 10.45 the borate total concentration was held constant at $[B]_t = 5.0 \times 10^{-3}$. With this constant value, the acid dissociation constant of $10^{-9.24}$ (24) for boric acid and pH values the $[H_3BO_3]$ concentration can be determined in Equation 14. A plot of k_i versus $[H_3BO_3]$ is shown in Figure 5. The intercept of this plot is the solvent dissociative rate constant which was found to be 0.042 s^{-1} . The slope of $110 \text{ M}^{-1} \text{ s}^{-1}$ represents the general acid rate constant for H_3BO_3 .

To determine the protonation rate constant, k_{H_3O} , experiments were performed between pH 6.53 and 7.16 with a constant TRIEN concentration and in the absence of borate buffer. Under these conditions Equation 15 is valid.

$$k_{obs} = k_d + k_{H_2T}[H_2T] + k_{H_3T}[H_3T] + k_{H_3O}[H_3O^+] \quad (15)$$

Equation 15 can be rearranged to give Equation 16.

$$k' = k_{obs} - k_d - k_{H_2T}[H_2T] - k_{H_3T}[H_3T] = k_{H_3O}[H_3O^+] \quad (16)$$

A plot of k' versus $[H_3O^+]$ is shown in Figure 6. The slope of this plot was resolved to give $k_{H_3O} = 1.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$.

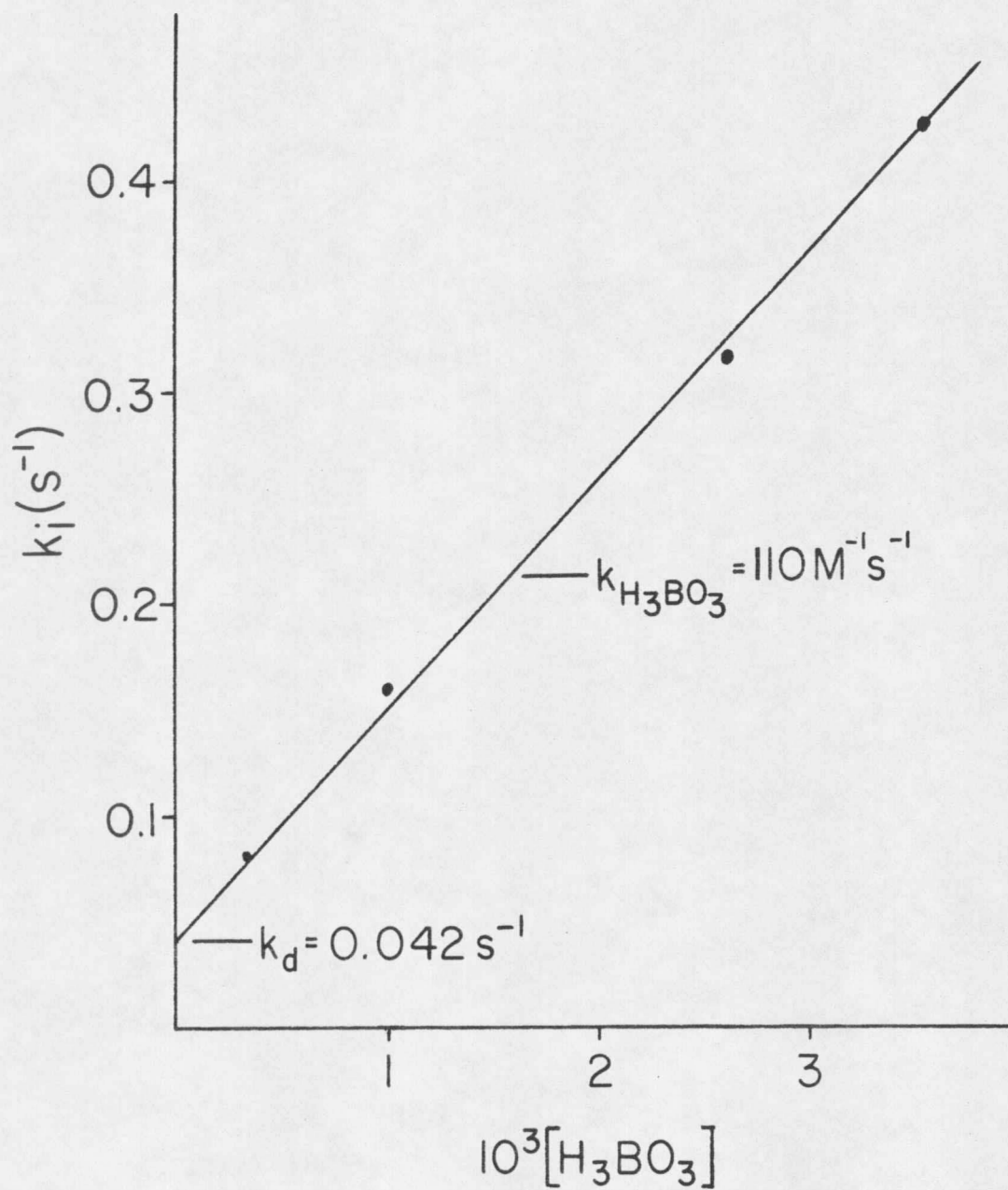


Figure 5. Dependence of k_1 on $[\text{H}_3\text{BO}_3]$ for the reaction of TRIEN with CuH_2DGEN .

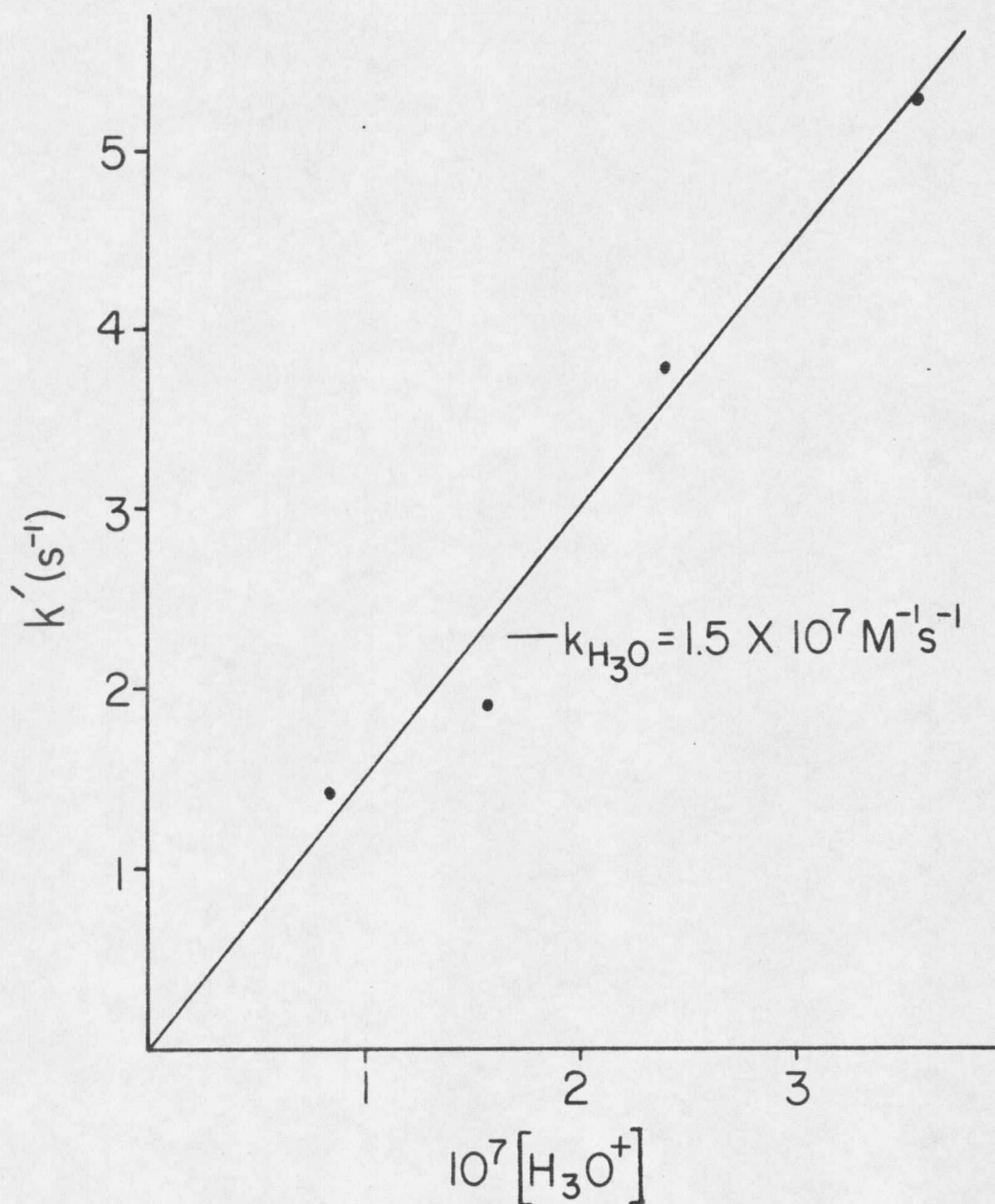


Figure 6. Dependence of k' on $[H_3O^+]$ (refer to Equation 16) for the reaction of TRIEN with CuH_2DGEN .

The complete rate expression for the reaction of TRIEN with CuH_2DGEN is given in Equation 17.

$$\frac{-d[\text{CuH}_2\text{DGEN}]}{dt} = \{k_d + k_{\text{H}_3\text{BO}_3}[\text{H}_3\text{BO}_3] + k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+] + k_{\text{T}}[\text{TRIEN}] + k_{\text{HT}}[\text{HTRIEN}^+] + k_{\text{H}_2\text{T}}[\text{H}_2\text{TRIEN}^{2+}] + k_{\text{H}_3\text{T}}[\text{H}_3\text{TRIEN}^{3+}]\}[\text{CuH}_2\text{DGEN}] \quad (17)$$

A summary of all rate constants is given in Table 5. To check the correlation between calculated and experimentally observed rate constants, a pH profile plot is offered in Figure 7. As this Figure illustrates, a minimum occurs at approximately pH 8.5. The increase in k_{obs} below pH 8.5 is due mainly to the general acid catalysis by $\text{H}_3\text{TRIEN}^{3+}$ and H_3O^+ . At pH values greater than 8.5, the increase in k_{obs} is associated with the nucleophilic reactivity of HTRIEN^+ and TRIEN.

Table 5. Rate constants for the reaction of TRIEN with CuH_2DGEN .

Constant	Value
k_d	$4.2 \times 10^{-2} \text{ s}^{-1}$
$k_{\text{H}_3\text{BO}_3}$	$(1.1 \pm 0.1) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$
$k_{\text{H}_3\text{O}^+}$	$(1.5 \pm 0.3) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
k_{T}	$(6.5 \pm 0.9) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$
k_{HT}	$(2.1 \pm 0.4) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$
$k_{\text{H}_2\text{T}}$	$(8.7 \pm 0.9) \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$
$k_{\text{H}_3\text{T}}$	$(2.1 \pm 0.5) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$

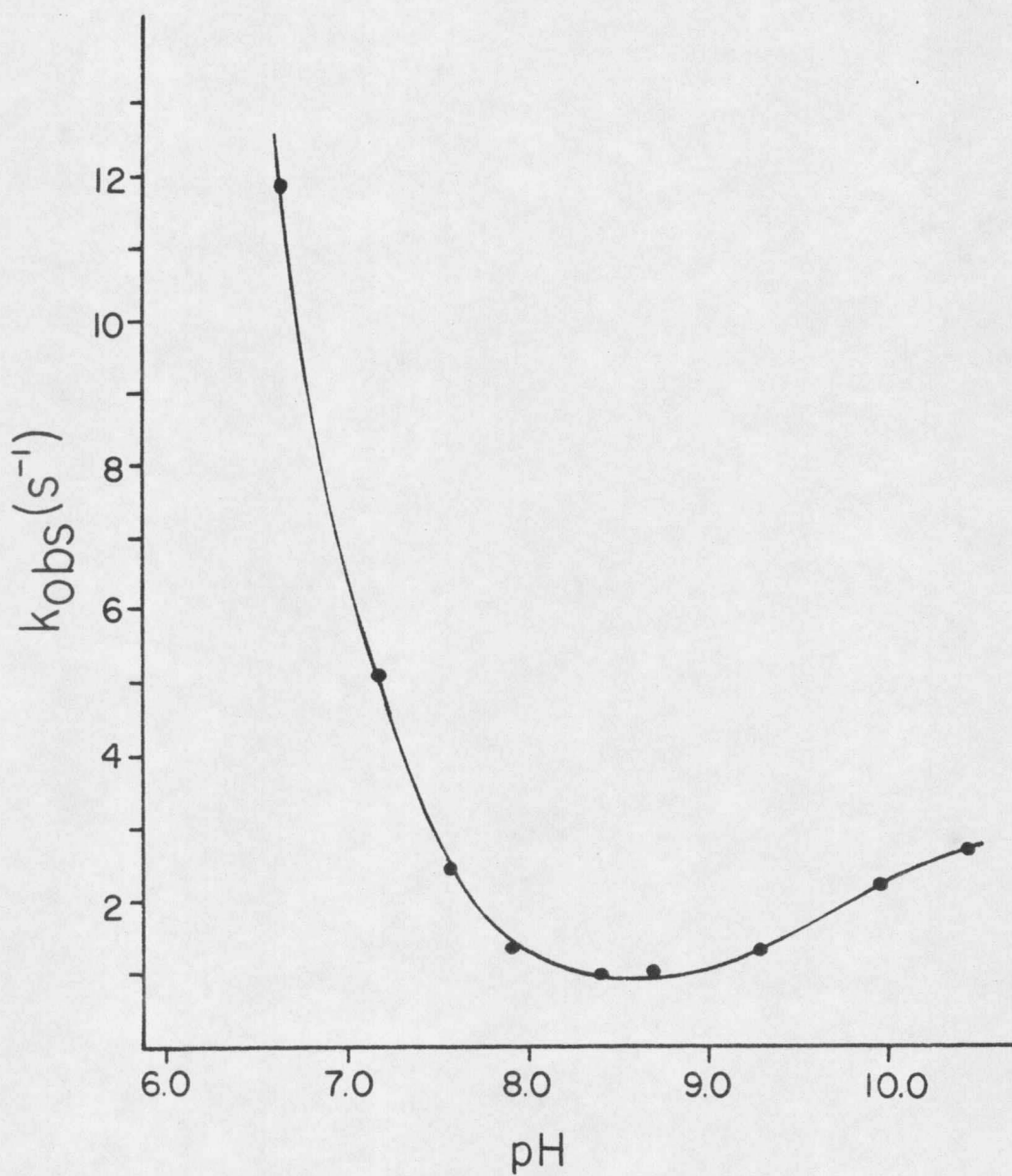


Figure 7. Dependence of observed rate constant on pH for the reaction of TRIEN with CuH_2DGEN .

General Rate Expression for the Reaction of CuH₂DGEN with EDTA

The reaction of CuH₂DGEN was studied over the pH range of 7.74 to 10.96. The reactions were monitored under pseudo first-order conditions in agreement with Equation 18.

$$\text{rate} = \frac{-d[\text{CuH}_2\text{DGEN}]}{dt} = k_{\text{obs}}[\text{CuH}_2\text{DGEN}] \quad (18)$$

Variation of EDTA_t concentration at constant pH resulted in a change in k_{obs} . All experimental k_{obs} values are an average of at least three kinetic runs. The dependence of k_{obs} on [EDTA]_t is shown in Figure 8. This [EDTA]_t dependence is similar to Equation 9, mentioned earlier for the TRIEN system, and substitution of [EDTA]_t into this equation results in Equation 19.

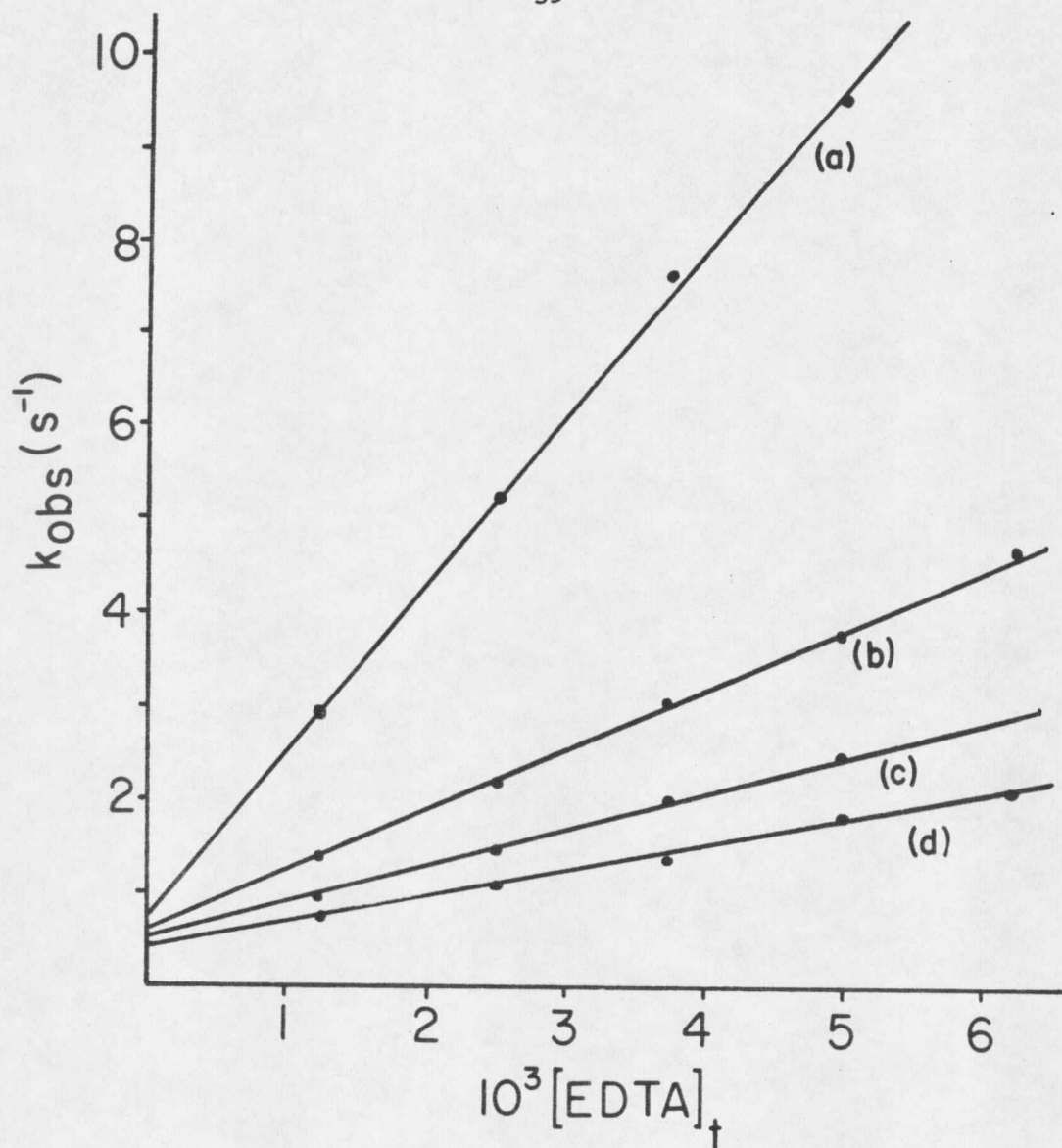
$$k_{\text{obs}} = k_i + k_s[\text{EDTA}]_t \quad (19)$$

Figure 8 indicates that an increase in pH results in the decrease of both k_i and k_s . Table 6 summarizes the experimental kinetic data.

From pH 7.75 to 10.96, EDTA_t is composed of the free ligand and several protonated forms indicated by Equation 20.

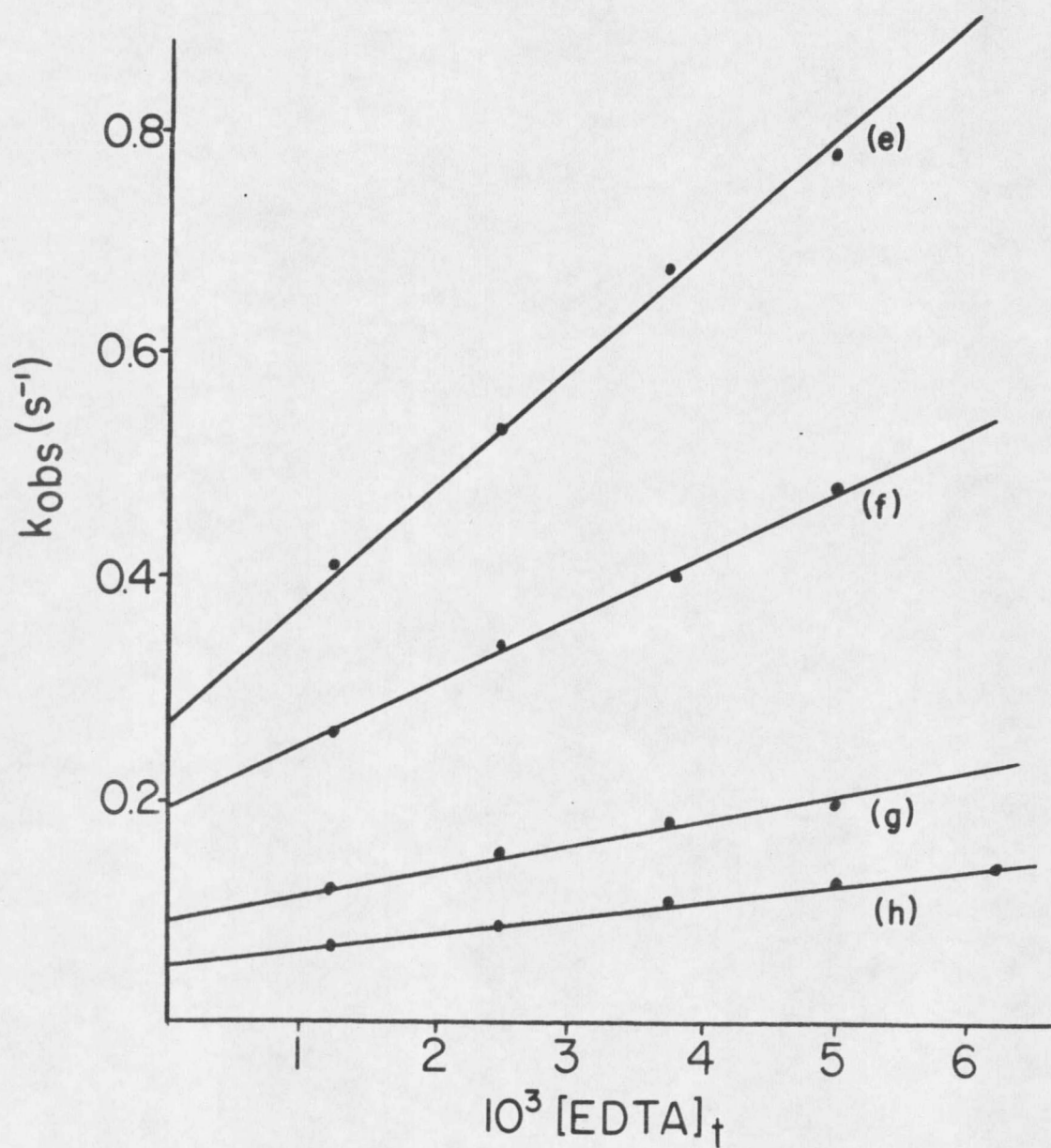
$$[\text{EDTA}]_t = [\text{EDTA}^{-4}] + [\text{HEDTA}^{-3}] + [\text{H}_2\text{EDTA}^{-2}] \quad (20)$$

The reaction was observed to be [EDTA]_t-dependent over the entire pH range. To determine the EDTA speciation of each experimental pH value, Equations 21-23 were used. Using the alpha values for each



	pH	$k_s (\text{M}^{-1} \text{s}^{-1})$	$k_i (\text{s}^{-1})$
(a)	8.03	1760	0.78
(b)	8.54	642	0.61
(c)	8.69	410	0.55
(d)	8.96	274	0.47

Figure 8. Dependence of k_{obs} on $[\text{EDTA}]_t$ for the reaction of EDTA with CuH_2DGEN .



	pH	$k_s (\text{M}^{-1} \text{s}^{-1})$	$k_i (\text{s}^{-1})$
(e)	0.54	99	0.27
(f)	9.98	58	0.19
(g)	10.44	24	0.090
(h)	10.96	13	0.054

Figure 8 (continued). Dependence of k_{obs} on $[\text{EDTA}]_t$ for the reaction of EDTA with $\text{CuH}_{-2}\text{DGEN}$.

Table 6. Concentrations and k_{obs} for the reaction of EDTA with CuH_2DGEN .

Run number ^a	pH	$10^3[\text{EDTA}]_t$	$k_{\text{obs}}(\text{s}^{-1})$
2-53-1	7.74	3.75	13.2
2-53-2	7.74	5.00	16.6
2-54-3	7.75	6.25	21.5
2-37-1	8.03	1.25	2.94
2-38-2	8.03	2.50	5.28
2-38-3	8.03	3.75	7.62
2-39-4	8.03	5.00	9.52
2-06-1	8.54	1.25	1.38
2-06-2	8.54	2.50	2.17
2-07-3	8.54	3.75	3.07
2-07-4	8.54	5.00	3.56
2-08-5	8.54	6.25	4.70
2-40-1	8.69	1.25	0.95
2-41-2	8.69	2.50	1.46
2-41-3	8.69	3.75	2.00
2-42-4	8.69	5.00	2.46
2-10-1	8.96	1.25	0.74
2-10-2	8.96	2.50	1.07
2-11-3	8.96	3.75	1.36
2-11-4	8.96	5.00	1.82
2-12-5	8.96	6.25	2.08
2-14-1	9.54	1.25	0.41
2-14-2	9.54	2.50	0.53
2-15-3	9.54	3.75	0.68
2-15-4	9.54	5.00	0.78
2-43-1	9.85	1.25	0.26
2-44-2	9.85	2.50	0.34
2-44-3	9.85	3.75	0.40
2-45-4	9.85	5.00	0.48
2-46-1	10.44	1.25	0.121
2-47-2	10.44	2.50	0.152
2-47-3	10.44	3.75	0.177
2-48-4	10.44	5.00	0.192
2-22-1	10.96	1.25	0.070
2-22-2	10.96	2.50	0.084
2-23-3	10.96	3.75	0.110
2-23-4	10.96	5.00	0.125
2-24-5	10.96	6.25	0.134

$$^a[\text{CuH}_2\text{DGEN}]_t = 4.38 \times 10^{-5}$$

EDTA species, the second-order rate constant, k_s , was resolved using Equation 24.

$$\alpha_Y = \frac{[\text{EDTA}^{-4}]}{[\text{EDTA}]_t} \quad (21)$$

$$\alpha_{\text{HY}} = \frac{[\text{HEDTA}^{-3}]}{[\text{EDTA}]_t} \quad (22)$$

$$\alpha_{\text{H}_2\text{Y}} = \frac{[\text{H}_2\text{EDTA}^{-2}]}{[\text{EDTA}]_t} \quad (23)$$

$$k_s = k_Y \alpha_Y + k_{\text{HY}} \alpha_{\text{HY}} + k_{\text{H}_2\text{Y}} \alpha_{\text{H}_2\text{Y}} \quad (24)$$

From evaluation of k_s values at high pH, it was determined that the unprotonated ligand, EDTA^{-4} , was an unreactive species, and Equation 24 could be simplified to give Equation 25.

$$k_s = k_{\text{HY}} \alpha_{\text{HY}} + k_{\text{H}_2\text{Y}} \alpha_{\text{H}_2\text{Y}} \quad (25)$$

The value of k_{HY} was resolved at pH 10.44 and 10.96, where the contribution from $[\text{H}_2\text{EDTA}^{-2}]$ is negligible. The value of k_{HY} was found to be $56 \text{ M}^{-1} \text{ s}^{-1}$. Using this constant and other known values in Equation 25, the data was resolved to give $k_{\text{H}_2\text{Y}} = 1.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. A comparison of the calculated and experimental k_s values is offered in Table 7.

Table 7. Calculated and experimental k_s values for the reaction of EDTA with CuH_2DGEN .

pH	$k_s(\text{CALC})$	$k_s(\text{EXP})$	% deviation	$k_i(\text{s}^{-1})$
	$\text{M}^{-1}\text{s}^{-1}$	$\text{M}^{-1}\text{s}^{-1}$		
7.74	3390	3320	2	0.38
8.03	1792	1770	1	0.78
8.54	655	642	2	0.61
8.69	400	408	2	0.55
8.96	257	274	6	0.47
9.54	94	99	5	0.27
9.85	62	58	7	0.19
10.44	28	24	17	0.090
10.96	11	13	15	0.054

The value of k_i increased with decreasing pH, indicating the participation of boric acid in the EDTA_t -independent pathway. The k_i dependence on $[\text{H}_3\text{BO}_3]$ can be evaluated through use of Equation 26.

$$k_i = k_d + k_{\text{H}_3\text{BO}_3}[\text{H}_3\text{BO}_3] \quad (26)$$

A plot of k_i versus $[\text{H}_3\text{BO}_3]$ is shown in Figure 9. The dissociative rate constant, k_d , was found to be 0.044 s^{-1} , while the general acid rate constant for boric acid was $k_{\text{H}_3\text{BO}_3} = 122 \text{ M}^{-1} \text{ s}^{-1}$.

To evaluate the protonation rate constant, $k_{\text{H}_3\text{O}^+}$, it was necessary to evaluate k_i between pH 7.74 to 8.69. In this pH region, k_i is dependent upon k_d , $k_{\text{H}_3\text{BO}_3}[\text{H}_3\text{BO}_3]$ and $k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+]$ and Equation 27 is valid.

$$k_i = k_d + k_{\text{H}_3\text{BO}_3}[\text{H}_3\text{BO}_3] + k_{\text{H}_3\text{O}^+}[\text{H}_3\text{O}^+] \quad (27)$$

Equation 27 can be rearranged to give Equation 28.

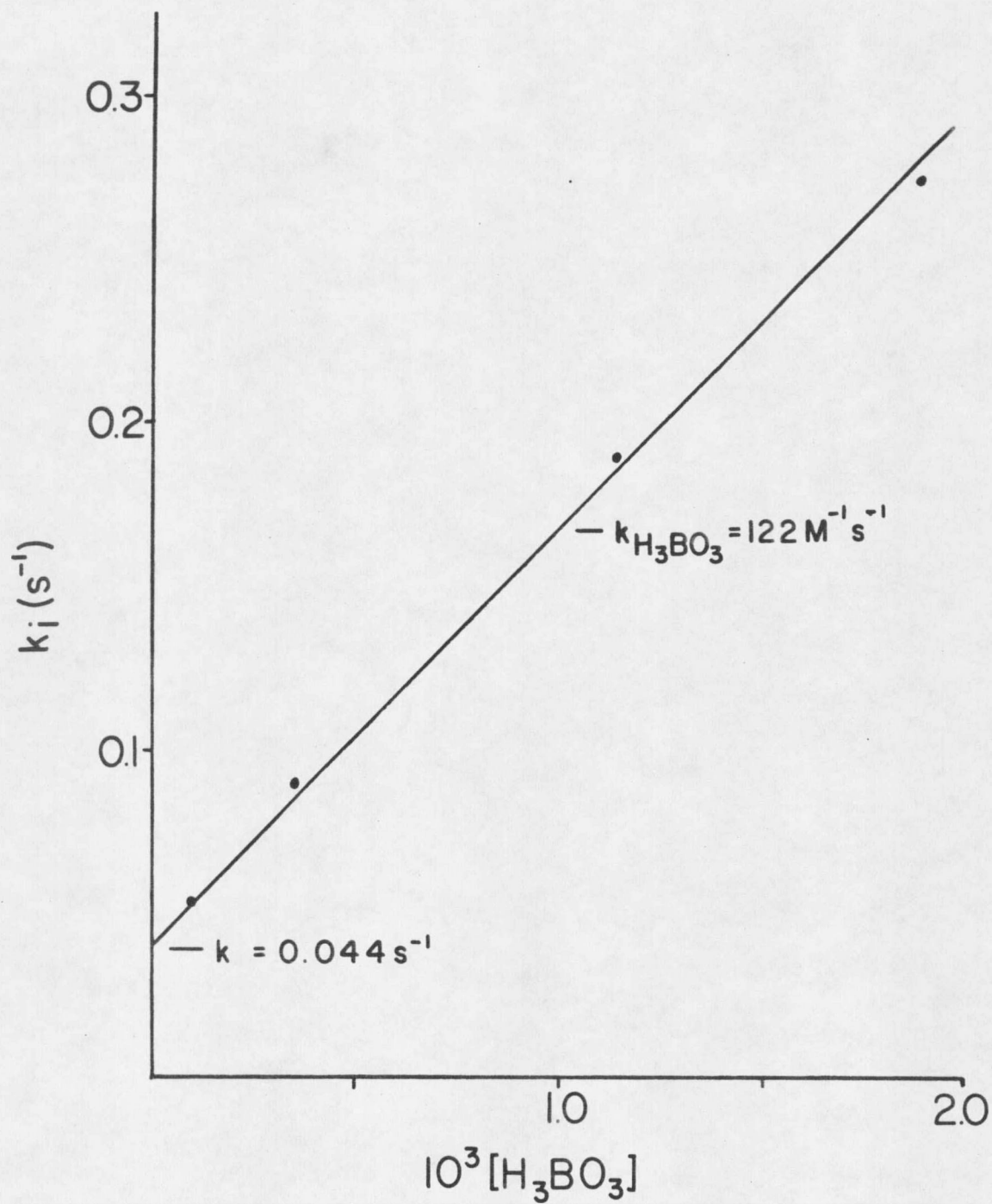


Figure 9. Dependence of k_i on $[\text{H}_3\text{BO}_3]$ for the reaction of EDTA with CuH_2DGEN .

$$k'' = k_i - k_d - k_{H_3BO_3}[H_3BO_3] = k_{H_3O}[H_3O^+] \quad (28)$$

Figure 10 illustrates the dependence of k'' on $[H_3O^+]$, and the slope of this plot was resolved to give $k_{H_3O} = 1.6 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. This experimentally determined k_{H_3O} value is in close agreement with the k_{H_3O} value found previously in the TRIEN reaction with CuH_2DGEN .

The resolved rate expression for the reaction of EDTA with CuH_2DGEN from pH 7.7 to 11 is given in Equation 29.

$$\frac{-d[\text{CuH}_2\text{DGEN}]}{dt} = \{k_d + k_{H_3BO_3}[H_3BO_3] + k_{H_3O}[H_3O^+] + k_{HY}[\text{HEDTA}^{-3}] + k_{H_2Y}[\text{H}_2\text{EDTA}^{-2}]\}[\text{CuH}_2\text{DGEN}] \quad (29)$$

The rate constants are summarized in Table 8, and the correlation between calculated and observed rate constants is shown in Figure 11. The solid line represents the predicted curve.

Table 8. Rate constants for the reaction of EDTA with CuH_2DGEN .

Constant	Value
k_d	$4.4 \times 10^{-2} \text{ s}^{-1}$
$k_{H_3BO_3}$	$(1.2 \pm 0.1) \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$
$k_{H_3O^+}$	$(1.6 \pm 0.2) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
k_{HEDTA}	$(5.6 \pm 1.1) \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$
$k_{\text{H}_2\text{EDTA}}$	$(1.1 \pm 0.3) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$

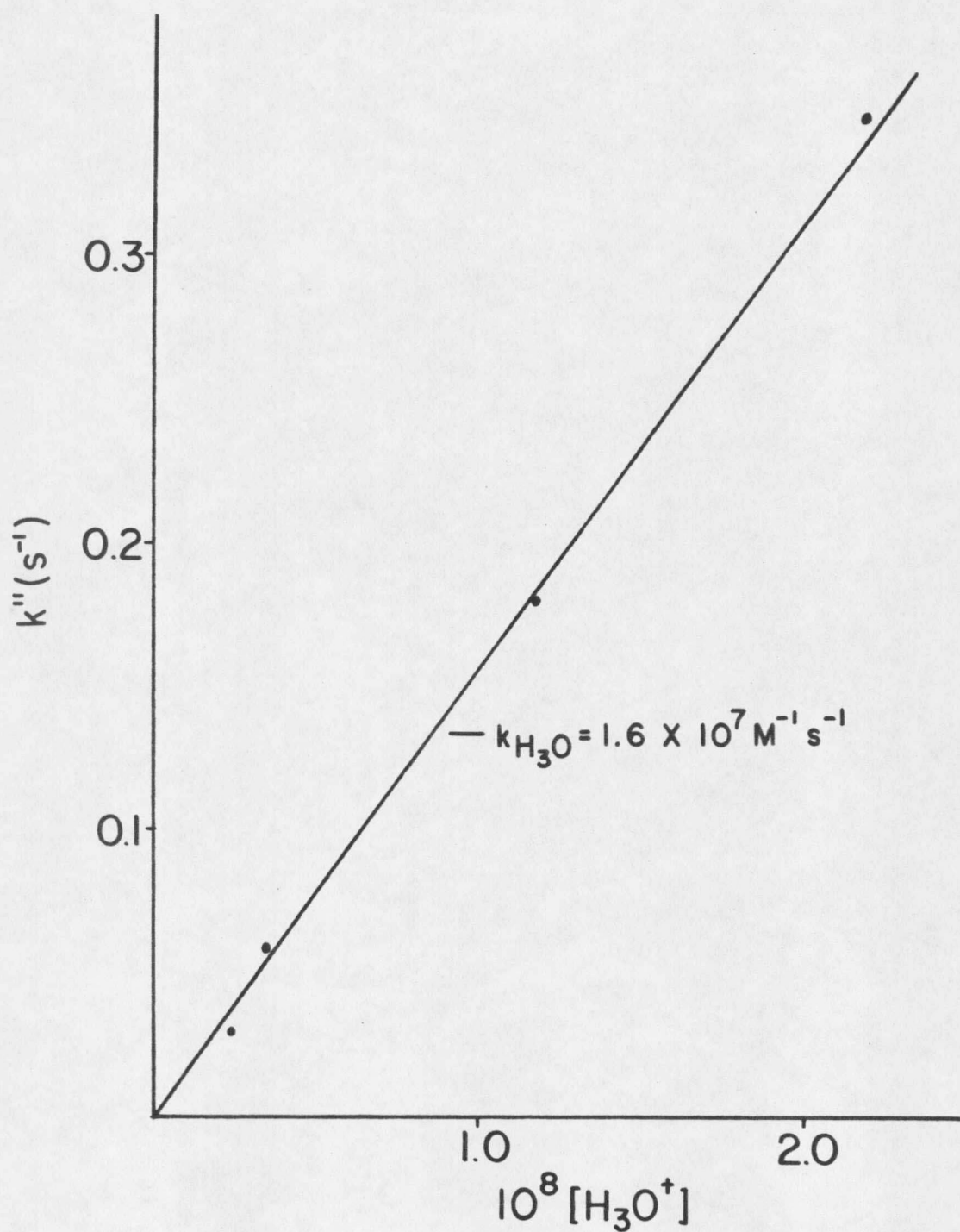


Figure 10. Dependence of k'' on $[H_3O^+]$ for the reaction of $EDTA_t$ with CuH_2DGEN (refer to Equation 28).

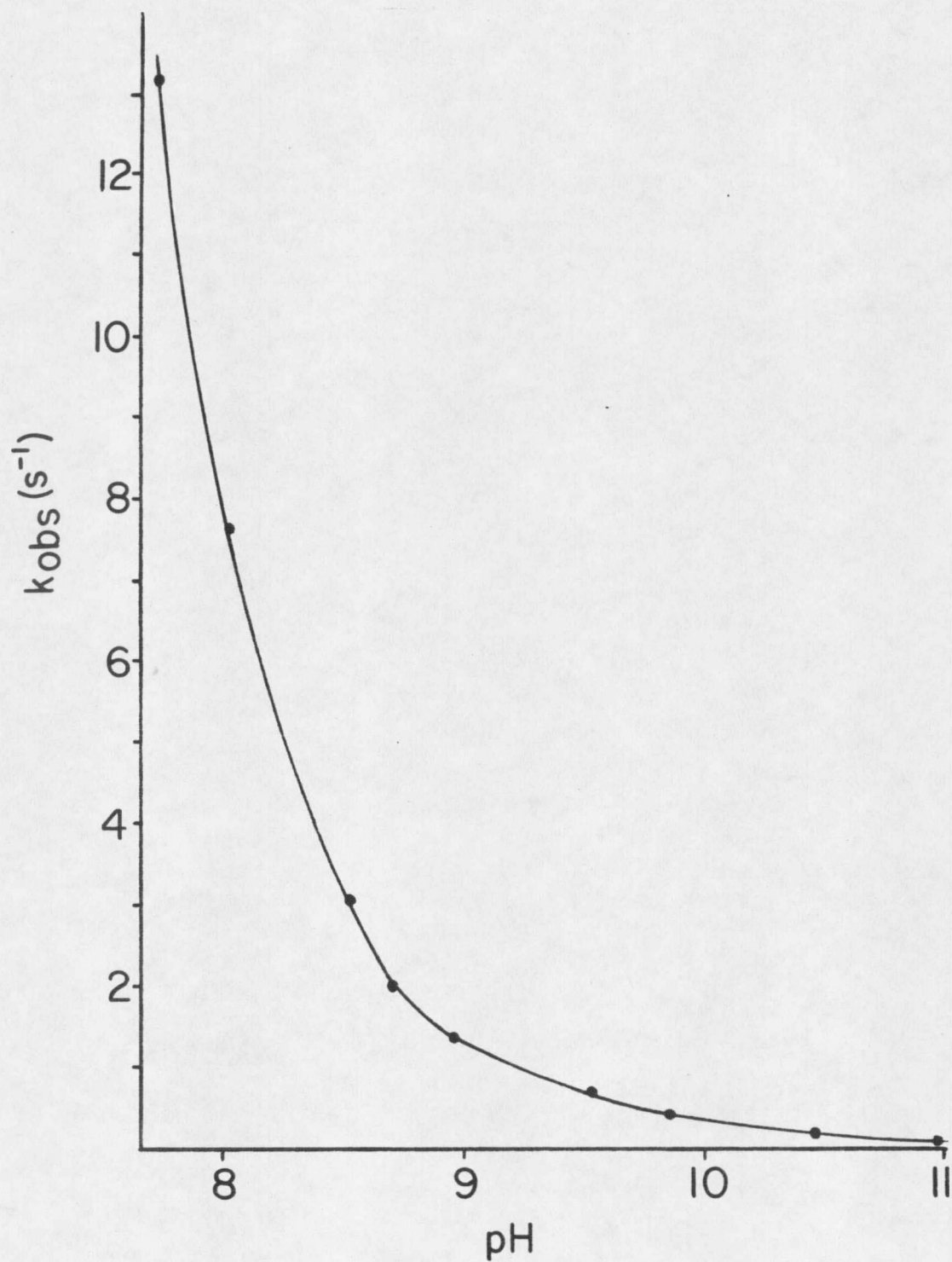


Figure 11. Dependence of observed rate constant on pH for the reaction of EDTA with CuH_2DGEN .

CHAPTER 5

DISCUSSION

The reaction of TRIEN and EDTA with CuH_2DGEN proceeds through a variety of parallel reaction pathways. A schematic representation of these possible pathways is shown in Figure 12. The following discussion will address these pathways and how they compare with other reactions of oligopeptide complexes.

Solvent Dissociation Pathway

Evidence for a solvent dissociative pathway was obtained from plots of k_1 versus $[\text{H}_3\text{BO}_3]$ shown in Figures 5 and 9. The intercepts of such plots represent the value of the solvent dissociative rate constant (k_d). For the reaction of TRIEN and EDTA with CuH_2DGEN the k_d term was found to be 0.043 s^{-1} . This constant is attributed to the molecular rearrangement of CuH_2DGEN to a form that can react directly with TRIEN or EDTA. This molecular rearrangement of CuH_2DGEN during solvent attack can lead to a reaction intermediate or a transition state in the mechanism (5). In the latter case, a concerted push-pull mechanism would exist with one water molecule acting as a nucleophile to push the peptide nitrogen away from the copper ion, while a second water molecule donates its proton to the very basic peptide group, thus assisting in pulling the peptide group away from the copper ion.

Many reactions of H_2O with oligopeptide complexes have been documented (6, 7, 10, 11). The rate constant for the reaction of H_2O

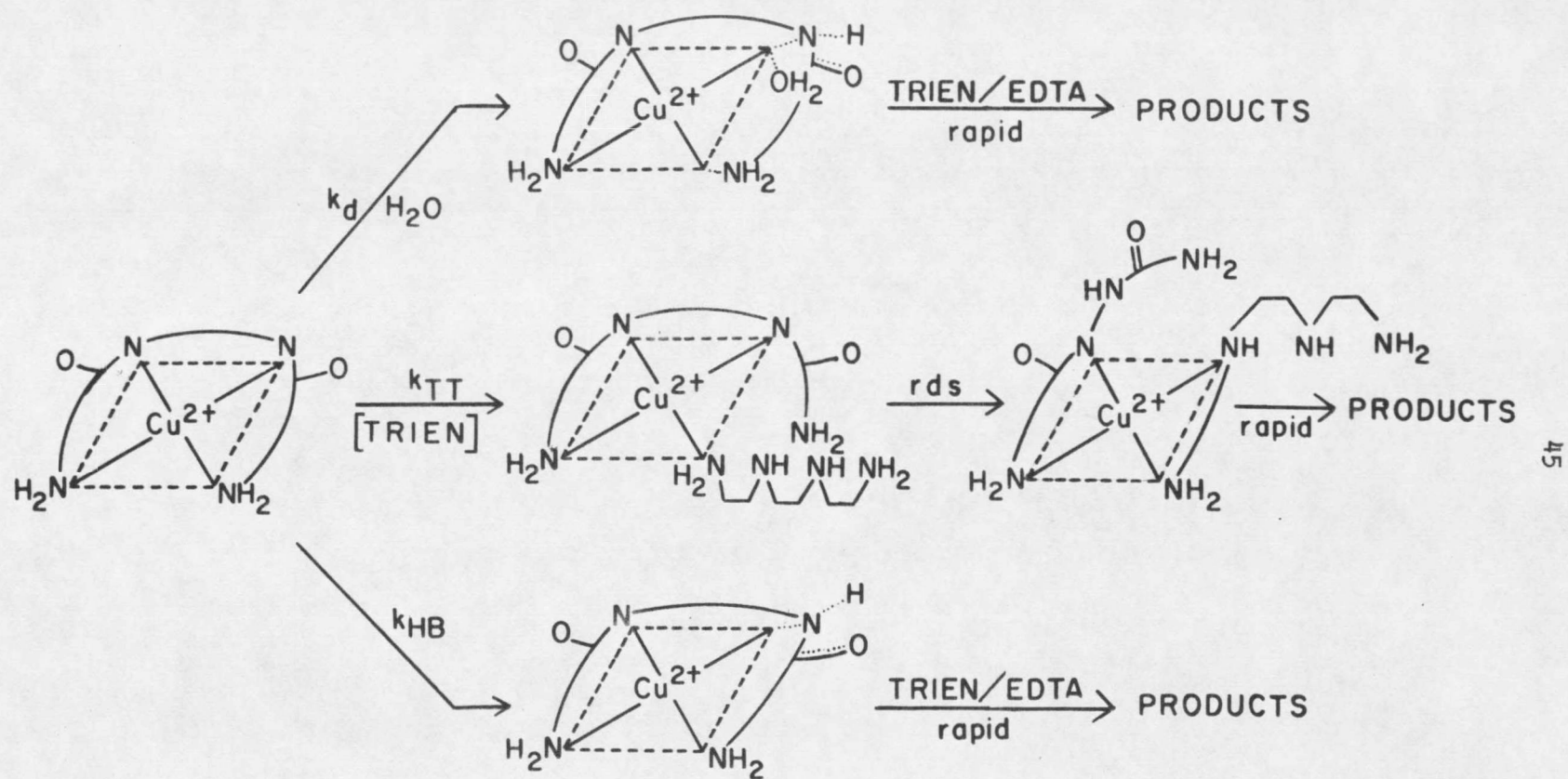
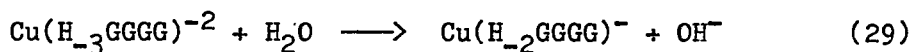


Figure 12. Schematic representation of the parallel reaction pathways of CuH₋₂DGEN.

with $\text{Cu}(\text{H}_2\text{GGG})^-$ is 0.12 s^{-1} (9). The reaction of H_2O with $\text{Cu}(\text{H}_3\text{GGGG})^{-2}$ is 16 s^{-1} ($2.9 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ for $55 \text{ M H}_2\text{O}$) (10). In the tetraglycine system it was first thought that this large k_d term was attributed to nucleophilic attack by H_2O . However, studies in D_2O have shown that the reaction of solvent with $\text{Cu}(\text{H}_3\text{GGGG})^{-2}$ exhibits normal isotope effects (29). Therefore, the H_2O pathway appears to be a proton-transfer, as depicted in Equation 29 (10).



The nickel(II) complex of DGEN was also found to have a solvent dissociative pathway with a k_d term of $2.4 \times 10^{-4} \text{ s}^{-1}$ (30). The larger k_d term of CuH_2DGEN is associated with its smaller crystal field stabilization energy, as compared to NiH_2DGEN .

Nucleophilic Pathway

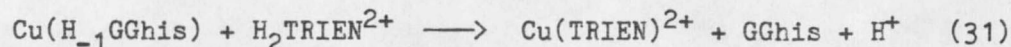
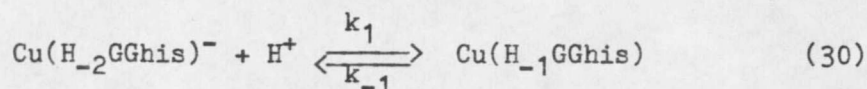
There are large differences in the kinetic behavior of copper(II) oligopeptide complexes in their reactions with TRIEN. The rate constant for the unprotonated TRIEN species reacting with $\text{Cu}(\text{H}_2\text{GGG})^-$ is $1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (9). In this study, the unprotonated TRIEN species reacted with CuH_2DGEN with a rate constant of only $650 \text{ M}^{-1} \text{ s}^{-1}$. In $\text{Cu}(\text{H}_2\text{GGG})^-$, only a carboxylate group need be displaced for a nucleophile to gain an equatorial site, whereas the corresponding reaction of CuH_2DGEN would require an amine nitrogen to be displaced. The amine nitrogen forms a much stronger bond to copper than does the carboxylate group, thus rendering the CuH_2DGEN complex less vulnerable to nucleophilic attack.

The monoprotinated TRIEN species was also found to react with CuH_2DGEN with a rate constant of $210 \text{ M}^{-1} \text{ s}^{-1}$. This is a three-fold reduction compared to the unprotonated TRIEN rate constant. This reduction may be reflected in the difference between the first and second acid dissociation constants for TRIEN (refer to Table 2). This difference suggests that the unprotonated TRIEN species should be a more effective nucleophile by approximately a factor of four, which is in close agreement with the reduction of the HTRIEN^+ rate constant compared to the rate constant for the unprotonated TRIEN species. Similar patterns have also been found for the reaction of TRIEN and HTRIEN^+ with $\text{Cu}(\text{H}_2\text{GGG})^-$ (9).

For the reaction of $\text{Cu}(\text{H}_2\text{GGG})^-$, the rate constant for $\text{H}_2\text{TRIN}^{2+}$ was experimentally found to be $1.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (9), which is approximately 100 times less reactive than the rate constant for the unprotonated TRIEN species. On the other hand, the rate constant for the reaction of $\text{H}_2\text{TRIN}^{2+}$ with CuH_2DGEN is $87 \text{ M}^{-1} \text{ s}^{-1}$, which is only seven times less reactive than the unprotonated TRIEN species. This comparison suggests that $\text{H}_2\text{TRIN}^{2+}$ reacts as a nucleophile with $\text{Cu}(\text{H}_2\text{GGG})^-$ but as a coordinating acid with CuH_2DGEN . A similar pattern was also observed for the reaction of TRIEN with $\text{Cu}(\text{H}_3\text{GGGG})^{-2}$ (10) where the rate constants are 490 and $62 \text{ M}^{-1} \text{ s}^{-1}$ for TRIEN and $\text{H}_2\text{TRIN}^{2+}$, respectively. In this study $\text{H}_2\text{TRIN}^{2+}$ was also classified as a coordinating acid rather than a nucleophile.

Further comparison of the reaction of TRIEN with CuH_2DGEN and $\text{Cu}(\text{H}_2\text{GGHis})^-$ illustrates that the presence of histidine as the third

amino acid residue in a given complex has a significant effect on the mechanism by which Cu^{2+} is transferred from the peptide complex to TRIEN. The nucleophilic displacement of the peptide ligand from $\text{Cu}(\text{H}_{-2}\text{GGhis})^-$ is very ineffective unless assisted by protonation forming $\text{Cu}(\text{H}_{-1}\text{GGhis})$ (7), as shown in Equations 30 and 31. Thus,



$\text{H}_2\text{TRIEN}^{2+}$, the predominant TRIEN species in solution at pH values where $\text{Cu}(\text{H}_{-1}\text{GGhis})$ is formed can effectively displace GGhis from $\text{Cu}(\text{H}_{-1}\text{GGhis})$, while the unprotonated TRIEN is relatively ineffective in displacing GGhis from $\text{Cu}(\text{H}_{-2}\text{GGhis})^-$.

The results obtained in this study indicate the proton-assisted mechanism is not required (in the rate determining step) for the displacement of DGEN from $\text{CuH}_{-2}\text{DGEN}$ by TRIEN. If $\text{CuH}_{-2}\text{DGEN}$ reacted by a proton-assisted nucleophilic mechanism, the dependence in TRIEN would have changed from first-order to zero-order as the TRIEN concentration increased. The linear plots of k_{obs} versus $[\text{TRIEN}]_t$ shown in Figure 4 verifies the non-existence of a proton-assisted mechanism.

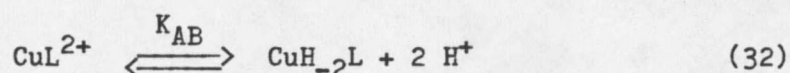
The reaction of EDTA with $\text{CuH}_{-2}\text{DGEN}$ was found to be independent of the unprotonated EDTA species. This suggests that EDTA^{-4} is unable to react in a direct nucleophilic manner. In a study

presented by Pagenkopf and Margerum (9), the unprotonated species of EDTA was ineffective at displacing triglycine from $\text{Cu}(\text{H}_2\text{GGG})^-$. Furthermore, it was postulated through use of space filling molecular models that the nucleophilic mechanism requires nitrogen coordination in the planar (equatorial) position. Those replacing ligands with tertiary nitrogens, such as EDTA, are sterically blocked, while those with primary nitrogens, as is the case with TRIEN, can react directly (9).

Protonation Pathway

The rapid rate with which general acids react with CuH_2DGEN has some very significant implications. The CuH_2DGEN complex, with its two terminal amine groups, reacts with acids (*i.e.*, $\text{H}_2\text{EDTA}^{-2}$, $\text{H}_3\text{TRIEN}^{3+}$ and H_3BO_3) approximately 50 times faster than the corresponding $\text{Cu}(\text{H}_2\text{GGG})^-$ complex. For example, the H_3BO_3 rate constant with $\text{Cu}(\text{H}_2\text{GGG})^-$ is $2.2 \text{ M}^{-1} \text{ s}^{-1}$ (28), while H_3BO_3 reacts with CuH_2DGEN with a rate constant of $116 \text{ M}^{-1} \text{ s}^{-1}$. Further evidence for the enhanced general acid reactivity pattern of CuH_2DGEN is documented in the large rate constant for $\text{H}_3\text{TRIEN}^{3+}$.

The difference between the general acid reactivity of CuH_2DGEN and $\text{Cu}(\text{H}_2\text{GGG})^-$ is reflected in the difference between their stability constants. The formation of a doubly-deprotonated copper(II) complex, similar to CuH_2DGEN and $\text{Cu}(\text{H}_2\text{GGG})^-$, can be represented by Equations 32 and 33. The value of K_{AB} for the



$$K_{AB} = \frac{[\text{CuH}_2\text{L}][\text{H}^+]^2}{[\text{CuL}^{2+}]} \quad (33)$$

formation of CuH_2DGEN is $10^{-13.8}$ (25) as compared to $10^{-12.0}$ for $\text{Cu}(\text{H}_2\text{GGG})^-$ (31). This comparison of stability constants for CuH_2DGEN and $\text{Cu}(\text{H}_2\text{GGG})^-$ shows that the carboxylate group increases the stability (toward acid catalysis) by a factor of 60.

The H_3O^+ protonation rate constant for CuH_2DGEN was found to be $1.6 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. This value falls within the range of other copper(II) oligopeptide H_3O^+ protonation constants (7, 9, 10, 11). One would expect the exceptionally strong acid (H_3O^+) to react with diffusion controlled rates. There are a number of reasons for this slow proton-transfer step: a) the pK_a of $\text{Cu}(\text{H}_n\text{L})\text{H}$ is small, and when ΔpK_a is negative {i.e., $\text{pK}_a(\text{HX})$ is greater} a slower proton-transfer step is expected (32); b) to accomplish the proton-transfer, some electronic and structural rearrangement is necessary, as was depicted in Figure 2. These rearrangements are required whether the proton goes initially to the peptide-oxygen or to the peptide-nitrogen.

The presence of an amino group at both terminal sites of CuH_2DGEN suggests three possible explanations for its increased reactivity with acids: a) strain in the terminal chelate rings, b) trans effects and c) symmetry considerations.

Examination of the crystal structure of $\text{Na}_2\text{Cu}(\text{H}_3\text{GGGG}) \cdot 10 \text{ H}_2\text{O}$ shows the metal-nitrogen bond distance to be longer for the terminal

nitrogens than for the non-terminal ones (33). Consequently, the bond distance of the terminal amino bonds of CuH_2DGEN is longer than the peptide-nitrogen bonds. This longer terminal bond length could possibly result in ring strain of the first and third chelated rings. It is possible that this resultant ring strain could enhance proton-transfer by facilitating the stretching of the metal-nitrogen bond as proton-transfer proceeds.

The second reasonable explanation for the greater proton-transfer of CuH_2DGEN is the trans effect (34). The trans effect may result in weakening the terminal amino bonds. This probable bond weakening is a result of another peptide group coordinated to copper in the trans position of the complex.

The symmetry of CuH_2DGEN is another possible explanation for its enhanced reactivity with acids. Most copper(II) oligopeptides {i.e., $\text{Cu}(\text{H}_2\text{GGG})^-$, $\text{Cu}(\text{H}_2\text{GGHis})^-$ and $\text{Cu}(\text{H}_3\text{GGGG})^{-2}$ } are asymmetrical. This asymmetry results in acid attack at the most basic peptide nitrogen. For example, nuclear magnetic resonance studies with triglycine (35) have shown that the peptide nitrogen nearest to the carboxylate group is the most basic. In the CuH_2DGEN complex either peptide nitrogen is susceptible to acid attack since both peptide nitrogens reside in identical environments. It is possible that this multidirectional acid attack at either peptide nitrogen results in the stimulated proton-transfer reactions with CuH_2DGEN . To fully evaluate this concept, more reactivity patterns of symmetrical complexes are needed.

The preceding discussion has developed the parallel reaction pathways of the CuH_2DGEN complex. It has been shown that CuH_2DGEN reacts with H_2O in a solvent dissociative pathway. This pathway results in a rearrangement of the complex to a form that can react readily with replacing ligands. The nucleophilic pathway by TRIE and HTRIE^+ could displace the original peptide (DGEN), while the unprotonated EDTA species was ineffective in displacing DGEN from CuH_2DGEN because it is sterically hindered from attack at a kinetically accessible planar position. Furthermore, the reaction with general acids such as $\text{H}_3\text{TRIE}^{3+}$, H_3BO_3 , HEDTA^{-3} and $\text{H}_2\text{EDTA}^{-2}$ is facilitated by proton-transfer to a peptide nitrogen. This proton-transfer results in cleavage of the copper-peptide nitrogen bond, and subsequent reaction with replacing ligand is rapid relative to the proton-transfer step.

CHAPTER 6

CONCLUSION

The kinetics and mechanisms for the reaction of CuH_2DGEN with TRIEN and EDTA have been studied as an aid to understand further metal-peptide complexes.

It has been shown that CuH_2DGEN reacts with H_2O in a solvent dissociative pathway. The rate of H_2O attack on CuH_2DGEN is much slower than the corresponding attack of H_2O on copper tetraglycine (1), but much faster than H_2O attack on copper glycylglycyl-L-histidine (7). From these comparisons it was postulated that the presence of a peptide-nitrogen in the terminal position greatly enhances the rate of solvent attack compared to the presence of an amine group in the terminal position.

The rate of nucleophilic attack by TRIEN is greatly reduced when the terminal group is an amine nitrogen. Similar effects are also observed when the terminal position is occupied by a peptide-nitrogen. When TRIEN can react by displacing an equatorial carboxylate group, the reaction is much faster compared to the displacement of an amine group or deprotonated peptide group. Furthermore, it has been shown that nucleophiles with tertiary nitrogens (such as EDTA) are ineffective in reacting directly with CuH_2DGEN because of steric requirements for attack at a kinetically accessible equatorial site.

The kinetics for the reaction of CuH_2DGEN with acids has shown that proton-transfer is more rapid than proton-transfer to $\text{Cu}(\text{H}_2\text{GGG})^-$ or $\text{Cu}(\text{H}_2\text{GGHis})^-$. It has been postulated that the

enhanced reactivity of CuH_2DGEN with acids is caused by a combination of four effects: a) stability constant of the complex, b) strain in the terminal chelated rings, c) trans effect and d) symmetry considerations.

The kinetics and mechanisms of the transfer of Cu(II) between peptide and amino acid complexes are important to an understanding of the possible limiting rates, specificity and catalysis in the biological transport of this metal ion. Understanding the basic chemistry and mechanisms of such complexes as CuH_2DGEN may aid in developing models for other peptide and amino acid complexes of copper.

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