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Spectrophotometric Studies of Complex Formation of Alpha Aminohydroxamic Acids

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SPECTROPHOTOMETRIC STUDIES OF COMPLEX FORMATION OF ALPHA AMINOHYDROXAMIC ACIDS

by

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INTRODUCTION

The purpose of this research was to prepare the α-aminohydroxamic acids, and to develop a method to study the complexes of these compounds with several metal ions and to calculate the stability constants of the complexes.

α-Aminohydroxamic acids are of interest because of the antitumor activity exhibited by the structurally related compound hydroxyurea. It was hoped that the compounds synthesized would have anticancer activity. It was anticipated that the biological test data of these compounds would demonstrate a correlation between the stability constants and biological activity. It was also hoped that the results of this research would shed some light on future investigation on this series of compounds.

HISTORICAL

In 1917, Jones and Sneed[1] prepared glycine hydroxamic acid from ethylglycinate and hydroxylamine. The procedure is briefly described as follows:

Proper amounts of ethylglycinate were treated at about -10°C with an alcoholic solution of hydroxylamine. The hydroxylamine was added slowly and the flask was shaken vigorously after each addition. A clear solution resulted from which a white solid began to separate in the course of an hour. The mixture was placed in an ice-box for 12 hours. The contents of the flask were collected, dried, and recrystallized from aqueous methanol. The yield was almost quantitative.

Phenylglycine hydroxamic acid is another α-aminohydroxamic acid which was prepared later by Dunn and coworkers.[2]

The complexes of α-aminohydroxamic acids have not been investigated extensively. The copper and nickel complexes of glycine hydroxamic acid have been isolated as reported by Ley and Männchen,[3] however no stability constant calculations were made. The stability constant of the 2:1 complex of glycine hydroxamic acid with copper has been studied polarographically by Cieleszky and coworkers.[4]

No attempt to calculate the stability constants of α-aminohydroxamic acids by the spectrophotometric method has been reported.

EXPERIMENTAL

The Preparation of Alpha Aminohydroxamic Acids

All melting points expressed in degrees centigrade were measured on Thomas Hoover capillary melting point apparatus and were corrected. Thin layer chromatography was done on Silica Gel-G which was manufactured by Brinkmann Instruments, Inc., Westbury, L.I., New York; spots were detected by iodine vapor. Infrared spectra were obtained from a Beckmann IR-8 spectrophotometer. Elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tennessee. All analytical samples were dried in vacuo over phosphorus pentoxide for at least twenty-four hours prior to sending them in for analysis.

General Procedure for the Preparation of Alpha Amino Acid Ester Hydrochlorides.--A suspension of an alpha amino acid in excess absolute alcohol cooled in an ice-bath, was saturated with a stream of anhydrous hydrogen chloride. The HCl gas was passed through the solution until the amino acid dissolved and then for another five minutes to ensure complete saturation. The resulting solution contained a small amount of crystallized solids. This solution was allowed to stand overnight at room temperature with occasional shaking and then concentrated under

reduced pressure to give a white product. The infrared spectra showed the C=O absorption expected for esters. The crude product thus obtained was used directly for the preparation of alpha aminohydroxamic acid without further purification. The results obtained were shown in Table 1.

Preparation of Glycine Hydroxamic Acid (I).--The method of Jones and Sneed[1] was used in the preparation of α-aminohydroxamic acids. A solution of 6.73 g (0.12 mole) of potassium hydroxide in 100 ml of absolute methanol was added slowly to a solution of 16.7 g (0.12 mole) of ethylglycinate hydrochloride in 100 ml of absolute methanol at 30-40°. The reaction mixture was shaken vigorously during the addition and then allowed to cool in an ice-bath for five minutes. The potassium chloride from the reaction mixture was collected on a Celite filter. The filtrate was concentrated under reduced pressure to a volume of about 30 ml. The precipitate after concentration was filtered. A concentrated solution of about 70 ml of hydroxylamine was prepared by treating a solution of 13.9 g (0.2 mole) of hydroxylamine hydrochloride in 150 ml of absolute methanol with a solution of 11.2 g (0.2 mole) of potassium hydroxide in 100 ml of absolute methanol at 30-40°. The hydroxylamine solution was slowly added to the ethylglycinate solution at 0°. The whole reaction mixture was then placed overnight in the

Table I

The Preparation of Alpha Amino Acid Ester, Hydrochlorides

Alpha amino acid	Amount g.	Solvent	Amount ml.	Yield of product %	m.p. Exptl.	m.p. Lit.[5]
Phenylglycine	15.1	Ethanol	100	98.2	200-205°	202°
D,L-alanine	23.0	Methanol	150	99.2	154-160°	158°
D,L-phenylalanine	33.0	Methanol	150	98.9	158-162°	158-162°
1-aminocyclopentanecarboxylic acid	19.4	Methanol	100	95.2	202-205°	---

refrigerator. The resulting precipitate was collected by vacuum filtration to give 8.3 g, 77.6% yield, of crude product. Two recrystallizations from 50% pyridine-water gave white crystals, m.p. 140-141°, lit.[1] 140°, which gave one sharp spot on a thin layer chromatographic plate using a mixture of acetone and methanol as solvent. Anal. Calc. for $C_2H_6N_2O_2$: C, 26.66; H, 6.71; N, 31.10. Found: C, 26.52; H, 6.64; N, 31.08

Preparation of D,L-Phenylglycine Hydroxamic Acid (II). --In a manner analogous to that used in the preparation of I, compound (II) was prepared from 21.57 g (0.1 mole) of ethyl phenylglycinate hydrochloride and 13.9 g (0.2 mole) of hydroxylamine hydrochloride. A yield of 14.7 g, 61% of crude product, was obtained. A sample submitted for analysis was recrystallized once from deionized water and gave white crystals, m.p. 169-170°. Anal. Calc. for $C_8H_{10}N_2O_2$: C, 57.81; H, 6.06; N, 16.86. Found: C, 58.0; H, 6.0; N, 16.76.

Preparation of D,L-Alanine Hydroxamic Acid (III). --In a manner analogous to that used in the preparation of I, compound (III) was prepared from 20.94 g (0.15 mole) of methyl α-alaninate hydrochloride and 20.86 g (0.3 mole) of hydroxylamine hydrochloride. A yield of 12.25 g, 78.4% of crude product, was obtained. A sample submitted for

analysis was recrystallized twice from 50% pyridine-water and gave white crystals, m.p. 166-167°. Anal. Calc. for $C_3H_8N_2O_2$: C, 34.60; H, 7.74; N, 26.91. Found: C, 34.45; H, 7.74; N, 26.77.

Preparation of D,L-Phenylalanine Hydroxamic Acid (IV). --In a manner analogous to that used in the preparation of I, compound (IV) was prepared from 21.57 g (0.1 mole) of methyl α-phenylalaninate hydrochloride and 13.9 g (0.2 mole) of hydroxylamine hydrochloride. A yield of 13.0 g, 72% of crude product, was obtained. A sample submitted for analysis was recrystallized twice from 50% pyridine-water and gave white crystals, m.p. 182-183°. Anal. Calc. for $C_9H_{12}N_2O_2$: C, 60.00; H, 6.71; N, 15.55. Found: C, 59.81; H, 6.63; N, 15.26.

Spectrophotometric Measurements

Materials.--All perchlorates of copper, nickel, and cobalt were obtained from the G. Fredrick Smith Chemical Co., Columbus, Ohio and used directly without further drying or purification. The metal perchlorate solutions of proper concentration were prepared by dissolving the proper weight of the perchlorates in deionized water at room temperature and titrated by the method of ion exchange as described below.

The standardization of the metal solutions[6] were performed as follows. Each perchlorate to be used in the complexation titrations was made approximately 0.1 N by weighing 0.05 of a mole of the hydrated metal perchlorate and dissolving it in 500 ml of deionized water. Subsequently, 4 ml aliquots of these metal solutions were passed through 5 ml of Amberlite IR-120 (H^+) ion exchange resin in a column. The effluent contained an equivalent amount of perchloric acid which was titrated with a standard base. From the titration, the molarities of the metal ion solutions were calculated. This analysis was suggested by Serjeant.[7] The concentrations of the metal solutions were determined spectrophotometrically using the solutions of known concentration as standard solutions which were titrated before by the method of ion exchange. The following equation based on Beer's Law was used in calculating the concentration of the metal ions in the unknown solutions.

$$C' = (A'/A)C \qquad (1)$$

Where A is the absorbance of the standard solution at concentration C in mole/ℓ, and A' is the absorbance of the unknown solution at concentration C' in mole/ℓ.

The α-aminohydroxamic acids used were obtained by vacuum drying over phosphorus pentoxide. Solutions of

the acids at room temperature were prepared by dissolving the proper weight of the acids in solution which was 0.1 N in perchloric acid and 0.1 N in sodium perchlorate.

Apparatus.--Measurements of absorbance were made with a Cary Model 14 spectrophotometer and/or a Beckmann Model DB spectrophotometer using a tungsten lamp and Pyrex cells of 10-mm light path. Temperature measurements were made directly on the solutions in the cells, and, unless otherwise specified, were at 25° ± 0.5°C for all determinations.

The pH measurements were made with a Beckmann Zeromatic pH meter using a combination electrode. This was calibrated against a standard buffer, pH 7.

Procedure.--After each solution was prepared, the absorbance was measured immediately. Preliminary experiments showed that the pH of the solution decreased when it stood at room temperature for one hour. The pH adjustments were made using a 30% potassium hydroxide solution.

The ionization constants of the acids were obtained potentiometrically.[8]

RESULTS

Absorption Spectra

In Fig. 1 are shown typical absorption spectra. Curve II is for a solution which is 0.0406 M in copper perchlorate and 0.0812 M in glycine hydroxamic acid at pH 5.8, and Curve III is for a solution which is 0.0406 M in copper perchlorate and 0.0406 M in glycine hydroxamic acid at pH 5.8. A 0.0914 M glycine hydroxamic acid solution or a 0.0914 M copper perchlorate solution (curve I) showed no appreciable absorption through this range. Since the wavelengths at maximum absorbance for curve II and III were considerably shifted from that for curve I, it is evident that the complexes which were formed with glycine hydroxamic acid absorbed much more strongly than copper perchlorate, particularly in the region of 580 mμ, and 550 mμ respectively.

The wavelengths at maximum absorbance, λ_{max}, for the mixtures of various α-aminohydroxamic acids and various metal perchlorates are shown in Table II. It should be noted that in the case of complex formation of various α-aminohydroxamic acids with nickel(II) and cobalt(II) ions, the maximum absorbances occurred at the same wavelengths for both 1:1 and 2:1 mixtures at a given pH despite the

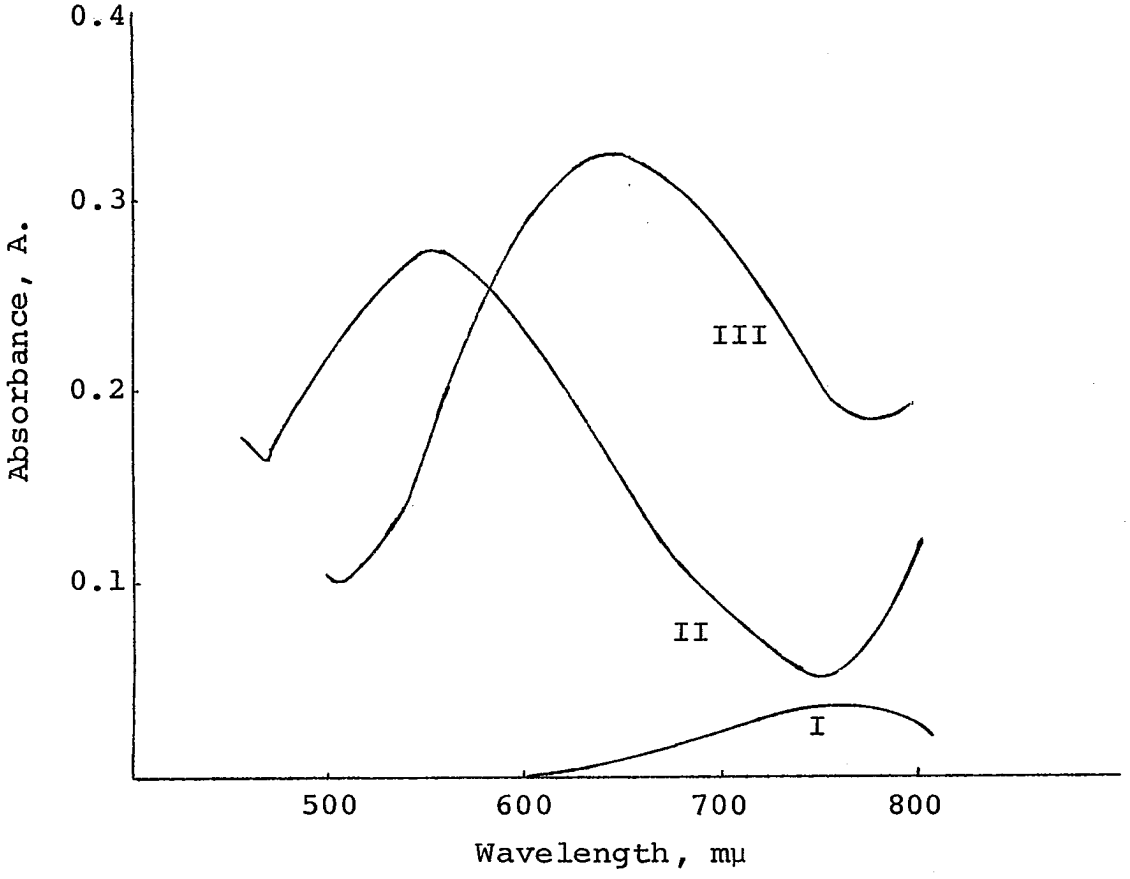

Fig. 1.--Absorption spectra of solutions, pH 5.8 : I, 0.0914 M $[Cu^{++}]$; II, 0.0406 M $[Cu^{++}]$ + 0.0812 M $[CH_2(NH_2)CONHOH]$; III, 0.0406 M $[Cu^{++}]$ + 0.0406 M $[CH_2(NH_2)CONHOH]$.

Table II

The Wavelengths at Maximum Absorbance

Ligand	Metal Ions	Molar Ratio* of Ligand to Metal	pH	λ_{max} mμ	Color
Glycine hydroxamic acid	Cu(II)	1:1	5.8	640	Green
		2:1	5.8	550	Purple
	Ni(II)	1:1	6.0	500	Pink
		2:1	6.0	500	Pink
	Co(II)	1:1	6.8	500	Pink
		2:1	6.8	500	Pink
D,L-phenylglycine hydroxamic acid	Cu(II)	1:1	3.95	640	Green
	Ni(II)	1:1	5.9	500	Pink
		2:1	5.9	500	Pink
	Co(II)	1:1	5.6	500	Pink
		2:1	5.6	500	Pink
D,L-alanine hydroxamic acid	Cu(II)	1:1	5.8	640	Green
		2:1	5.8	550	Purple
	Ni(II)	1:1	5.45	500	Pink
		2:1	5.45	500	Pink
	Co(II)	1:1	7.0	500	Pink
		2:1	7.0	500	Pink

*It should be made clear that this refers to the analytical concentrations of acid and metal, and not necessary to the composition of the complex.

fact that the maximum absorbances were different. The colors of the two solutions concerned were identical. These observations indicate, therefore, that either a 1:1 complex only, or a 2:1 complex only, was formed at that given pH, unless the molar extinction coefficients of the two complexes are identical over the range of wavelength covered. The identification of the composition of complexes will be discussed in more detail later.

Observation of Effect of pH on Complex Formation

Several methods have been employed for the detection of the presence of complexes in aqueous solution. In our investigation however, the complexes formed were observed by the formation of a color different from that of a solution of either metal perchlorates or α-aminohydroxamic acids. Preliminary experiments showed that the color of solutions of α-aminohydroxamic acids with metal ions changed as the pH of the solution changed. The pH used for the studies of complex formation was therefore selected by carefully adding either a 30% potassium hydroxide solution or concentrated (70%) perchloric acid to the ligand-metal solution until a change of color was observed which might indicate the presence of complexes. The following results were obtained (Table III).

Table III

Observation of Complexes

Ligand	Metal Ions	Color	Complexes predicted
Glycine hydroxamic acid	Cu(II)	Green and Purple	2
	Ni(II)	Pink	1
	Co(II)	Pink	1
D,L-phenylglycine hydroxamic acid	Cu(II)	Green and Purple*	2
	Ni(II)	Pink	1
	Co(II)	Pink	1
D,L-alanine hydroxamic acid	Cu(II)	Green and Purple	2
	Ni(II)	Pink	1
	Co(II)	Pink	1

It was observed that for α-aminohydroxamic acids with Cu(II) ion, the color of the solution containing the acid and the metal ion in the molar ratios $\leq$ 1 was green at pH $\leq$ 6 and that in the molar ratios $\geq$ 2 was purple at pH $\geq$ 6. It appeared that at pH of approximate 6, both complexes could be detected by the corresponding colors present in the solution. It should be noted, however, that identification of the composition of the complex formed can be carried out at two different pHs as done earlier by Foley and Anderson[9] in this field.

*The solution of phenylglycine hydroxamic acid with Cu(II) ion precipitated as the color of the solution changed to purple.

In order to observe the pH or the region of pH in which more than one complex was obtained, a solution of glycine hydroxamic acid with Cu(II) ion in the molar ratio 2:1, pH 2.0 was prepared. The pH of the solution was gradually increased using a 30% potassium hydroxide solution. A change of color from blue to green at pH 3.05 and then to purple at pH 5.8 was observed. The absorption spectra were then obtained at pH 5.8 for both 1:1 and 2:1 mixtures. The results are shown in Figure 1.

In the case of the complex formation of α-amino-hydroxamic acids with nickel(II) and cobalt(II) ions, it is assumed that no more than one complex was formed since the color of the solution showed no appreciable change through the whole pH range. In this case, only a single pH was used for the investigation of complex formation. The possibility that the colors of the solutions with acid:metal ratios of 1:1 and 2:1 are identical, or that colorless complexes may exist in the solution must be considered.

Composition of Complex

The composition of complexes was determined by the Job's method of continuous variations[10,11] and confirmed by the molar ratio and the slope ratio methods which are described later. Job's method of continuous variations

is based on the variation of the absorbance of solutions containing different ratios of metal ion and ligand, while simultaneously maintaining a constant total concentration of reactants. Job has pointed out that the method of continuous variations was not generally applicable when more than one complex was formed. However, Vosburgh and Cooper[11] showed that the method of continuous variations would be more useful if it could be readily ascertained in any particular case whether or not more than one complex is formed from a pair of components. In order to show that Job's procedure is applicable to the system in which more than one complex is formed, the following theoretical treatment[11,12] was made.

Consider, for example, that the interaction of a metal ion with a ligand results in the successive formation of the metal complexes ML and ML_2, according to the following reactions:

$$M + L = ML, \quad k_1 = [ML]/[M][L] \tag{2}$$

$$ML + L = ML_2, \quad k_2 = [ML_2]/[ML][L] \tag{3}$$

If a solution of the ligand, L, is mixed with a solution of the metal ion, M, so that the total concentration of ligand plus metal is maintained constant, the following equations hold:

$$[M] = M_t - [ML] - [ML_2]$$
$$[L] = L_t - [ML] - 2[ML_2] \quad (4)$$
$$\text{and } M_t + L_t = \text{constant}$$

where M_t and L_t are the total molarities of species containing M and L. The absorbance, A, of the solution at a given wavelength represents the total absorption of all species in the solution:

$$A = l\,(a_1[M] + a_2[L] + a_3[ML] + a_4[ML_2]) \quad (5)$$

where l is the length of the light path through the solution, and a_1, a_2, a_3 and a_4 are the molar absorptivities of M, L, ML and ML_2. Let us now introduce a function Y, which represents the difference in the absorbance A, of Equation 5 and the corresponding absorbance that would have resulted if no reaction had occurred when solutions of M and L were mixed:

$$Y = l\,(a_1[M] + a_2[L] + a_3[ML] + a_4[ML_2]) - l\,(a_1M_t + a_2L_t) \quad (6)$$

where the ligand is optically transparent, Equation 6 may be simplified by setting $a_2 = 0$. If the cell path l is 1 cm., the function Y is defined by the relationship

$$Y = a_1[M] + a_3[ML] + a_4[ML_2] - a_1M_t \quad (7)$$

Differentiation of Equation 7 with respect to x, the mole fraction of the chelating agent, and rearrangement give

$$dY/dx = a_3 d[ML]/dx + a_4 d[ML_2]/dx - a_1 (d M_t/dx - d[M]/dx) \quad (8)$$

Differentiation of Equation 4 with respect to x and rearrangement give

$$dM_t/dx - d[M]/dx = d[ML]/dx + d[ML_2]/dx \quad (9)$$

Substitution of Equation 9 into 8 and rearrangement give

$$dY/dx = (a_3 - a_1)\ d[ML]/dx + (a_4 - a_1)\ d[ML_2]/dx \quad (10)$$

Equation 10 represents the basis of the Job's method of continuous variations. It is apparent from Equation 10 that the maximum or minimum value of Y need not coincide with a maximum in [ML] or $[ML_2]$ since it is not necessary that both d[ML]/dx and $d[ML_2]/dx$ be zero simultaneously when dY/dx is zero. It can be seen from Equation 10, however, that a maximum in Y (i.e., dY/dx = 0) will correspond to a maximum formation of ML or ML_2 if the following conditions hold:

I). For maximum formation of ML (i.e., dY/dx = d[ML]/dx = 0)

a). $a_3 > a_4$ and/or $[ML] > [ML_2]$

b). $a_4 = a_1$ and $a_3 \neq a_1$

c). $a_3 = a_4$ and $[ML] > [ML_2]$

II). For maximum formation of ML_2 (i.e., $dY/dx = d[ML_2]/dx = 0$)

d). $a_4 > a_3$ and all of M has been presumably converted to ML.

If the condition a) holds, Equation 10 approaches

$$dY/dx = (a_3 - a_1)\, d[ML]/dx \tag{11}$$

since a large value of a_3, relative to a_4 should result in making the first term on the right side of Equation 10 much larger than the second. Thus, a maximum in Y would correspond to maximum formation of ML.

If the condition b) holds, Equation 7 is greatly simplified and becomes

$$Y = (a_3 - a_1)\, [ML] \tag{12}$$

Thus Y is only a function of [ML], independent of $[ML_2]$ since a_3 and a_1 are finite quantities. Then Equation 10 reduces to:

$$dY/dx = (a_3 - a_1)\, d[ML]/dx \tag{13}$$

and the maximum in Y corresponds to the maximum in ML.

If the condition c) holds, Equation 10 becomes

$$dY/dx = (a_3 - a_1)\ (d[ML]/dx + d[ML_2]/dx) \qquad (14)$$

when ML is a maximum, both $[ML_2]$ and $d[ML_2]/dx$ should be relatively small, especially if the lower chelate is the more stable, and consequently the maximum in Y corresponds approximately to a maximum in [ML].

If condition d) holds, Y' is defined[11] as the difference between the measured absorbance and the absorbance calculated on the assumption that all of M has been converted to ML:

$$Y' = a_3[ML] + a_4[ML_2] - a_3M_t \qquad (15)$$

The assumption of the presence of a negligible concentration of free metal ion is generally valid, if the complexes are sufficiently stable. Under these conditions,

$$dY'/dx = (a_4 - a_3)\ d[ML_2]/dx \qquad (16)$$

if a wavelength can be found where $a_4 > a_3$, a maximum in Y' corresponds to a maximum in $[ML_2]$.

Application of these principles to a particular system has been reported by Jonassen and Dexter.[13] As shown in Figure 1 and Table II, for solutions of glycine hydroxamic acid and Cu(II) ion at pH 5.8 the absorption maximum

shifts toward shorter wavelengths as the concentration of ligand is increased, and a sharp change in the spectrum results when the ligand concentration is increased from a ligand:Cu(II) ratio of 1:1 to 2:1. A further increase in the ligand:Cu(II) ratio at constant Cu(II) concentration results in no further change in the spectrum compared to that of the 2:1 ratio of ligand:Cu(II) (see Table II). In fact, the near coincidence of the 2:1 and 3:1 data indicates that no colored complex ion containing more than two moles of ligand per mole of Cu is produced.

It is evident from Figure 1 that light having a wavelength of 580 mμ should be suitable for determining the existence of a 1:1 Cu(II) chelate species, since the molar absorptivities of the 1:1 and 2:1 chelates are approximately equal. Equation 10 should apply, and a maximum in the absorption increment with respect to the fraction of glycine hydroxamic acid, x, should correspond to the maximum formation of Cu(GHA) complex. From Table II, the wavelength of 640 mμ should also be suitable for this purpose since $a_3 > a_4$ at this wavelength and ML rather than ML_2 is presumably the predominant species in the solution thus satisfying condition a). A spectrophotometric study at this wavelength was performed. It was found, however, that a plot of Y versus the mole fraction of ligand, at 640 mμ, did not give a curve of the expected form.

The result is shown in Figure 2. The following assumption was made concerning the mechanism of complex formation of glycine hydroxamic acid with Cu(II) ion at 640 mμ.

The irregularity which occurred in curve A (ligand: metal ratio $\leq$ 1:1), Figure 2 was assumed to be the result of a high concentration of the species, ML, as shown by the following reactions:

$$M + L \rightleftarrows ML \tag{17}$$

$$ML + L \rightleftarrows ML_2 \tag{18}$$

However, curve B (ligand:metal ratio $\geq$ 2:1) was considered to be predominantly representative of the species, ML_2, according to the following reactions:

$$M + L \rightleftarrows ML \tag{19}$$

$$ML + L \rightleftarrows ML_2 \tag{20}$$

This case is considered to be different from that reported by Vosburgh and Cooper[11] because condition a) is not applicable to the formation of 1:1 complex of Cu(II) ion with the ligand at 640 mμ and the other wavelengths where $a_3 > a_4$ but $[ML_2]$ is relatively larger than [ML] for the solutions with ligand:metal ratios $\geq$ 2:1. In addition, Job's method could not be used to determine the formation of 1:1 complex of Cu(II) ion with D,L-alanine hydroxamic acid and D,L-phenylglycine hydroxamic acid at wavelengths

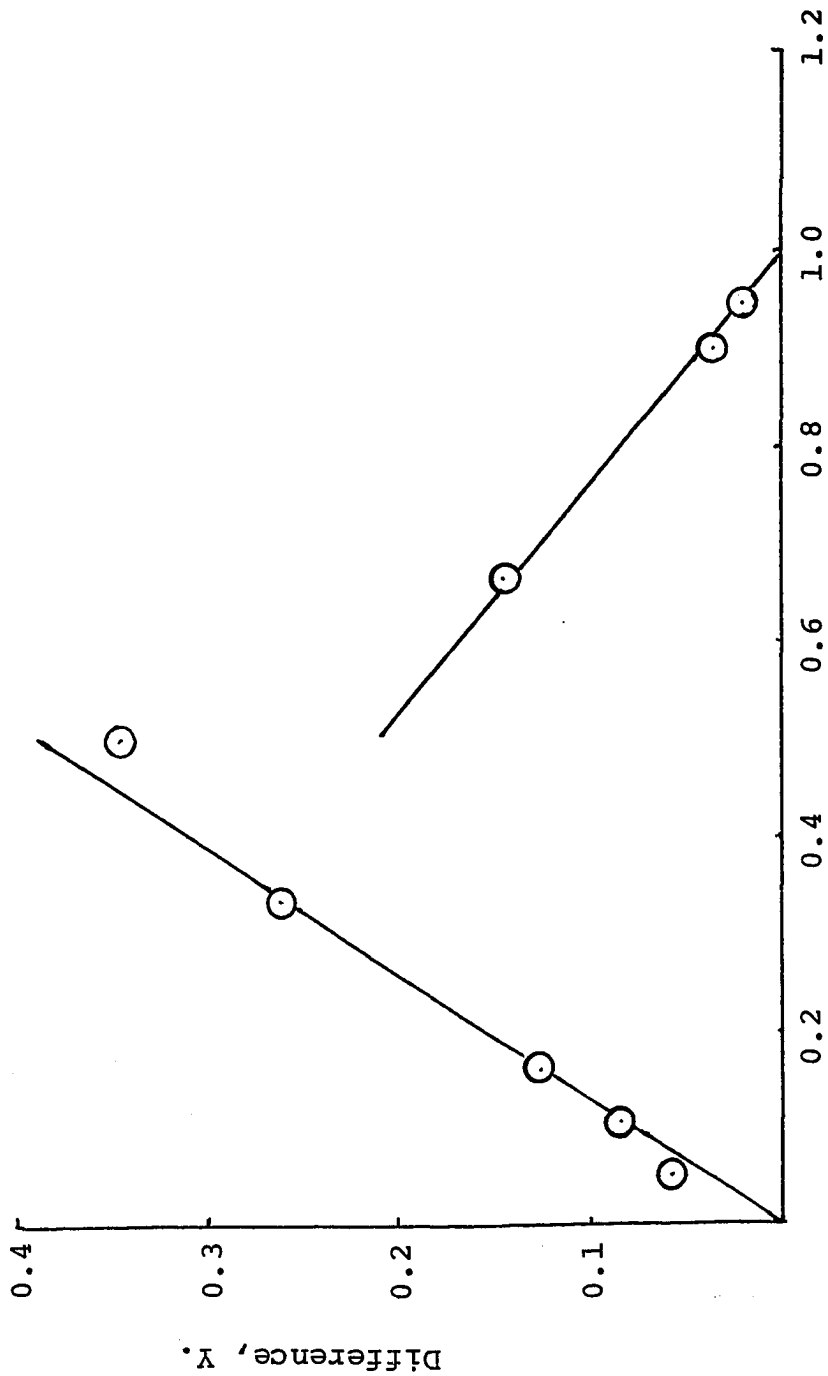

Fig. 2.--Method of continuous variations, pH 5.8, λ = 640 mμ.

where $a_3 > a_4$ because $[ML_2] > [ML]$ at ligand:metal ratios $\geq$ 2:1.

However, measurements at 550 mμ should be suitable for determining the existence of the $Cu(GHA)_2$ species, since in this case, a_4 for the 2:1 form is greater than a_3 for the 1:1 compound (Figure 1) and condition d) holds for this case. As shown in Figure 3, plot of Y' versus the composition at 550 mμ gives a maximum at mole fraction = 0.625 indicative of a 2:1 complex. Deviation from the expected value of 0.667 is due to the above assumption made in deriving Equation 15. The Y' and Y values plotted against the fraction of the ligand for Cu(II) chelates are shown in Figures 3, 4, and 5 whereas those for nickel(II) and cobalt(II) chelates are shown in Figures 6, 7, and 8 and Figures 9, 10, and 11 respectively. The results are summarized in Table IV.

It should be recalled that since the mixture containing the ligand and Ni(II) or Co(II) ion in the molar ratios 1:1 and 2:1 at a given pH showed very similar colors and adsorption spectra (i.e., there is no intersection between two curves at which wavelength the molar absorptivities of a 1:1 complex is approximately equal to that of a 2:1 complex). In order to verify that there may exist only a single complex at the given pH, several wavelengths were selected for the spectrophotometric studies.

The composition of complexes was confirmed using the molar ratio method[15] and the slope ratio.[16] In Figure 3a and 3b are shown the molar ratio plots for the Cu(II)-glycine hydroxamic acid system. Examples of application of the slope ratio method to the determination of the composition of complexes were shown in Figures 3c, 3d, 5c, 5d, 6c, 6d, 10c and 10d. The results obtained were in good agreement with those obtained by Job's methods of continuous variations.

Table IV

Composition of the Complexes

Ligand*	Metal Ion	λmμ	pH	X**	Complex formed
GHA	Cu(II)	580	5.8	0.495	Cu(GHA)
		550	5.8	0.625	$Cu(GHA)_2$
	Ni(II)	540	6.0	0.68	$Ni(GHA)_2$
		500	6.0	0.64	$Ni(GHA)_2$
	Co(II)	540	6.8	0.64	$Co(GHA)_2$
		500	6.8	0.65	$Co(GHA)_2$
PGHA	Cu(II)	580	3.95	0.5	Cu(PGHA)
	Ni(II)	800	5.4	0.655	$Ni(PGHA)_2$
		500	5.4	0.645	$Ni(PGHA)_2$
	Co(II)	800	5.6	0.65	$Co(PGHA)_2$
		550	5.6	0.675	$Co(PGHA)_2$
		500	5.6	0.68	$Co(PGHA)_2$
AHA	Cu(II)	580	5.8	0.5	Cu(AHA)
		550	5.8	0.625	$Cu(AHA)_2$
	Ni(II)	800	5.45	0.63	$Ni(AHA)_2$
		500	5.45	0.68	$Ni(AHA)_2$
		510	5.45	0.68	$Ni(AHA)_2$
	Co(II)	800	7.0	0.67	$Co(AHA)_2$
		600	7.0	0.625	$Co(AHA)_2$
		500	7.0	0.66	$Co(AHA)_2$

*Ligand : α-aminohydroxamic acid

GHA : Glycine hydroxamic acid

PGHA : D,L-phenylglycine hydroxamic acid

AHA : D,L-alanine hydroxamic acid

**X : mole fraction of maximum concentration

The Determination of the Stability Constants

Theoretical.--The continuous-variations and molar-ratio methods have been used, as reported by Reilley and Sawyer,[14] for the determination of the stability constant of a system where only a single complex is formed. Application of these methods to a system where more than one complex is formed has not been reported. Attempt was therefore made to achieve this purpose. Here, we will consider only Job's method of continuous variations.

Consider, for example, the stepwise formation of two complex species, ML and ML_2 (Equation 2 and 3). As shown earlier, the maximum formation of ML is independent of the concentration of ML_2 whereas the maximum formation of ML_2 is independent of the concentration of ML, if condition can be attained such that the concentration of the predominant species is much larger than that of the other complex. For such a system calculation of the stability constant for the system is not very different[11] from that for a system where only one complex is formed and can be done separately for either ML or ML_2 species by treating the system as though only one complex were formed. Thus, the method of Reilley and Sawyer also can be extended to the system where more than one complex is formed and the derivation of equations follows directly.

The extrapolated value, A_e, near the "equivalent point" on the continuous-variation plot corresponds to the total absorbance of the complex if the complex formation were complete. Actually the complex is slightly dissociated in this region, and the absorbance read is therefore somewhat lower.

Assuming that a complex, ML_n (n = 1, 2) is formed, the ratio of the true absorbance to the extrapolated absorbance is the mole fraction of complex actually formed:

$$A/A_e = [ML_n]/\underline{C} \tag{21}$$

where $\underline{C}$ is the total analytical concentration of the metal or ligand (whichever is the limiting concentration at the point in question). Then

$$[ML_n] = (A/A_e)\underline{C} \tag{22}$$

Consider that two complexes, ML and ML_2 are formed simultaneously according to Equations 2 and 3:

$$M + L = ML, \; k_1 = [ML]/[M][L] \tag{2}$$

$$ML + L = ML_2, \; k_2 = [ML_2]/[ML][L] \tag{3}$$

in which L represents $RCH(NH_2)CONHO^-$ where R may be H, C_6H_5- or CH_3- group. According to the following ionizations which have been investigated[6] earlier, however,

if (a), (b) and (c) were

$$\underset{(a)}{RCH(NH_3^+)CONHOH} = \underset{(b)}{RCH(NH_3^+)CONHO^-} + H^+ \qquad (23)$$

$$\underset{(b)}{RCH(NH_3^+)CONHO^-} = \underset{(c)}{RCH(NH_2)CONHO^-} + H^+ \qquad (24)$$

represented by $(H_2L)^+$, HL and L^- respectively, the total analytical concentration of metal, M_t and ligand, L_t can be obtained to give

$$M_t = [M] + [ML] + [ML_2] \qquad (25)$$

$$\begin{aligned} L_t &= [H_2L] + [HL] + [L] + [ML] + 2[ML_2] \\ &= ([H]^2/K_1K_2 + [H]/K_1 + 1)\ [L] + [ML] + 2[ML_2] \\ &= P[L] + [ML] + 2[ML_2] \end{aligned} \qquad (26)$$

Rearrangement of Equation 26 yields

$$[L] = (L_t - [ML] - 2[ML_2])/P \qquad (27)$$

where $P = 1 + [H]/K_1 + [H]^2/K_1K_2$ and K_1, K_2 are the ionization constants corresponding to Equations 24 and 23 respectively. Electrostatic charges are omitted here for simplicity.

I). For maximum formation of ML, Equations 25 and 27 are reduced to:

$$[M] = M_t - [ML] \tag{28}$$

$$[L] = (L_t - [ML])/P \tag{29}$$

Combination of Equations 22, 28, 29 and 2 gives the stepwise stability constant, k_1 of ML:

$$k_1 = \frac{[ML]}{[M][L]} = \frac{(A/A_e)\underline{C}P}{(M_t - \frac{A}{A_e}\underline{C})\ (L_t - \frac{A}{A_e}\underline{C})} \tag{30}$$

It should be noted, however, that Equation 30 can be extended to the system where only a single complex, ML_n (n = 1, 2, 3, etc.) is formed. Thus, we obtain a general equation:

$$k_{on} = \frac{(A/A_e)\underline{C}P^n}{(M_t - \frac{A}{A_e}\underline{C})\ (L_t - n\frac{A}{A_e}\underline{C})^n} \tag{31}$$

The log value of k_{on} is usually used to represent the stability constant of the complex:

$$\log k_{on} = \frac{(A/A_e)\underline{C}}{(M_t - \frac{A}{A_e}\underline{C})\ (L_t - n\frac{A}{A_e}C)^n} + n \text{ Log } \bar{P} \tag{32}$$

II). For maximum formation of ML_2, due to the assumption as given in the condition d) (p. 20), we may consider only Equation 3. Thus, Equation 27 is generally valid and Equation 25 is reduced to:

$$[ML] = M_t - [ML_2] \tag{33}$$

Combination of Equations 22, 33, 27 and 3 gives the stepwise stability constant, k_2 of ML_2:

$$k_2 = \frac{[ML_2]}{[ML][L]} = \frac{(A/A_e)\underline{C}\ P}{(M_t - \frac{A}{A_e}\underline{C})\ (L_t - M_t - \frac{A}{A_e}\underline{C})} \qquad (34)$$

The log value of k_2 is obtained to give

$$\log k_2 = \log \frac{(A/A_e)\underline{C}}{(M_t - \frac{A}{A_e}\underline{C})\ (L_t - M_t - \frac{A}{A_e}\underline{C})} + \log P \qquad (35)$$

The values of pK_a's of α-aminohydroxamic acids were given in Table V.

Table V

The Ionization Constants of Alpha aminohydroxamic Acids

Alpha Aminohydrosamic Acids	pK_1	pK_2
Glycine[6] (I)	9.16	7.21
D,L-phenylglycine[6] (II)	9.00	6.44
D,L-alanine (III)	9.24	7.14

Sample Calculation.--The stability constants of complexes were calculated from the continuous-variations data using Equations 32 and 35. In addition, the stability constants of complexes were also calculated for Cu-GHA system from the molar-ratio data. The results obtained are shown in Table VI. It is apparent, however, that the results

obtained from the molar-ratio data were in good agreement with those obtained from the continuous-variations data. Typical calculations are shown below.

(1). Stability constant calculation based on the continuous-variations data (Figure 3).

a). For a 1:1 complex, CuL^+, where L^- represents the glycine hydroxamate ion, the stability constant k_{01} = [CuL]/[Cu][L], is obtained as follows.

pH = 5.8

x = mole fraction of ligand in question = 0.5

V_x = volume of ligand stock used in 25 ml of the solution = 4.5 ml

V_m = volume of metal stock used in 25 ml of the solution = 4.5 ml

C = concentration of the stock solution of metal or ligand = 0.0254 mole/ℓ

$L_t = (0.0254)\ (4.5)/25 = 4.58 \times 10^{-3}$ mole/ℓ

$M_t = (0.0254)\ (4.5)/25 = 4.58 \times 10^{-3}$ mole/ℓ

$\underline{C} = L_t = M_t = 4.58 \times 10^{-3}$ mole/ℓ

A = 0.246

A_e = 0.276

K_1 = 9.16

K_2 = 7.21

Thus for n = 1, using Equation 32 we obtain

$$\log k_{01} = 9.00$$

(2). Stability constant calculation based on the molar-ratio data (Figure 3a).

b.)

pH = 5.8

R_x = molar-ratio of ligand to metal in question = 1.0

V_x = 4 ml

V_m = 4ml

C = 0.0254 mole/ℓ

L_t = (0.0254) (4.0)/25 = 4.08×10^{-3} mole/ℓ

M_t = 4.08×10^{-3} mole/ℓ

$\underline{C}$ = 4.08×10^{-3} mole/ℓ

A = 0.240

A_e 0.275

K_1 = 9.16

K_2 = 7.21

Thus for n = 1, using Equation 32 we obtain

$$\log k_{01} = 8.91$$

Table VI

The Log of Stability Constants

Ligand	Metal Ions	pH	$\lambda_{m\mu}$	Log k_{01}	Log k_2	Log k_{02}
GHA	Cu(II)	5.80	580	9.00		
				8.91*		
			550		9.05	
					9.29*	
	Ni(II)	6.00	540			13.90
			500			14.08
	Co(II)	6.80	540			11.20
			500			11.28
D,L-PGHA	Cu(II)	3.95	550	11.89		
	Ni(II)	5.40	500			14.48
	Co(II)	5.65	550			14.31
			500			14.21
D,L-AHA	Cu(II)	5.80	580	8.80		
			550		8.85	
	Ni(II)	5.45	500			14.61
			410			14.67
	Co(II)	7.00	500			11.41

*The values calculated from the molar-ratio data.

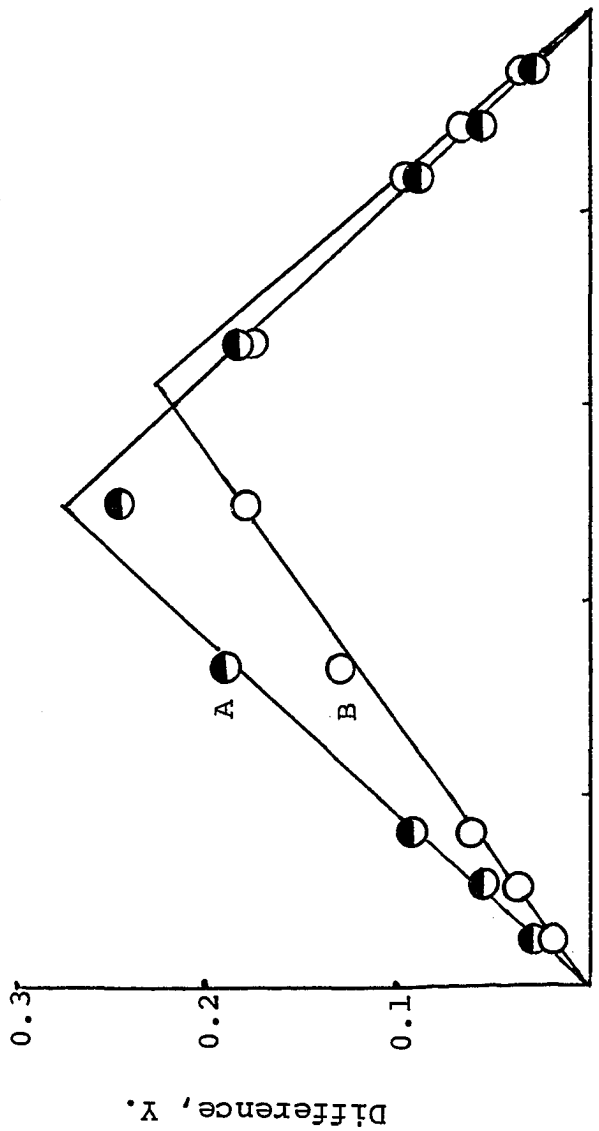

Fig. 3.--Method of continuous variations, pH 5.8:
A, λ = 580 mμ; B, λ = 550 mμ.

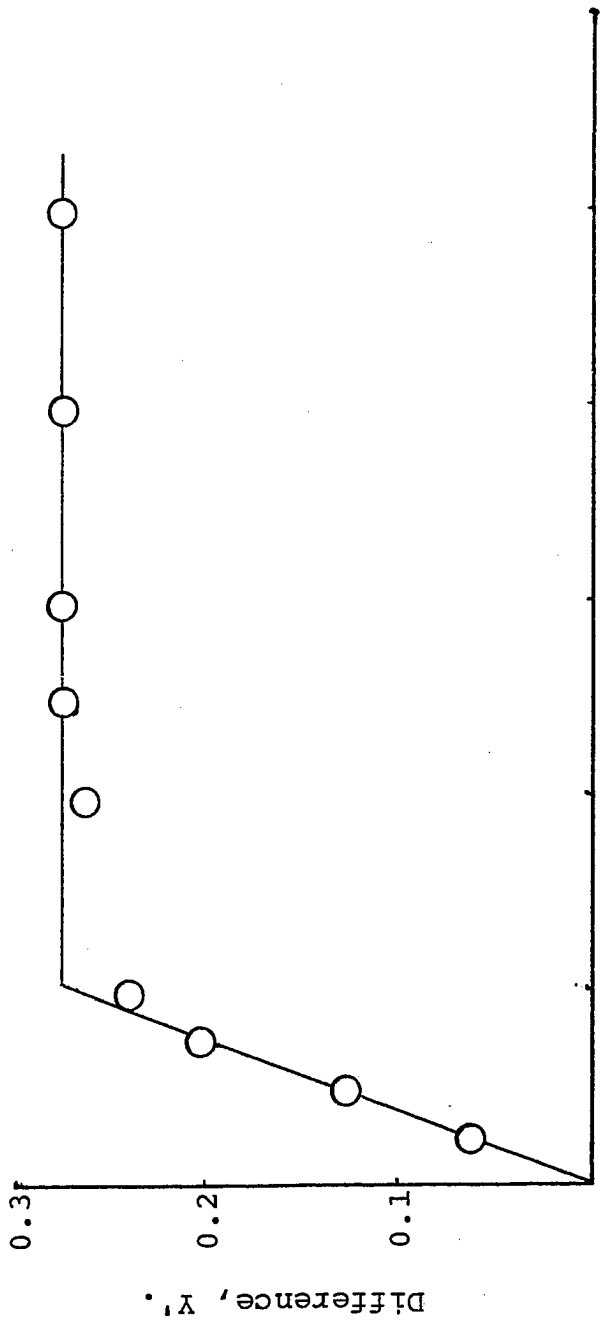

Fig. 3a.--Molar ratio method, pH 5.8, λ = 580 mμ.

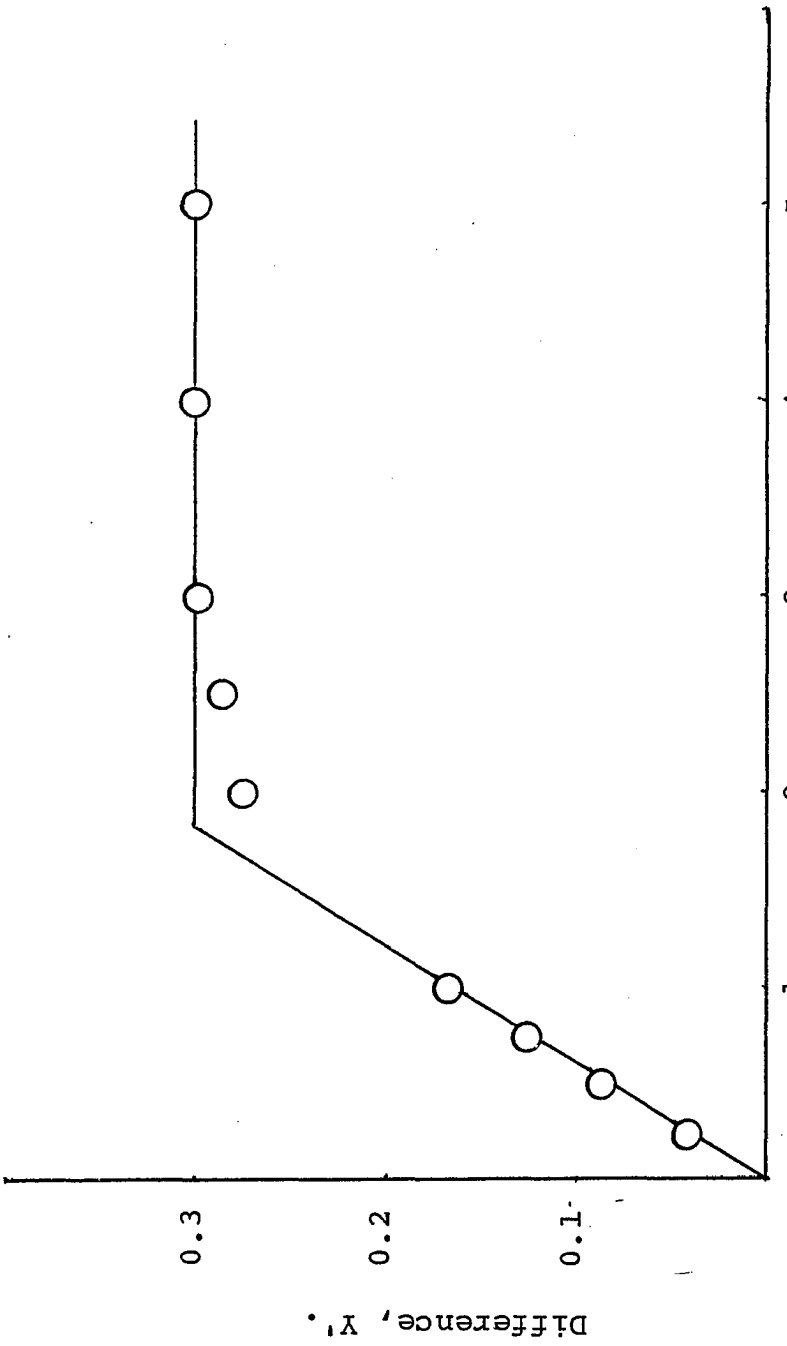

Fig. 3b.--Molar ratio method, pH 5.8, λ = 550 mμ.

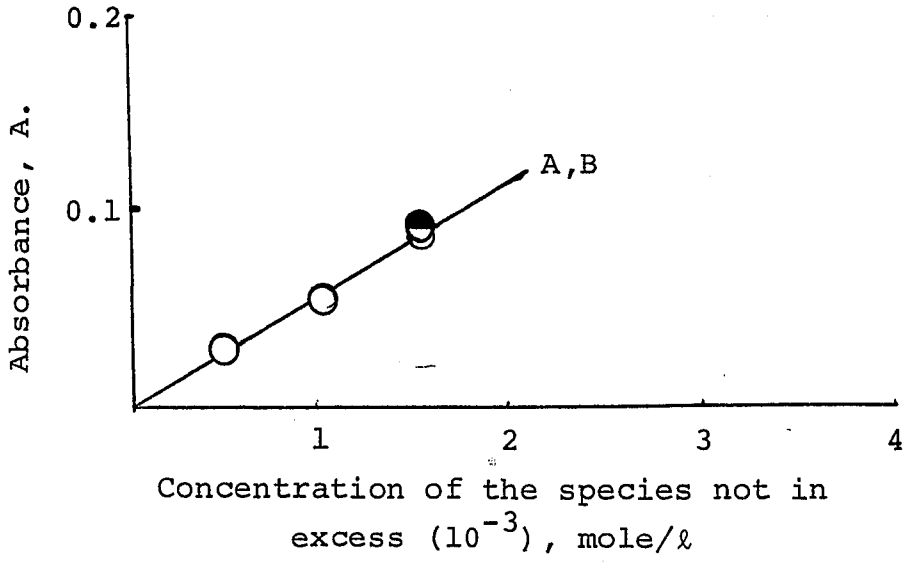

Fig. 3c.--Slope ratio method, Cu (II) + GHA at pH 5.8 and λ = 580 mμ: A, GHA in excess and B, Cu (II) ion in excess. A : B = 1 : 1

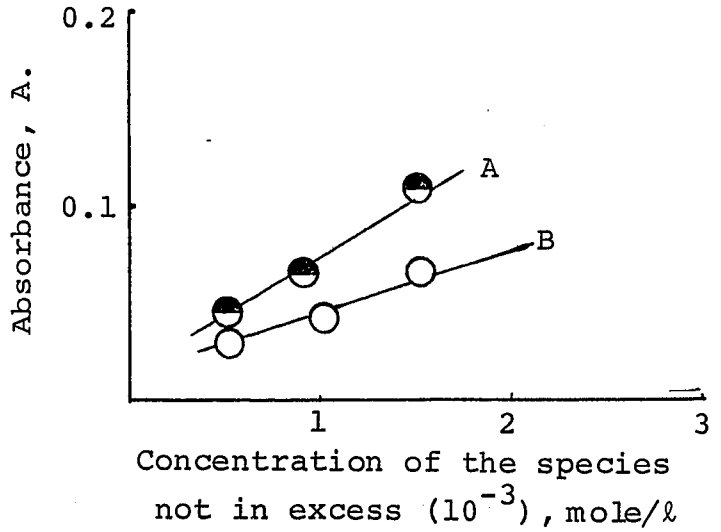

Fig. 3d.--Slope ratio method, Cu (II) + GHA at pH 5.8 and λ = 550 mμ: A, GHA in excess; B, Cu (II) ion in excess. A : B = 1.8 : 1

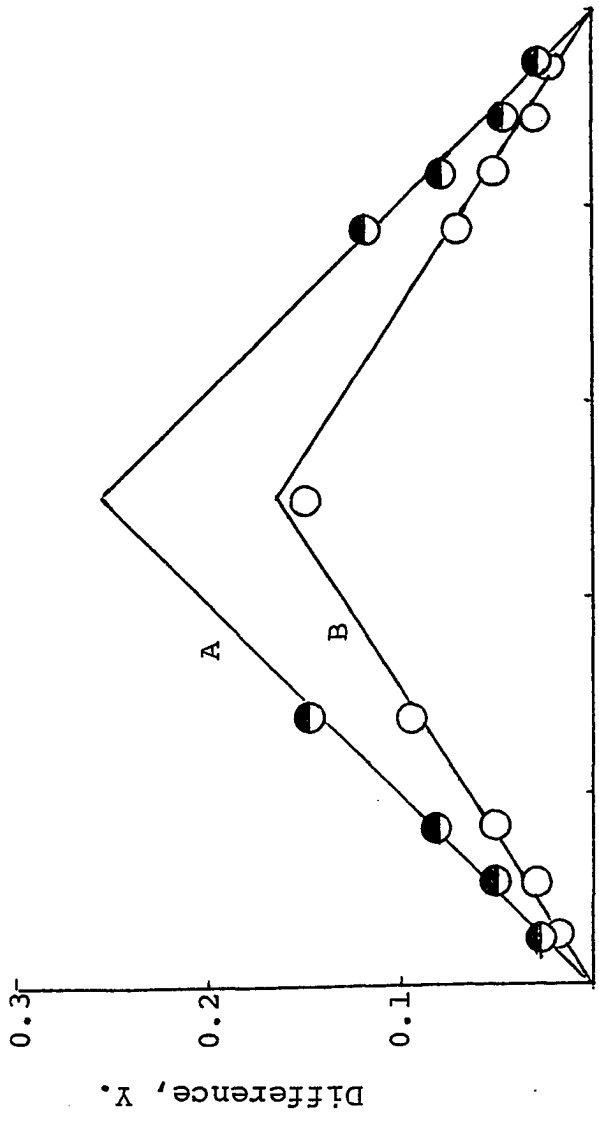

Fig. 4.--Method of continuous variations, pH 3.95: A, λ = 580 mμ; B, λ = 550 mμ. (Curve A is obtained only for further identification of the complex formed.)

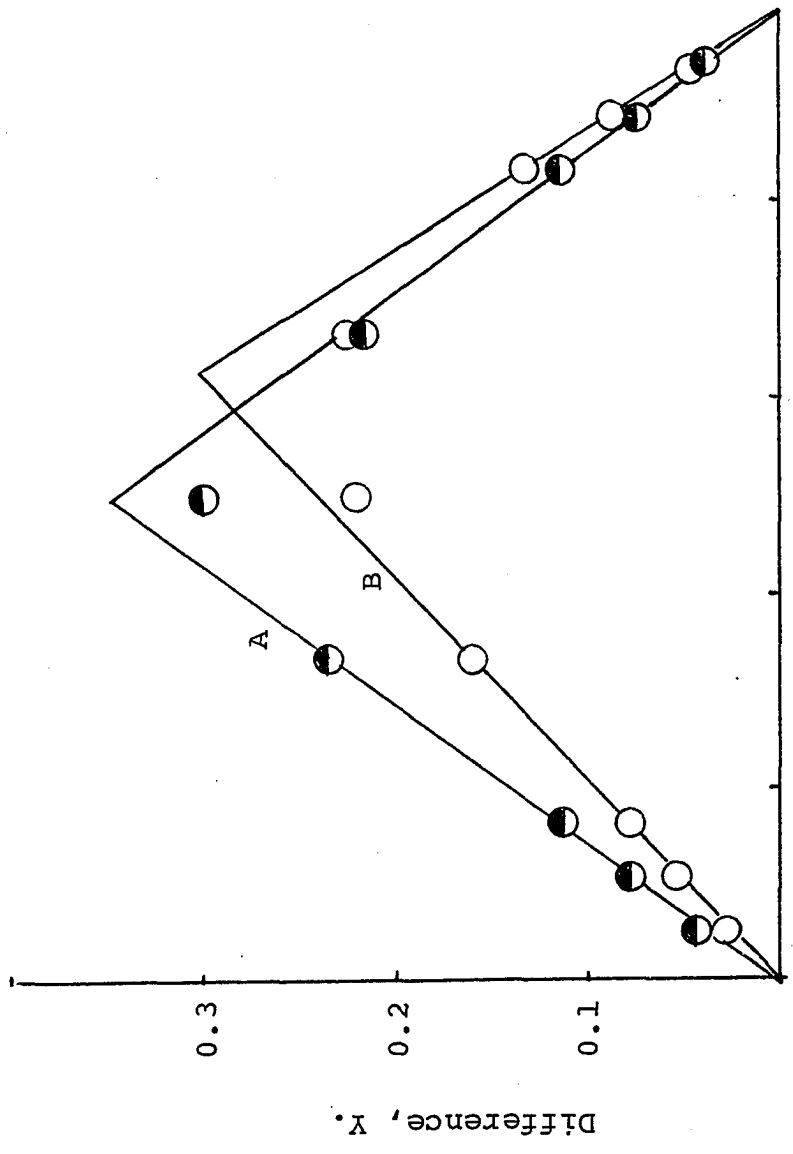

Fig. 5.--Method of continuous variations, pH 5.8 : A, λ = 580 mμ; B, λ = 550 mμ.

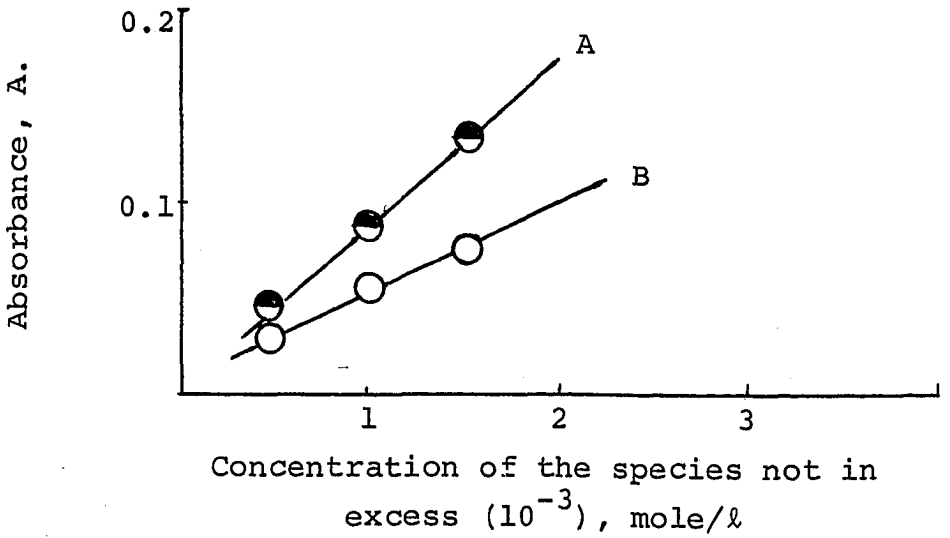

Fig. 5d.--Slope ratio method, Cu (II) + α-AHA at pH 5.8 and λ = 550 mμ. A : B = 1.8 : 1

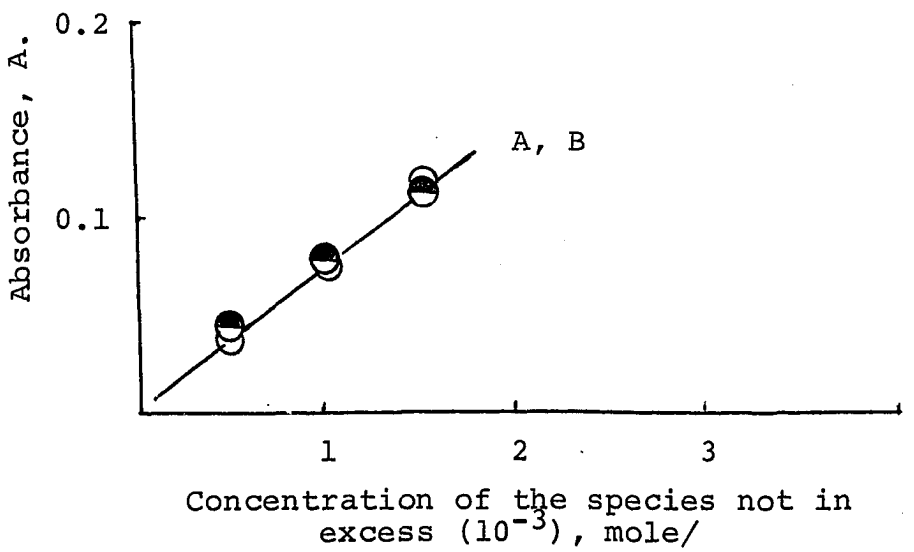

Fig. 5c.--Slope ratio method, Cu (II) + α-AHA at pH 5.8 and λ = 580 mμ. A : B = 1 : 1

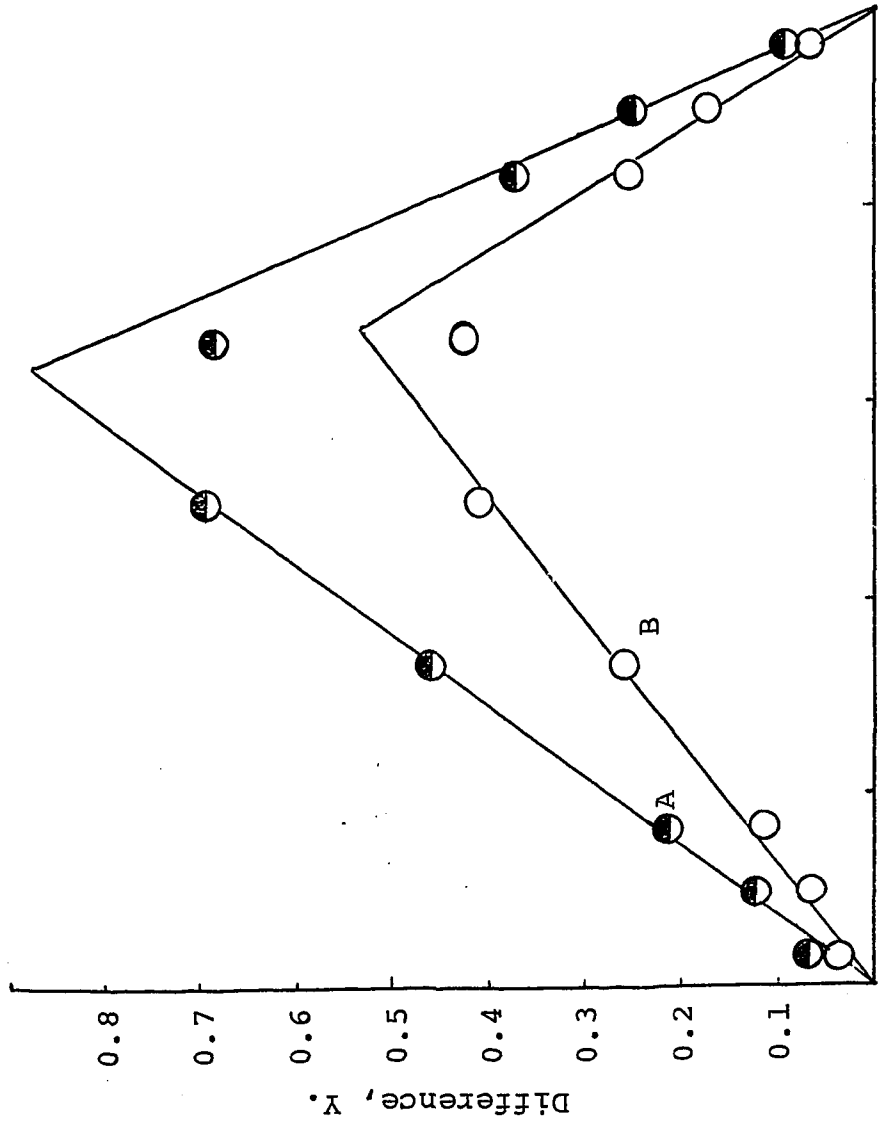

Fig. 6.--Method of continuous variations, pH 6.0 : A, λ = 500 mμ; B, λ = 540 mμ.

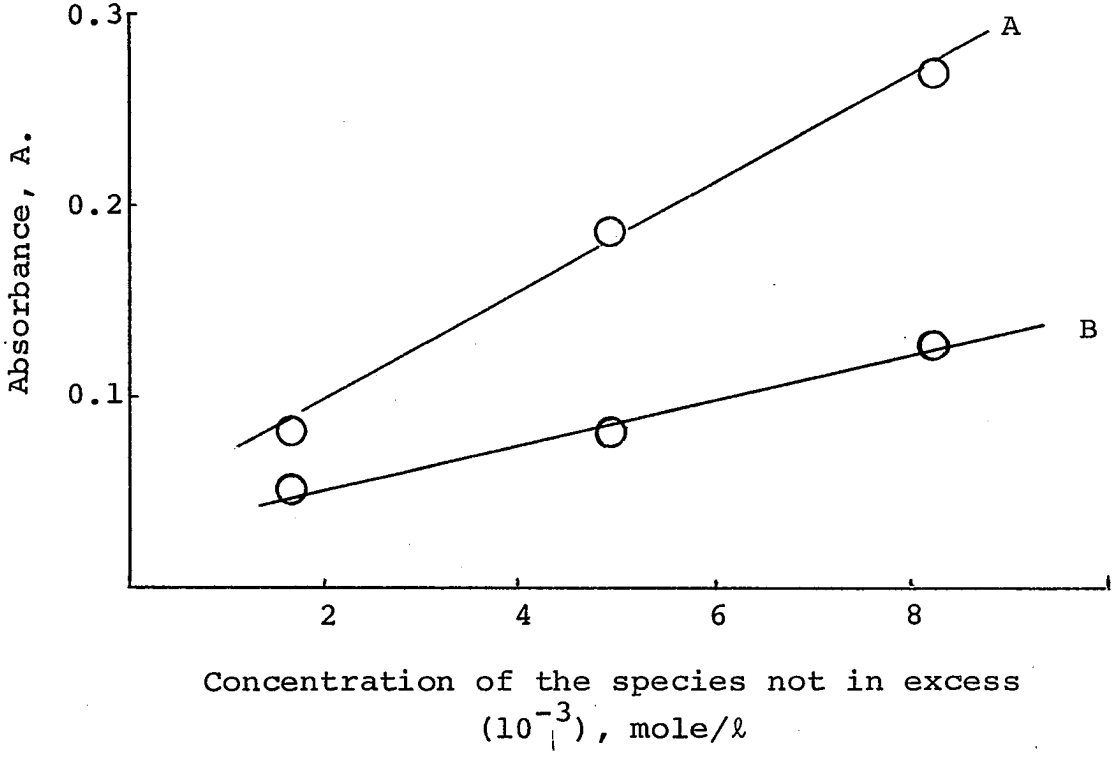

Fig. 6c.--Slope ratio method, Ni (II) + GHA at pH 6.0 and λ = 540 mμ. A : B = 2.2 : 1

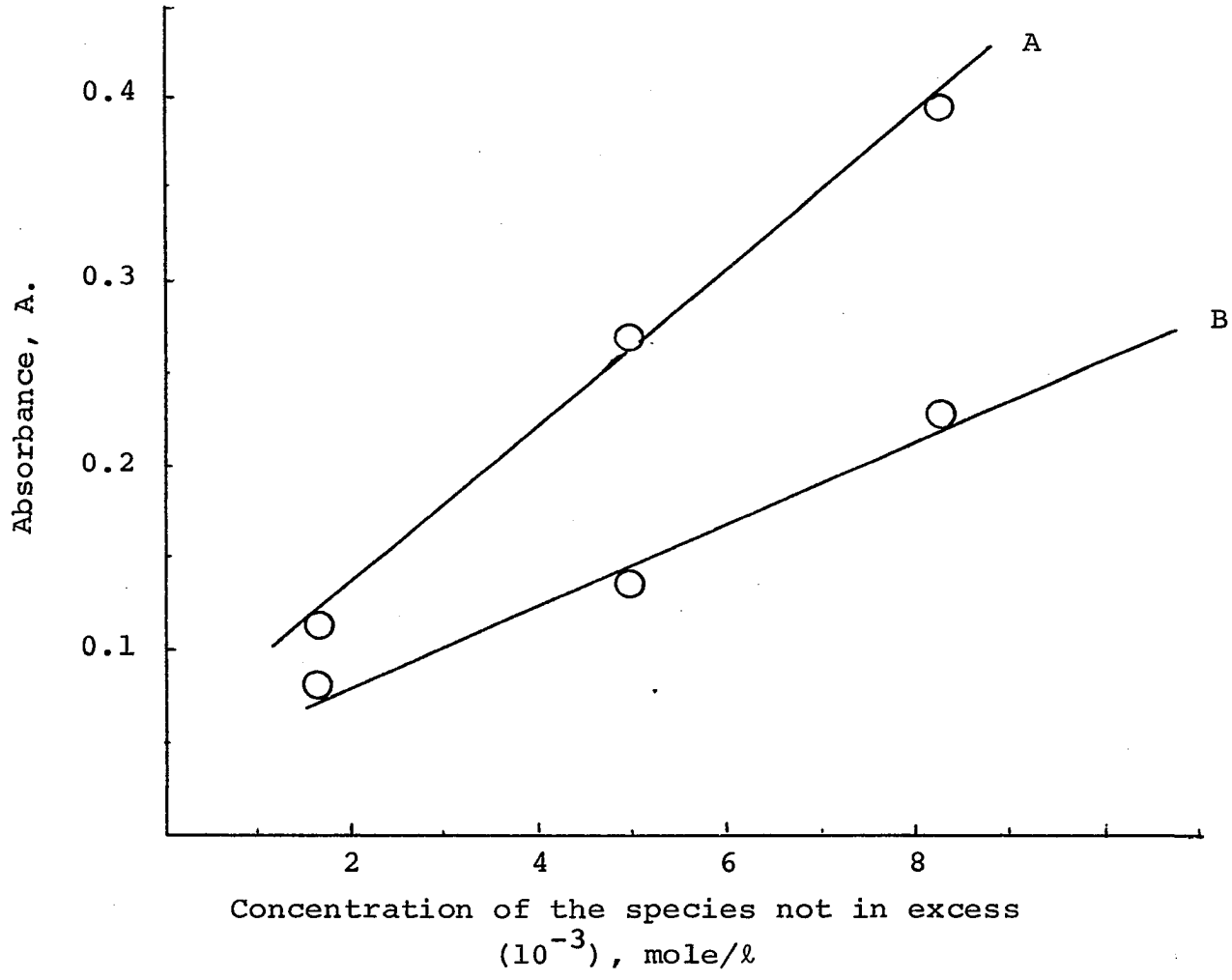

Fig. 6d.--Slope ratio method, Ni (II) + GHA at pH 6.0 and λ = 500 mμ. A : B = 1.8 : 1

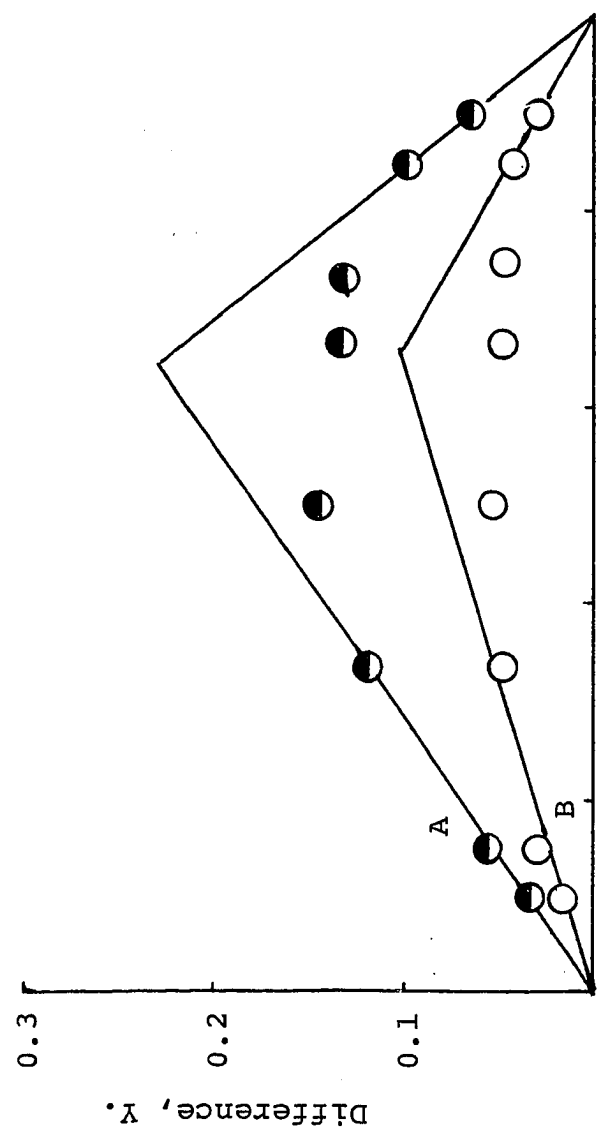

Fig. 7.--Method of continuous variations, pH 5.4 : A, λ = 500 mμ; B, λ = 800 mμ.

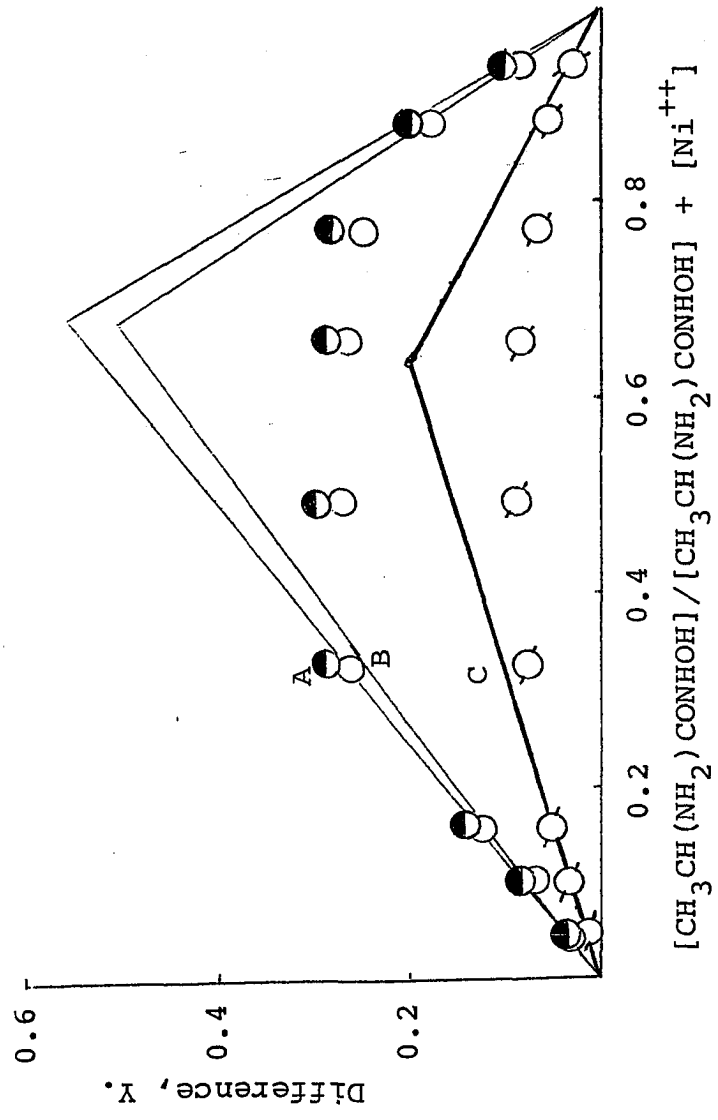

Fig. 8.--Method of continuous variations, pH 5.45 : A, λ = 500 mμ; B, λ = 410 mμ; C, λ = 800 mμ.

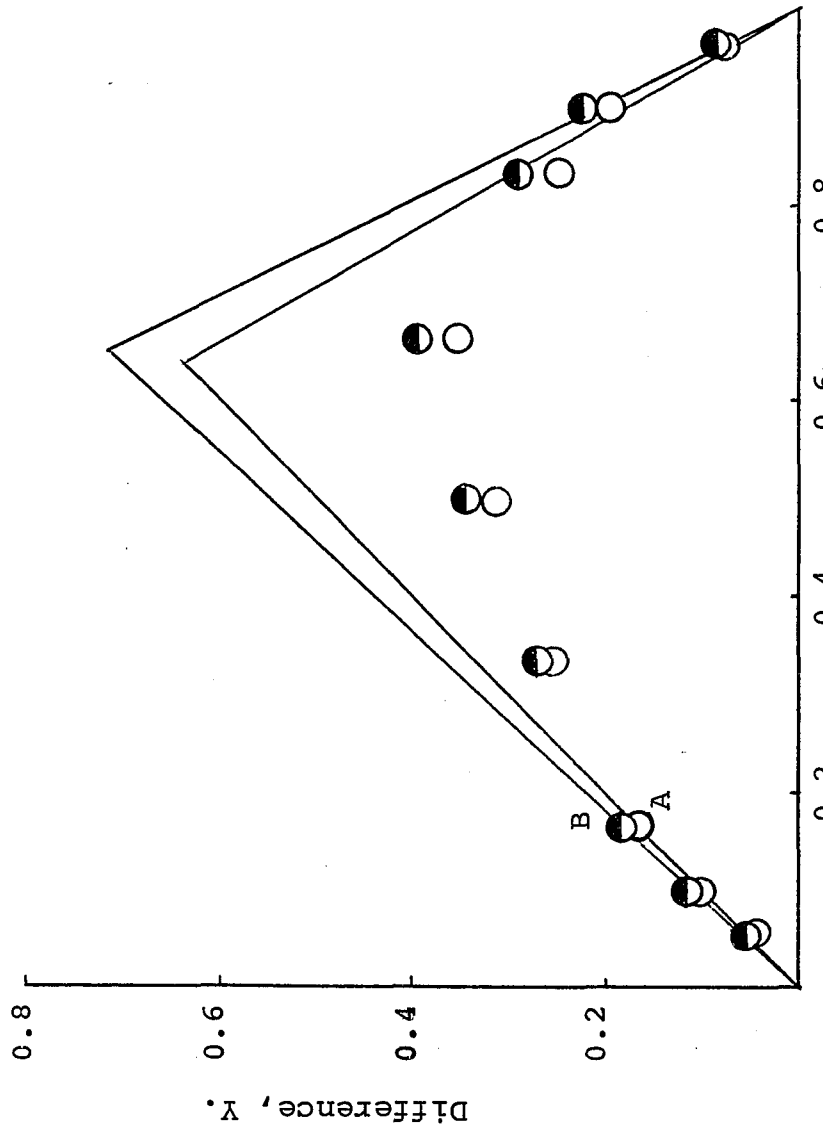

Fig. 9.--Method of continuous variations, pH 6.8 : A, λ = 540 mμ; B, λ = 500 mμ.

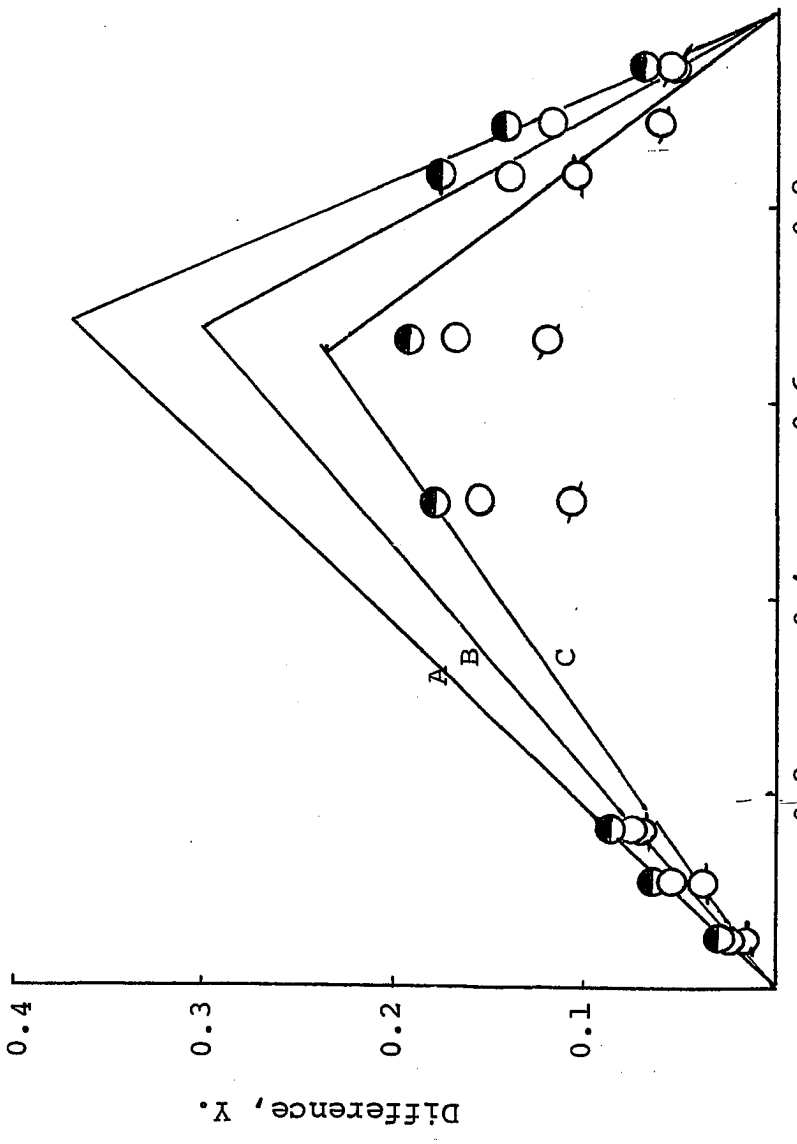

Fig. 10.--Method of continuous variations, pH 5.65 : A, λ = 500 mμ; B, λ = 550 mμ; C, λ = 800 mμ.

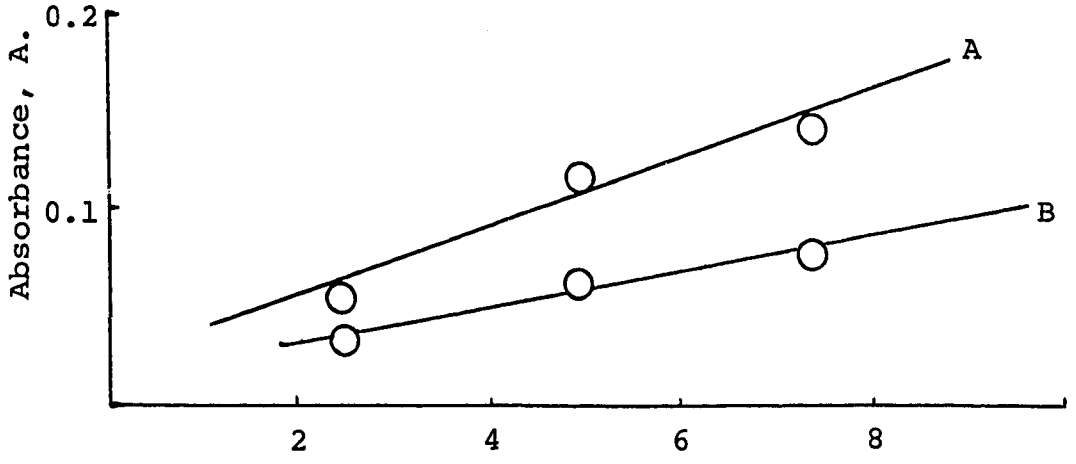

Fig. 10c.--Slope ratio method, Co (II) + PGHA at pH 5.6 and λ = 550 mμ : A, PGHA in excess; B, Co (II) ion in excess. A : B = 1.8 : 1

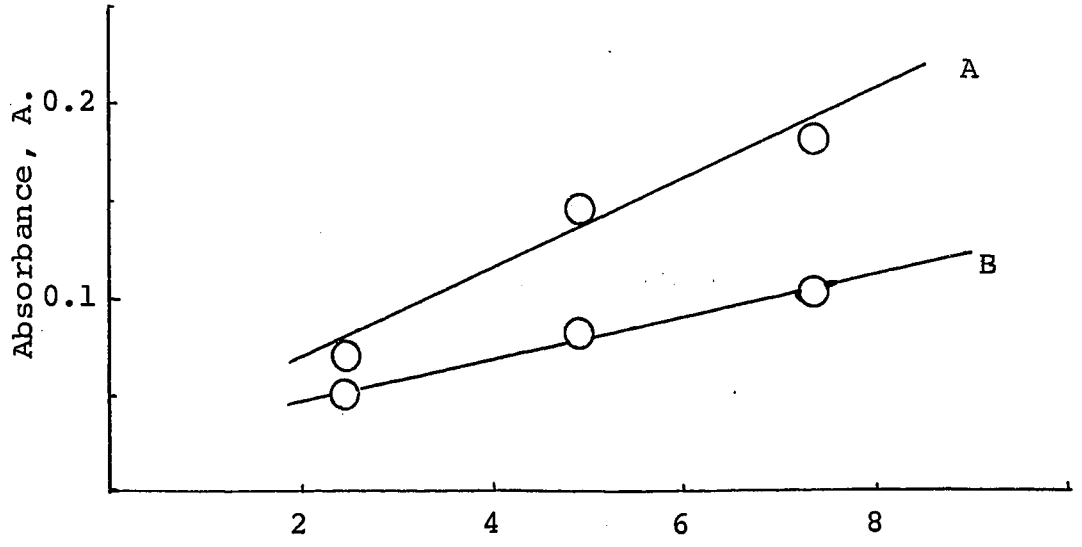

Fig. 10d.--Slope ratio method, Co (II) + PGHA at pH 5.6 and λ = 500 mμ : A, PGHA in excess; B, Co (II) in excess. A : B = 2 : 1

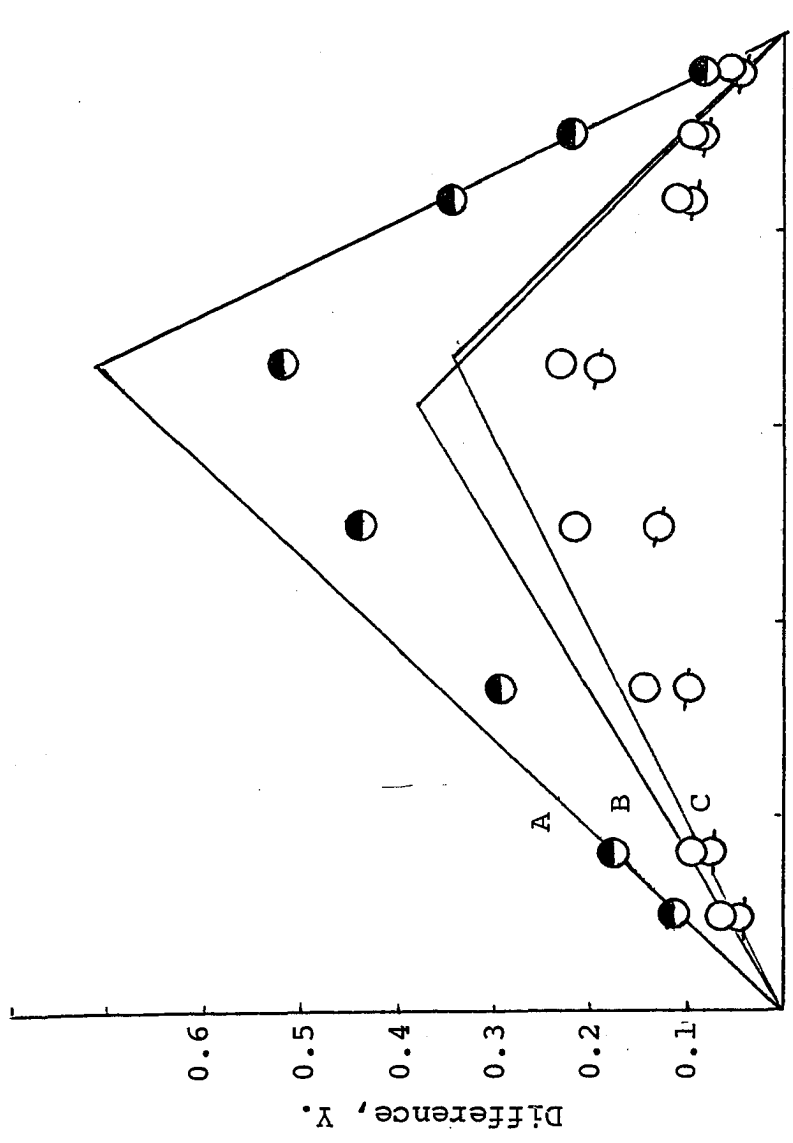

Fig.11.--Method of continuous variations, pH 7.0 :
A, λ = 500 mμ; B, λ = 600 mμ; C, λ = 800 mμ.

SUMMARY

The theory of the method of continuous variations as given by Job is reviewed for the cases in which more than one complex is formed from a given pair of components.

The method has been applied to the complex formation at a given pH at which more than a single complex is formed. The fact that the stability constant of the 2:1 copper(II) complex obtained at a pH of 5.8 was less than that reported by Cieleszky and coworkers,[4] might indicate that the stability of the complex is a function of pH. The complex formation was observed to be pH-dependent. When only a single complex is formed, the results are independent of the frequency of the light used. When more than one complex is formed, the results obtained depend on the frequencies of the light used, and for valid conclusions the frequencies must be carefully selected.

Both nickel(II) and cobalt(II) ions unite with α-aminohydroxamic acids in a 1:2 proportion at a given pH. Copper(II) ion, however, forms the complexes with α-aminohydroxamic acids in the molar ratios of 1:1 and 1:2 at a given pH. The stability constants of the complexes were calculated.

The method to prepare α-aminohydroxamic acids was described.

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VITA

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