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The Study of the Metal Complexes of Alpha Aminohydroxamic Acids

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THE STUDY OF THE METAL COMPLEXES OF ALPHA AMINOHYDROXAMIC ACIDS

by

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INTRODUCTION

The objectives of this investigation were to develop: 1) compounds similar to hydroxy urea, a compound known to have anti-tumor activity, 2) a method to prepare α-aminohydroxamic acids and to study the complexes of these compounds with several metal ions, 3) computer programs to determine the pK's[1] of the hydroxamic acids and the stepwise stability constants[2] for the ligands and several metal ions.

The values obtained for the stability constants were compared with values for the stability constants of acetohydroxamic acid and α-aminocarboxylic acids in an attempt to find which sites in the ligand formed bonds with the metal ions. These compounds will also be screened for anti-tumor activity and if found active, a correlation of stability constants and biological activity will be undertaken.

HISTORICAL

Hydroxamic acids were discovered by Lossen in 1869.[3] He reported that the reaction between diethyl oxalate and hydroxylamine gave an acidic compound which he named oxalohydroxamic acid. In 1917, Jones and Sneed[4] prepared glycinehydroxamic acid from ethyl glycinate and hydroxylamine. Another α-aminohydroxamic acid which was prepared later by Dunn and coworkers[5] was phenylglycinehydroxamic acid.

The complexes of α-aminohydroxamic acids have not been studied extensively. Ley and Männchen[6] isolated the copper and nickel complexes of glycinehydroxamic acid. However, they made no attempt to calculate the stability constants of these complexes.

EXPERIMENTAL

Apparatus

The apparatus used to determine the pK_a's and the stability constants consisted of a cell, a Beckman research pH meter, a miniature combination glass electrode, and a constant temperature bath maintained at 25.0 ± 0.1°C. The cell was composed of two beakers: a 150 ml electrolytic beaker, which held the sample, and a 400 ml beaker, which served as a cooling jacket. They were sealed together so that water from a constant temperature bath could be circulated between them. The 150 ml beaker was fitted with a stopper through which a thermometer, a combination miniature glass electrode, a burette, and a sintered glass gas dispersion tube were placed. The dispersion tube was used to pass nitrogen over the solution. The solution was stirred by a magnetic stirrer.

All the melting points were corrected and taken in a magnetically stirred silicone oil bath.

Standardization of the Metal Solutions

The perchlorates of the metals to be used in the complexation titrations were made approximately 0.1 M by weighing about 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 over 5 ml of amberlite IR-120 (H^+) ion exchange resin in a column. The eluent contained an equivalent amount of perchloric acid which was titrated with a standard base. From the

titrations, the molarities of the metal ion solutions were calculated. The metal perchlorate solutions standardized by this method were ferric, nickel, strontium, magnesium, copper, chromium, zinc, barium, cobaltous, vanadyl, and manganous. This analysis was suggested by Serjeant.[7]

The Preparation of Hydroxamic Acids

ETHYL 1-AMINOCYCLOPENTANECARBOXYLATE - 25.8 g, 0.20 mole, of 1-aminocyclopentanecarboxylic acid was suspended in 800 ml of absolute alcohol and hydrogen chloride gas was passed through the solution for 30 min, dissolving all the amino acid. This solution was heated under reflux on a steam bath for four hours. Then the excess solvent was removed by rotary evaporation. The residue was dissolved in a minimum amount of water and treated with 300 ml of concentrated ammonia and extracted with 700 ml of benzene. The ester was separated from the benzene layer by rotary evaporation and the residue was distilled at reduced pressure, bp 42-3° (3 mm); 22.2 g (71%); $n_D^{25°}$ 1.511.

The analysis of this ester was carried out on the ester hydrochloride. This was prepared by suspending the ester in absolute benzene and bubbling dry hydrogen chloride through the solution for three minutes. An analytical sample of ethyl 1-aminocyclopentanecarboxylate hydrochloride was obtained after two recrystallizations from 95% ethanol, mp 227-8°. Anal. Calcd for $C_8H_{16}O_2N_2Cl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 49.47; H, 8.26; N, 7.46.

ETHYL PHENYLGLYCINATE - A modification of the method of Marvel and Noyes[8] was used. Twenty-five grams, 0.17 mole, of phenylglycine were suspended in 450 ml of absolute ethyl alcohol and dry hydrogen chloride gas was passed through the suspension. All the amino acid dissolved after 30 min. The resulting solution was heated under reflux on a steam bath for four hours. The excess ethyl alcohol was removed by rotary evaporation and the residue dissolved in a minimum amount of water. The aqueous solution was treated with 200 ml of concentrated ammonia and extracted with 500 ml of benzene. The excess benzene was separated from the ester by rotary evaporation. The ester was distilled at reduced pressure, bp 113-4° (5 mm), yielding 21.2 g (71%).

PHENYLGLYCINEHYDROXAMIC ACID - A solution of 13.9 g, 0.20 mole, of hydroxylamine hydrochloride in 50 ml of absolute methanol was mixed slowly with a solution of 11.2 g, 0.20 mole, of potassium hydroxide in 30 ml absolute methanol. The temperature of both solutions was kept at approximately 40°. The resulting solution was allowed to stand at room temperature for 30 min with intermittent stirring. This solution was then placed in an ice bath and cooled to 0°. The potassium chloride was filtered from the reaction mixture with a Celite filter. The filtrate was added slowly over a 30 min period to a solution of 17.9 g, 0.10 mole, of ethyl phenylglycinate in 10 ml of absolute methanol, which had also been cooled to 0°. This mixture was then placed in the refrigerator for four days. The resulting precipitate was collected by vacuum filtration. The solid was extracted with a 100 ml portion of

deionized water. The resulting solution was cooled and the white crystals which formed were collected by filtration, mp 178-9° (dec) yielding 6.7 g (41%). Anal. Calcd for $C_8H_{10}O_2N_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.79; H, 5.97; N, 16.66.

GLYCINEHYDROXAMIC ACID - To a solution of 27.9 g, 0.20 mole, of ethyl glycinate hydrochloride in 50 ml of methanol was added a solution of 11.2 g, 0.20 mole, of potassium hydroxide in 30 ml of absolute methanol. During the addition the amino acid ester solution was kept at approximately 40° with an ice bath. After the addition, the solution was allowed to stand for 30 min. The solution was then vacuum filtered using celite.

A second solution consisted of 27.9 g, 0.40 mole, of hydroxylamine hydrochloride in 50 ml of absolute methanol. This was treated at 40° with 22.5 g, 0.40 mole, of potassium hydroxide in 55 ml of methanol. After the addition the mixture was allowed to stand for 30 min, then it was cooled to 0° and was filtered through celite.

The solution of the amino acid ester and the hydroxylamine were slowly added over a 30 min period. The resulting solution was refrigerated for four days. During this time the product precipitated and was collected by filtration. The crude product was recrystallized twice from deionized water (mp 143-4° (dec)) yielding 6.1 g (56%). Anal. Calcd for $C_2H_6O_2N_2$: C, 26.66; H, 6.71; N, 31.10. Found: C, 26.72; H, 6.53; N, 30.86.

DERIVATION OF EQUATIONS

The determination of stability constants for a system in which a complex is formed involves first the calculation of the pK_a's for the ligand and second the calculation of the ligand ion concentration, [L], and the average number of ligands per metal ion, $\bar{n}$, for every point on the formation curve of the complex. The derivations for the acid dissociation constant and stability constants follow.

In the titration of an α-aminohydroxamic acid two ionizable groups are found. The approximate values of pK and pK' are 7 and 9. If the zwitterion represents the neutral form, the ionizations observed would correspond to equations 1 and 2.

$$\underset{\overset{|}{NH_3^+}}{RCHCONHOH} \rightleftarrows \underset{\overset{|}{NH_3^+}}{RCHCONHO^-} + H^+ \qquad (1)$$

$$\underset{\overset{|}{NH_3^+}}{RCHCONHO^-} \rightleftarrows \underset{\overset{|}{NH_2}}{RCHCONHO^-} + H^+ \qquad (2)$$

This gives reasonable values for the pK_a's--7 for reaction 1 and 9 for reaction 2.

If, however, the non-polar intermeate is involved, the reactions would follow equations 3 and 4.

$$\underset{\overset{|}{NH_3^+}}{RCHCONHOH} \rightleftharpoons \underset{\overset{|}{NH_2}}{RCHCONHOH} + H^+ \qquad (3)$$

$$\underset{\overset{|}{NH_2}}{RCHCONHOH} \rightleftarrows \underset{\overset{|}{NH_2}}{RCHCONHO^-} + H^+ \qquad (4)$$

These equations would lead to the assignment of pK_a's of 7 for equation 3 and 9 for equation 4 which contradict previous values for hydroxamic acids and amine. Hence, the pK_a of the hydroxamic proton was found by titration of the hydroxamic acid in water with 0.0912 M hydrochloric acid. The pH was recorded after each small

increment of acid was added. The procedure suggested by Albert and Serjeant[9] involved the determination of the ratio of the protonated species, HA, to the unprotonated species, A. This ratio was then corrected for the hydroxyl ion concentration at the recorded pH. The log of this ratio was taken, then added to the pH to give the value of the pK_a.

$$pK_a = pH + \log (HA/A) \qquad (5)$$

Similarly, to find the pK_a of the basic function of the molecule, the compound was titrated with 0.0985 M sodium hydroxide, and the volume of titrant and the corresponding pH readings were noted. The ratio of the protonated to unprotonated species was found. The ratio of protonated, HA, to unprotonated, A, species was corrected for the hydrogen ion concentration at each point in the titration. The log of this ratio was added to the pH to give the pK_a'.

$$pK_a = pH + \log (HA/A) \qquad (6)$$

After the pK_a's were calculated, the next step in finding the stability constants was to determine [L] and $\bar{n}$. Data for the calculation of [L] were obtained from the titration of the ligand and metal ion with 0.0985 sodium hydroxide. First, the ligand ion concentration, $[I_o]$, was calculated by dividing the number of moles of ligand by the total volume of the solution. Next, the analytical metal ion concentration, $[M_o]$, was calculated by dividing the number of moles of metal ion by the total volume of the solution.

The metal ion concentration, $[M_o]$, was then corrected for hydrolysis. The relationship which was used can be derived in

the following way. The free metal ion in solution is equal to the analytical concentration of the metal ion present minus the amount of metal in the form of the hydroxo complexes. The formula for this was:

$$[M_f^{+n}] = [M_o] - [MOH^{+n-1}] - [M(OH)_2^{+n-2}] \quad (7)$$

From the stepwise formation constants, the concentrations of the hydroxyl complexes were derived in terms of $[M_f^{+n}]$:

$$K_1 = \frac{[MOH^{+n-1}][H^+]}{[M_f^{+n}][H_2O]} \quad (8)$$

$$K_2 = \frac{[M(OH)_2^{+n-2}][H^+]}{[MOH^{+n-1}][H_2O]} \quad (9)$$

Solving for $[MOH^{+n-1}]$ from equation 8 and $[M(OH)_2^{+n-2}]$ from equation 9, one arrived at:

$$[MOH^{+n-1}] = \frac{K_1[H_2O][M_f^{+n}]}{[H^+]} \quad (10)$$

$$[M(OH)_2^{+n-2}] = \frac{K_2[H_2O][MOH^{+n-1}]}{[H^+]} \quad (11)$$

Since for a given point in the titration the hydrogen ion concentration and the water concentration were constant and K_1, K_2 were characteristic of the metal ion present, equations 10 and 11 were simplified by the substitution of a constant, A, for $\frac{K_1[H_2O]}{[H^+]}$ in equation 10 and B for $\frac{K_2[H_2O]}{[H^+]}$ in equation 11. Equation 11 was further simplified by substituting the value of $[MOH^{+n-1}]$ obtained in equation 10. This reduced the equation to:

$$[M(OH)_2^{+n-2}] = AB[M_f^{+n}] \quad (12)$$

Next, by substituting the values obtained for the hydroxyl complexes in the material balance equation, equation 7,

one obtained:

$$[M_f^{+n}] = [M_o] - A\,[M_f^{+n}] - AB\,[M_f^{+n}] \qquad (13)$$

Rearranging the equation and solving for $[M_f^{+n}]$ yielded:

$$[M_f^{+n}] = \frac{[M_o]}{1 + A + AB} \qquad (14)$$

The free ligand ion concentration was then obtained from the equation:

$$[L^-] = \frac{[L_o] - [KOH] - [H^+] + [OH^-]}{P} \qquad (15)$$

where $P = \frac{[H^+]}{K_a} + \frac{2[H^+]^2}{K_a K_a'}$. K_a and K_a' were found by taking the antilog of the pK_a's. K_a was calculated from the largest pK and K_a' from the smallest.

To calculate $\bar{n}$, one used the values of $[L_o]$, $[L^-]$, and $[M_f^{+n}]$. The relationship of these quantities is given below:

$$\bar{n} = \frac{[L_o] - S\,[L^-]}{[M_f^{+n}]} \qquad (16)$$

where $S = 1 + \frac{[H^+]}{K_a} + \frac{[H^+]^2}{K_a K_a'}$.

Having determined values for $\bar{n}$ and $[L]$ for every point in the titration, the stability constants were then calculated. J. A. Chapoorian et al.[10] described a general method for determining stability constants using a high speed digital computer. They defined $\bar{n}$ as the average number of ligands complexed per metal atom. Using the following equation:

$$\bar{n}_j = \frac{\sum_{n=1}^{N} n\,\beta_n\,[L]_j^n}{1 + \sum_{n=1}^{N} \beta_n\,[L]_j^n} \qquad (17)$$

where β_n was the product of the stepwise stability constants, N was the number of complexed species in solution, n was all the integers from 1 to N, j denoted the point on the graph from 1 to N, and [L] was the free ligand ion concentration, it was possible to derive the actual equations needed to find the stability constants.

Taking equation 17, dividing it by [L], and setting the result equal to a minimum value, R_j, gave the following equation:

$$R_j = \frac{\sum_{n=1}^{N} n \beta_n [L]_j^{n-1}}{1 + \sum_{n=1}^{N} \beta_n [L]_j^n} - \frac{\bar{n}_j}{[L]_j} \qquad (18)$$

The least-squares method for the calculation of β_n required that the value of the sum of the squares of the R_j's be a minimum. The solution to the function of the sum of the R_j's squared is found from the partial derivatives of this function taken with respect to the β's and set equal to zero. This amounted to an array of linear equations in the unknowns of β_1, β_2, β_3 ... β_n.

To derive these linear equations, equation 18 was rewritten with a common denominator to obtain the next equation:

$$R_j = \frac{\sum_{n=1}^{N} n \beta_n [L]_j^{n-1} - \frac{n_j}{[L]_j} \left(1 + \sum_{n=1}^{N} \beta_n [L]_j^n\right)}{1 + \sum_{n=1}^{N} \beta_n [L]_j^n} \qquad (19)$$

The partial of this equation was too difficult to solve readily. However, the solution was simplified by holding the denominator constant and solving the partial of the numerator of equation 19 for the individual β's. This was accomplished by initially assuming values for β which were substituted in the denominator

of the equation to obtain a numerical constant, Q. The numerator, then, reduced to an expansion of the values of $\bar{n}$ and [L]:

$$P_j = \frac{\bar{n}_j}{[L]} + \beta_1 (\bar{n}_j - 1) + \beta_2 (\bar{n}_j - 2) [L] + \ldots + \beta_N (\bar{n}_j - N) [L]_j^{N-1} \quad (20)$$

Since R_j equaled $\frac{P_j}{Q_j}$, the solutions were found as the partial of the sum of the $\left(\frac{P_j}{Q_j}\right)^2$ in terms of β_n where n = 1, 2, 3, ... N.

Substituting letters for the terms of $\bar{n}$ and [L] in equation 20, for the case in which N = 3, equation 21 was obtained:

$$P_j = A + \beta_1 C + \beta_2 D + \beta_3 E \quad (21)$$

Squaring this equation resulted in the following equation:

$$P_j^2 = A^2 + 2AC\beta_1 + 2AD\beta_2 + 2AE\beta_3 + 2CD\beta_1\beta_2 + 2CE\beta_1\beta_3 + C^2\beta_1^2 + D^2\beta_2^2 + 2DE\beta_2\beta_3 + E^2\beta_3^2 \quad (22)$$

Taking the partial derivative of equation 22 in terms of β_1 and setting it equal to zero formulated the next equation:

$$2AC + 2CD\beta_2 + 2CE\beta_3 + 2C^2\beta_1 = 0$$

Similarly, taking the partial derivatives of equation 20 with respect to β_2 and β_3 gave the following:

$$2AD + 2CD\beta_1 + 2D^2\beta_2 + 2DE\beta_3 \quad (24)$$

and

$$2AE + 2CE\beta_1 + 2DE\beta_2 + 2E^2\beta_3 \quad (25)$$

Equations 23-25 gave the set of linear equations which, when divided by Q^2, were solved for values of β_1, β_2, and β_3.

These new values of β_1, β_2, and β_3 were compared with the β's previously used to define the numerical constant, Q. When the β's agreed within the given tolerance, a satisfactory answer had been reached. If, however, the β's just calculated did not

agree within the limits, these β's were substituted for the previous β's. Q, then, was reevaluated and the simultaneous equations were again solved for β. This process was continued until satisfactory agreement was reached.

RESULTS

The pK_{a_1} and pK_{a_2} for glycinehydroxamic acid are 9.16 and 7.21, respectively. For phenylglycinehydroxamic acid, pK_{a_1} is 9.00 and pK_{a_2} is 6.44.

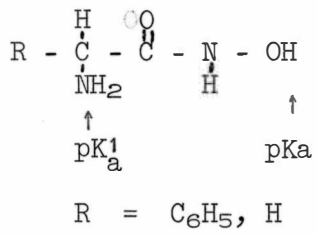

Figure 1. Structure diagram showing which functional group corresponds to each pK_a

The behavior of both ligands with the metal ions studied is listed in Table I.

The stability constants of the complexes of glycinehydroxamic acid and phenylglycinehydroxamic acid are listed in Table II. All values are corrected for hydrolysis.

Some comparison with some values for the stability constants of acetohydroxamic acid (Table III) and glycine (Table IV) show a marked increase in the stability of the complexes of the α-aminohydroxamic acids.

The general trend in the data is consistant with data previously reported in the literature for complexes of the metals of the first transition series, i.e. the strength of the complexes increases through the first transition series until copper(II) is reached. The copper(II) complex is the most stable complex formed. Zinc, the last element of the first transition series, shows a marked decrease in the stability of the complex.

Table I

Colors of the Complexes

Hydroxamic Acid	Mg^{+2}	Sr^{+2}	Ba^{+2}	Mn^{+2}	Cr^{+3}	Fe^{+3}	Co^{+2}	VO^{+2}	Ni^{+2}	Cu^{+2}	Zn^{+2}
Glycine	cl[a]	cl	cl	tan	ppt[b] light green	dark red brown	orange	ppt blue green	orange	green	cl
Phenylglycine	cl	cl	cl	tan	ppt light green	dark red brown	orange	ppt blue green	orange	green	cl

[a]colorless [b]precipitate

Table II

The Log of Stability Constants

Hydroxamic Acid		Mg^{+2}	Sr^{+2}	Ba^{+2}	Mn^{+2}	Cr^{+3}	Fe^{+3}	Co^{+2}	VO^{+2}	Ni^{+2}	Cu^{+2}	Zn^{+2}
Glycine	β_1	2.30	1.93	1.66	3.18	ppt[a]	ppt	6.24	ppt	6.72	11.27	5.60
	β_2	3.04	3.58	3.23	6.03	ppt	ppt	11.33	ppt	12.97	21.16	10.01
Phenylglycine	β_1	2.35	1.85	1.90	2.64	ppt	ppt	4.84	ppt	6.98	11.10	4.60
	β_2	3.72	3.50	3.61	6.24	ppt	ppt	9.68	ppt	13.30	19.14	10.28

[a]precipitate

Table III

The Stability Constants of Acetohydroxamic Acid and Several Metal Ions[11]

Metal	Mn	Co	Ni	Cu	Zn
β_1	4.0	5.1	5.3	7.9	5.4
β_2	6.9	8.9	9.3	---	9.6

Table IV

The Stability Constants of Glycine and Several Metal Ions[12]

Metal	Mn	Co	Ni	Cu	Zn
β_1	3.45	4.95	6.12	6.51	5.33
β_2	6.46	8.94	11.15	13.42	9.72

APPENDIX

Program to Calculate the pK_a of the Amino Group

The program for determining the pK_a of the amino group of the α-aminohydroxamic acids was calculated from the following data. The first card had the name of the hydroxamic acid to be titrated on it. The second card contained data characteristic of the hydroxamic acid to be titrated. The final set of cards contained the pH and volume of base at different points in the titration.

The name card had no set form, the name was punched in any way. The standard form of the second card was: the sample weight, .XXXX; the molecular weight, XXX.XXX; the initial volume of the hydroxamic acid solution, XX.XX; the normality of the base, X.XXXX; and the number of points from the titration curve. The last set of cards has the following form: volume of base, XX.XX; and pH, XX.XXX.

The symbols used in the NIPEBA program were:

A - the weight of sample

AM - molecular weight of the compound

AV - volume in which sample was placed

X(1,I) - the volume of base added to this point is the titration

X(2,I) - the pH at this point in the titration

X(3,I) - the total volume of the solution. The sum of the initial volume and the base volume.

X(4,I) - the concentration of the ligand

X(5,I) - the concentration of the base added

X(6,I) - the unreacted ligand

X(7,I) - the log of hydroxyl ion concentration at the pH of this point

X(8,I) - the hydroxyl ion concentration

X(9,I) - the ratio of the concentration of the unreacted to the reacted corrected for the hydroxyl ion concentration at this pH

X(11,I) - the pK_a for this point

AVE - the average value of the pK_a

SD - the standard deviation in the pK_a

Immediately following are the computer program and a set of sample data for which the pK_a has been calculated.

```
THE PROGRAM TO CALCULATE THE PKA FROM BASE TITRATION DATA
      DIMENSION X(13,50)
 10 READ820
820 FORMAT(80H                                                          /
    N                                                                   )
     PUNCH820
     READ1,A,AM,AV,BN,N
   1 FORMAT(F5.5,F6.3,F5.3,F5.4,I2)
      S=N
     READ2,(X(1,I),X(2,I),I=1,N)
   2 FORMAT(F5.3,F6.4)
     DO 3 I=1,N
     X(3,I)=(AV+X(1,I))*.001
     X(4,I)=(A/AM)/X(3,I)
      X(5,I)=(BN*X(1,I))/(1000.*X(3,I))
     X (6,I)=X(4,I)-X(5,I)
     IF(X(6,I)-.00095)9,9,8
   9 S=S- 1.
     X(11,I)=0.
     GO TO 3
 8   X(7,I)=(X(2,I)-14.9265)*2.302585
      X(8,I)=EXP(X(7,I))
     X(9,I)=(X(6,I)+X(8,I))/(X(5,I)-X(8,I))
      X(10,I)=LOG(X(9,I))/2.302585
     X(11,I)=X(10,I)+X(2,I)
   3 CONTINUE
      PUNCH6
   6 FORMAT(8HBASE VOL,3X,2HPH,3X,7HVOL SOL,2X,8HTOT CONC,2X,8HCONC REA
```

```
     1,2X,8HCONC UNR)
      PUNCH 4,((X(I,J),I=1,6),J=1,N)
    4 FORMAT(F7.3,F8.4,F9.6,F10.7,F10.7,F10.7)
       PUNCH7
    7 FORMAT(1X,5HLN OH,7X,2HOH,4X,9HCONC CORR,3X,6HLOG CC,2X,3HPKA)
      DO14J=1,N
      IF(X(6,J)-.00095)21,21,16
   21 PUNCH15,X(6,J)
   15 FORMAT(14HDATA DISCARDED,4X,8HCONC UNR,F10.7)
      GOTO14
   16 PUNCH5, (X(I,J),I=7,11)
    5 FORMAT(F8.4,F10.7,F11.7,F8.4,F8.4)
   14 CONTINUE
       SX12=0.
       DO 11 I=1,N
   11 SX12=SX12+X(11,I)
       AVE=SX12/S
      D2=0.
      DO13I=1,N
      IF(X(6,I)-.00095)13,13,17
   17 D=(AVE-X(11,I))
      D2=D2+D*D
   13 CONTINUE
      SD=SQRT(D2/(S-1.))
      AVE=AVE+.005
       PUNCH 12, AVE,SD
   12  FORMAT (10X,13HAVERAGE PKA =,F6.2,1H(,F6.2,1H))
      PUNCH821
```

```
821 FORMAT(79X,1H-)
      GOTO10
      END
```

BASE TIT.GLYCINEHYDROXAMIC AC RUN1

037340900885000000098516

0.6008.468

0.7008.522

0.8008.568

0.9008.608

1.0008.647

1.2008.745

1.4008.859

1.6008.929

1.8009.009

2.0009.087

2.2009.168

2.8009.421

3.0009.522

3.2009.597

3.4009.712

3.6009.826

BASE TIT.GLYCINEHYDROXAMIC AC RUN1

BASE VOL	PH	VOL SOL	TOT CONC	CONC REA	CONC UNR
.600	8.4680	.050600	.0081913	.0011679	.0070233
.700	8.5220	.050700	.0081752	.0013599	.0068152
.800	8.5680	.050800	.0081591	.0015511	.0066079
.900	8.6080	.050900	.0081430	.0017416	.0064014
1.000	8.6470	.051000	.0081271	.0019313	.0061957
1.200	8.7450	.051200	.0080953	.0023085	.0057867
1.400	8.8590	.051400	.0080638	.0026828	.0053810
1.600	8.9290	.051600	.0080326	.0030542	.0049783
1.800	9.0090	.051800	.0080016	.0034227	.0045788
2.000	9.0870	.052000	.0079708	.0037884	.0041823
2.200	9.1680	.052200	.0079402	.0041513	.0037889
2.800	9.4210	.052800	.0078500	.0052234	.0026265
3.000	9.5220	.053000	.0078204	.0055754	.0022449
3.200	9.5970	.053200	.0077910	.0059248	.0018662
3.400	9.7120	.053400	.0077618	.0062715	.0014903
3.600	9.8260	.053600	.0077329	.0066156	.0011172

LN OH	OH	CONC CORR	LOG CC	PKA
-12.7298	.0000029	6.0310866	.7803	9.2483
-12.6055	.0000033	5.0262250	.7012	9.2232
-12.4995	.0000037	4.2726167	.6306	9.1986
-12.4074	.0000040	3.6865061	.5666	9.1746
-12.3176	.0000044	3.2177212	.5075	9.1545
-12.0920	.0000056	2.5151624	.4005	9.1455
-11.8295	.0000072	2.0138675	.3040	9.1630
-11.6683	.0000085	1.6373642	.2141	9.1431
-11.4841	.0000102	1.3448036	.1286	9.1376

-11.3045	.0000123	1.1108403	.0456	9.1326
-11.1180	.0000148	.9195700	-.0364	9.1315
-10.5354	.0000265	.5105266	-.2919	9.1290
-10.3029	.0000335	.4111394	-.3860	9.1359
-10.1302	.0000398	.3238919	-.4895	9.1074
-9.8654	.0000519	.2479694	-.6056	9.1063
-9.6029	.0000675	.1809310	-.7424	9.0835

AVERAGE PKA = 9.15(.04)

Program to Calculate the pK_a of the Hydroxamic Acid

The program for determining the pK_a of the hydroxamic acids required the following data: 1) a card which gave the name of the acid to be titrated; 2) a card with data characteristic of the hydroxamic acid; and 3) a set of cards with the data from every point in the titration.

Although the first card could be punched in any form, the second card had to follow a set form: the sample weight, .XXXX; the molecular weight, XXX.XXX; the initial volume of the hydroxamic acid solution, XX.XX; the normality of the base used, X.XXXX; and the number of points from the titration curve, XX.

The rest of the cards which contained data from the titration curve had the form: the volume of base added, XX.XX; and the pH, XX.XXX.

This NIPEAC program used the following symbols:

A - the sample weight

AM - the molecular weight of the sample

AV - the volume of the sample solution

X(1,I) - the volume of acid added

X(2,I) - the pH at this volume of acid

X(3,I) - the total volume at this point in the titration

X(4,I) - the concentration of the sample

X(5,I) - the concentration of acid added

X(6,I) - the amount of unprotenated sample

X(7,I) - the ln of hydrogen ion concentration

X(8,I) - the concentration of hydrogen ions

X(9,I) - the ratio of unprotenated sample to protenated sample. This ratio is corrected for the hydrogen ion concentration at this pH.

X(11,I) - the pK for this point in the titration

AVE - average pK

SD - the standard deviation in the pK_a

The actual computer program, a set of data, and the answers to this set of data follow.

```
THE PROGRAM TO CALCULATE THE PKA FROM ACID TITRATION DATA
      DIMENSION X(11,50)
   10 READ820
  820 FORMAT(80H                                                        /
     N                                                                  )
      PUNCH820
      READ1,A,AM,AV,BN,N
    1 FORMAT(F5.5,F6.3,F5.3,F5.4,I2)
       S=N
      READ2,(X(1,I),X(2,I),I=1,N)
    2 FORMAT(F5.3,F6.4)
      DO 3 I=1,N
      X(3,I)=(AV+X(1,I))*.001
      X(4,I)=(A/AM)/X(3,I)
       X(5,I)=(BN*X(1,I))/(1000.*X(3,I))
      X (6,I)=X(4,I)-X(5,I)
      IF(X(6,I)-.00095)9,9,8
    9 S=S- 1.
      X(11,I)=0.
      GO TO 3
    8 X(7,I)=-X(2,I)*2.302585
       X(8,I)=EXP(X(7,I))
      X(9,I)=(X(5,I)-X(8,I))/(X(6,I)+X(8,I))
       X(10,I)=LOG(X(9,I))/2.302585
      X(11,I)=X(10,I)+X(2,I)
    3 CONTINUE
       PUNCH6
    6 FORMAT(8HACID VOL,3X,2HPH,3X,7HVOL SOL,2X,8HTOT CONC,2X,8HCONC REA
```

```
     1,2X,8HCONC UNR)
      PUNCH 4,((X(I,J),I=1,6),J=1,N)
    4 FORMAT(F7.3,F8.4,F9.6,F10.7,F10.7,F10.7)
       PUNCH7
    7 FORMAT(1X,5HLN H+,7X,2HH+,4X,9HCONC CORR,3X,6HLOG CC,2X,3HPKA)
      DO14J=1,N
      IF(X(6,J)-.00095)21,21,16
   21 PUNCH15,X(6,J)
   15 FORMAT(14HDATA DISCARDED,4X,8HCONC UNR,F10.7)
      GOTO14
   16 PUNCH5, (X(I,J),I=7,11)
    5 FORMAT(F8.4,F10.7,F10.7,F8.4,F8.4)
   14 CONTINUE
       SX12=0.
       DO 11 I=1,N
   11 SX12=SX12+X(11,I)
       AVE=SX12/S
      D2=0.
      DO13I=1,N
      IF(X(6,I)-.00095)13,13,17
   17 D=(AVE-X(11,I))
      D2=D2+D*D
   13 CONTINUE
      SD=SQRT(D2/(S-1.))
      AVE=AVE+.005
       PUNCH 12, AVE,SD
   12  FORMAT (10X,13HAVERAGE PKA =,F6.2,1H(,F6.2,1H))
      PUNCH821
```

```
821 FORMAT(79X,1H-)
      GOTO10
      END
```

TIT. OF 166 GLYCINEHYDROXAMIC ACID RUN 1 ACID

037340900885000000098512

0.7 7.832

0.9 7.738

1.1 7.654

1.3 7.576

1.5 7.494

1.7 7.417

1.9 7.335

2.1 7.252

2.3 7.162

2.6 7.025

2.7 6.977

2.9 6.864

TIT. OF 166 GLYCINEHYDROXAMIC ACID RUN 1 ACID

ACID VOL	PH	VOL SOL	TOT CONC	CONC REA	CONC UNR
.700	7.8320	.050700	.0081752	.0013599	.0068152
.900	7.7380	.050900	.0081430	.0017416	.0064014
1.100	7.6540	.051100	.0081112	.0021203	.0059908
1.300	7.5760	.051300	.0080796	.0024961	.0055835
1.500	7.4940	.051500	.0080482	.0028689	.0051792
1.700	7.4170	.051700	.0080170	.0032388	.0047782
1.900	7.3350	.051900	.0079861	.0036059	.0043802
2.100	7.2520	.052100	.0079555	.0039702	.0039852
2.300	7.1620	.052300	.0079251	.0043317	.0035933
2.600	7.0250	.052600	.0078799	.0048688	.0030110
2.700	6.9770	.052700	.0078649	.0050464	.0028184
2.900	6.8640	.052900	.0078352	.0053998	.0024354

LN H+	H+	CONC CORR	LOG CC	PKA
-18.0338	0.0000000	.1995438	-.6999	7.1320
-17.8174	0.0000000	.2720677	-.5653	7.1726
-17.6239	0.0000000	.3539254	-.4510	7.2029
-17.4443	0.0000000	.4470426	-.3496	7.2263
-17.2555	0.0000000	.5539138	-.2565	7.2374
-17.0782	0.0000000	.6778295	-.1688	7.2481
-16.8894	0.0000000	.8232202	-.0844	7.2505
-16.6983	0.0000000	.9961979	-.0016	7.2503
-16.4911	0.0000000	1.2054366	.0811	7.2431
-16.1756	0.0000000	1.6168781	.2086	7.2336
-16.0651	.0000001	1.7903994	.2529	7.2299
-15.8049	.0000001	2.2170202	.3457	7.2097

AVERAGE PKA = 7.22(.03)

Stability Constant Program

The program to calculate the β's for the complexes required the following data: 1) a single card with the name of the ligand and metal ion being studied; 2) a second data card comprised of data characteristic of the ligand and metal ion being studied; and 3) as many data cards as there were points on the formation curve, each recorded with the volume of base added and the corresponding pH reading.

The first card had no specified form. However, the second card had an exact form. No decimals were punched in the numbers placed on the card as the program had the necessary information for placing the decimal point in the proper position. The actual input for this card (where X is any digit) is as follows: the sample weight, .XXXXX; the molecular weight, XXX.XXX; pK_1, XX.XX; pK_2, XX.XX; (where pK_1 is greater than pK_2) metal solution concentration, X.XXXX; volume of metal ion solution, X.XX; initial volume of ligand, XX.XX; base normality, X.XXXX; the number of points from the formation curve, XX.; the 95% confidence level test number, X.XX; the first hydrolysis constant, -XX.XX; and the second hydrolysis constant, -XX.XX. If the hydrolysis constants were omitted, the hydrolysis effects on the metal ion concentration would be ignored. The form for the data cards made from each point in the titration is as follows: the volume, XX.XXX; and the pH, XX.XXX.

When a set of data was being run for the first time, the console switch number one was placed on. This allowed the program to punch on cards the values obtained for the volume of base, the pH, $\bar{n}$, the metal ion concentration, and the concentration of ligand. The first sense switch was also used in the program to type the number of times the solution for the β's had been approximated and to give the particular β's for each approximation. The second console switch was used to discard the set of data and to shift control to read the next set of data.

The symbols used in the NIPIN subroutine were:

FN - the number of samples minus one

SV - the initial total volume of the metal solution and the ligand solution

TV - the total volume for each point in the titration, i.e. SV and the volume of base added to this point in the titration

CL - the analytical concentration of the ligand at this point of the titration

CM - the analytical concentration of the metal ion to this point in the titration (either corrected or uncorrected for hydrolysis)

CONCH - the hydrogen ion concentration found from the pH at this point in the titration

CONCOH - the hydroxyl ion concentration found from the pH and the ionization constant of water at 25°

X(I,1) - the volume of base added to this point in the titration

X(I,2) - the pH at this volume

X(I,3) - the concentration of L^- for this point

X(I,4) - $\bar{n}$ for this point

NN - the next integer after the largest $\bar{n}$

The symbols used in the NIPEBS program were:

B1 - the value of β_1

B2 - the value of β_2

B3 - the value of β_3

PLB1 - the log of the previous B1

PLB2 - the log of the previous B2

PLB3 - the log of the previous B3

TPB1 - the log of B1

TPB2 - the log of B2

TPB3 - the log of B3

A(I,I) - the A matrix used to set up the coefficients for the linear equations which solve for the β s

SD1 - the standard deviation of B1

SD2 - the standard deviation of B2

SD3 - the standard deviation of B3

The symbols used in the NIPOUT program were:

E1 - the 95% confidence level for B1

E2 - the 95% confidence level for B2

E3 - the 95% confidence level for B3

The actual computer program follows with a subsequent set of typical input data and the output from that data.

```
THE PROGRAM TO CALCULATE THE STABILITY CONSTANTS
    DIMENSIONX(23,4),A(3,5),Q(23)
    COMMONX,A,Q,NN,ZZ,PLB3,PLB2,PLB1,FN,TEST,N,SD3,SD2,SD1,IS,Y
  1 READ820
820 FORMAT(80H                                                  /
   N                          )
    PUNCH822
822 FORMAT(//)
    PUNCH820
    ZZ=0.
    TCO=15.
    TC=0.
    Y=10000.
    R=.0005
    CALL NIPIN
    IF(ZZ-9999.)695,659,695
659 IF(IS-N)658,1,1
658 READ3,(X(I,1),I=IS,N)
  3 FORMAT(F5.3)
    GO TO 1
695 NN=ZZ+1.
112 GOTO(111,113,116,117,117),NN
111 ACCEPT830,NN
830 FORMAT(I2)
    IF(NN-1)112,84,112
 84 CALL NIPEB1
    CALL NIPOUT
    GOTO1
```

```
117 PRINT128
128 FORMAT(23HN GREATER/EQUAL TO FOUR)
    GOTO1
116 B1=8.
    B2=15.
    B3=22.
    PLB1=8.
    PLB2=15.
    PLB3=22.
119 DO120I=1,4
    DO120J=1,4
120 A(I,J)=0.0
    DO100I=1,N
    Q(I)=1.+B1*X(I,3)+B2*X(I,3)*X(I,3)+B3*X(I,3)*X(I,3)*X(I,3)
    F=X(I,4)/X(I,3)/Q(I)
    C=(X(I,4)-1.)/Q(I)
    D=(X(I,4)-2.)*X(I,3)/Q(I)
    E=(X(I,4)-3.)*(X(I,3)*X(I,3))/Q(I)
    A(1,1)=A(1,1)+C*E
    A(1,2)=A(1,2)+D*C
    A(1,3)=A(1,3)+C*C
    A(1,4)=A(1,4)+E*C
    A(2,1)=A(2,1)+D*E
    A(2,2)=A(2,2)+D*D
    A(2,3)=A(2,3)+C*D
    A(2,4)=A(2,4)+E*D
    A(3,1)=A(3,1)+E*E
    A(3,2)=A(3,2)+D*E
```

```
      A(3,3)=A(3,3)+C*E
  100 A(3,4)=A(3,4)+E*E
  115 M=NN+1
      DO 7 I=1,NN
      II=I
    9 T=1000000000.
      NM=0
      IF(A(I,I))240,241,241
  240 V=-A(I,I)
      GO TO 19
  241 V=A(I,I)
   19 IF(V-T)20,21,21
   20 T=T*.1
      NM=NM+1
      IF(NM-100)19,11,19
   21 T=T*100.
      TT=(A(I,I)+T)-T
      IF(TT)110,11,110
   11 IF(NN-II)13,13,12
   13 PUNCH200
  200 FORMAT (19HNO UNIQUE SOLUTIONS  )
      QQ=EXP(1.)
      QQ=SQRT(1.)
      GOTO1
   12 II=II+1
      DO 8 J=1,M
    8 A(I,J)=A(I,J)+A(II,J)
      GOTO9
```

```
110   Z=A(I,I)
      DO71J=1,M
 71   A(I,J)=A(I,J)/Z
      DO 7 J=1,NN
      Z=A(J,I)
      DO 7 K=1,M
      IF (I-J) 14,7,14
 14   A(J,K)=A(J,K)-Z*A(I,K)
  7   CONTINUE
      TC=TC+1.
      IF(TC-TCO)259,260,260
260   TCO=TCO+5.
      Y=Y*10.
      R=R*10.
259   CONTINUE
      IF(SENSE SWITCH 3)700,129
700   PRINT701,TC
701   FORMAT(F4.0)
129   GOTO(111,142,125),NN
125   IF(A(1,4))171,172,172
171   G=-A(1,4)
      GO TO 173
172   G=A(1,4)
173   IF(A(2,4))174,175,175
174   H=-A(2,4)
      GO TO 176
175   H=A(2,4)
176   IF(A(3,4))177,178,178
```

```
177 H1=-A(3,4)
    GO TO 179
178 H1=A(3,4)
179 BL3=LOG(G)/2.302585
    BL2=LOG(H)/2.302585
    BL1=LOG(H1)/2.302585
    IF(SENSE SWITCH 4)190,123
190 BL3=BL3+R
    BL2=BL2+R
    BL1=BL1+R
123 IF(PLB3-BL3+Y-Y)118,121,118
121 IF(PLB2-BL2+Y-Y)118,122,118
122 IF(PLB1-BL1+Y-Y)118,201,118
118 B3=A(1,4)
    B2=A(2,4)
    B1=A(3,4)
    PLB3=BL3
    PLB2=BL2
    PLB1=BL1
    IF(SENSE SWITCH 1)151,119
151 PRINT132,BL3,BL2,BL1
    IF(SENSE SWITCH 2)1,119
113 B1=8.
    B2=15.
    PLB1=8.
    PLB2=15.
114 DO140I=1,3
    DO140J=1,3
```

```
140 A(I,J)=0.
    DO141I=1,N
    Q(I)=1.+B1*X(I,3)+B2*X(I,3)*X(I,3)
    F=X(I,4)/X(I,3)/Q(I)
    C=(X(I,4)-1.)/Q(I)
    D=(X(I,4)-2.)*X(I,3)/Q(I)
    A(1,1)=A(1,1)+D*C
    A(1,2)=A(1,2)+C*C
    A(1,3)=A(1,3)+F*C
    A(2,1)=A(2,1)+D*D
    A(2,2)=A(2,2)+D*C
141 A(2,3)=A(2,3)+F*D
    GOTO115
142  IF(A(1,3))161,162,162
161 G=-A(1,3)
    GO TO 163
162 G=A(1,3)
163 IF(A(2,3))164,165,165
164 H=-A(2,3)
    GO TO 166
165 H=A(2,3)
166 BL2=LOG(G)/2.302585
    BL1=LOG(H)/2.302585
    IF(SENSE SWITCH 4)146,145
146 BL2=BL2+R
    BL1=BL1+R
145 IF(PLB2-BL2+Y-Y)144,143,144
143 IF(PLB1-BL1+Y-Y)144,153,144
```

```
144 B2=A(1,3)
    B1=A(2,3)
    PLB1=BL1
    PLB2=BL2
    IF(SENSE SWITCH 1)152,114
152 PRINT132,PLB2,PLB1
    IF(SENSE SWITCH 2)1,114
153 SD1=0.
    SD2=0.
    B1=EXP(PLB1*2.302585)
    B2=EXP(PLB2*2.302585)
    PUNCH305
305 FORMAT(7HML BASE,4X,2HPH,10X,3H(L),9X,1HN,6X,5HLOGB2,3X,5HLOGB1)
    GO TO 155
201 SD1=0.
    SD2=0.
    SD3=0.
    B1=EXP(PLB1*2.302585)
    B2=EXP(PLB2*2.302585)
    B3=EXP(PLB3*2.302585)
    PUNCH306
306 FORMAT(7HML BASE,4X,2HPH,10X,3H(L),9X,1HN,6X,5HLOGB3,3X,5HLOGB2
   R,3X,5HLOGB1)
155 DO 400L=1,N
    A(3,5)=0
    A(2,5)=0
    A(1,5)=0
    Q(L)=1.+B1*X(L,3)+B2*X(L,3)*X(L,3)
```

```
      F=X(L,4)/X(L,3)/Q(L)
      C=(X(L,4)-1.)/Q(L)
      D=(X(L,4)-2.)*X(L,3)/Q(L)
      GO TO(111,280,281),NN
  281 Q(L)=1.+B1*X(L,3)+B2*X(L,3)*X(L,3)+B3*X(L,3)*X(L,3)*X(L,3)
      F=X(L,4)/X(L,3)/Q(L)
      C=(X(L,4)-1.)/Q(L)
      D=(X(L,4)-2.)*X(L,3)/Q(L)
      E=(X(L,4)-3.)*(X(L,3)*X(L,3))/Q(L)
      A(3,4)=F*E
      A(1,1)=C*E
      A(2,1)=D*E
      A(3,1)=E*E
      A(3,2)=D*E
      A(3,3)=C*E
  280 A(1,2)=D*C
      A(1,3)=C*C
      A(1,4)=F*C
      A(2,2)=D*D
      A(2,3)=C*D
      A(2,4)=F*D
      IF(NN-2)111,282,283
  282 DO 402J=1,NN
      A(1,5)=A(1,5)-((B1*A(J,3)+A(J,4))/(2.*A(J,2)))
  402 A(2,5)=A(2,5)-((B2*A(J,2)+A(J,4))/(2.*A(J,3)))
      GO TO 180
  283 DO 403J=1,NN
      A(1,5)=A(1,5)-(B2*A(J,2)+B1*A(J,3)+A(J,4))/(3.*A(J,1))
```

```
      A(2,5)=A(2,5)-(B3*A(J,1)+B1*A(J,3)+A(J,4))/(3.*A(J,2))
  403 A(3,5)=A(3,5)-(B3*A(J,1)+B2*A(J,2)+A(J,4))/(3.*A(J,3))
      GO TO 202
  180 IF(A(1,5))181,182,182
  181 G=-A(1,5)
      GO TO 183
  182 G=A(1,5)
  183 IF(A(2,5))184,185,185
  184 H=-A(2,5)
      GOTO 186
  185 H=A(2,5)
  186 TPB2=LOG(G)/2.302585
      TPB1=LOG(H)/2.302585
      SD2=SD2+((PLB2-TPB2)*(PLB2-TPB2))
      SD1=SD1+((PLB1-TPB1)*(PLB1-TPB1))
      TPB1=TPB1+.005
      TPB2=TPB2+.005
      PUNCH401,X(L,1),X(L,2),X(L,3),X(L,4),TPB2,TPB1
      GO TO 400
  202 IF(A(1,5))203,204,204
  203 G=-A(1,5)
      GO TO 205
  204 G=A(1,5)
  205 IF(A(2,5))206,207,207
  206 H=-A(2,5)
      GO TO 208
  207 H=A(2,5)
  208 IF(A(3,5))209,210,210
```

```
209 H1=-A(3,5)
    GO TO 211
210 H1=A(3,5)
211 TPB3=LOG(G)/2.302585
    TPB2=LOG(H)/2.302585
    TPB1=LOG(H1)/2.302585
    SD3=SD3+((PLB3-TPB3)*(PLB3-TPB3))
    SD2=SD2+((PLB2-TPB2)*(PLB2-TPB2))
    SD1=SD1+((PLB1-TPB1)*(PLB1-TPB1))
    TPB3=TPB3+.005
    TPB2=TPB2+.005
    TPB1=TPB1+.005
    PUNCH401,X(L,1),X(L,2),X(L,3),X(L,4),TPB3,TPB2,TPB1
401 FORMAT(F6.3,2X,F7.3,2X,E14.7,2X,F6.3,2X,3(F6.2,2X))
400 CONTINUE
    CALL NIPOUT
    GO TO 1
132 FORMAT(3(F8.4,5X))
    END
    SUBROUTINENIPIN
    DIMENSIONX(23,4),A(3,5),Q(23)
    COMMONX,A,Q,NN,ZZ,PLB3,PLB2,PLB1,FN,TEST,N,SD3,SD2,SD1,IS
    READ2,COMA,COMMW, PK1, PK2,SNM,AM,AV,BN,N,TEST,HK1,HK2
  2 FORMAT(F5.5,F6.3,2(F4.2),F5.4,F3.2,F4.2,F5.4,I2,F3.2,2F5.2)
    FN=N-1
    SV=AM+AV
    CPK1=EXP(-PK1*2.302585)
    CPK2=EXP(-PK2*2.302585)
```

```
      IF(HK1)676,675,676
      HK1=EXP(HK1*2.302585)
      IF(HK2)677,678,677
      HK2=EXP(HK2*2.302585)
      CONTINUE
      DO 10 I=1,N
      READ3,X(I,1),X(I,2)
      FORMAT(F5.3,F6.4)
      IS=I+1
      TV=SV+X(I,1)
      CL=((COMA/COMMW)/TV)*1000.
      CM=(SNM*AM)/TV
      CONCH=EXP (-(X(I,2)*2.302585))
      COR1=HK1*TV*55.34/(1000.*CONCH)
      COR2=HK2*TV*55.34/(1000.*CONCH)
      CM=CM/(1.+COR1+COR1*COR2)
      CR=((BN*X(I,1))/TV)
      CONCOH=EXP ((X(I,2)-13.9965)*2.302585)
      P=(CONCH/CPK1)+((2.*(CONCH**2))/(CPK1*CPK2))
      U=(CONCH/CPK1)+((CONCH**2)/(CPK1*CPK2))+1.
      X(I,3)=CL/P-CR/P-CONCH/P+CONCOH/P
      X(I,4)=(CL-(U*X(I,3)))/CM
      IF(SENSE SWITCH 1)699,698
9     PUNCH825,X(I,1),X(I,3),X(I,4),CM,CL
5     FORMAT(F7.2,2X,4(E14.7,1X))
      IF(X(I,4)-3.)695,694,694
4     PUNCH821
1     FORMAT(79X,1H-)
```

```
      GO TO 696
  695 IF(SENSE SWITCH 2)698,696
  696 ZZ=9999.
      RETURN
  698 IF(ZZ-X(I,4))4,10,10
    4 ZZ=X(I,4)
   10 CONTINUE
      RETURN
      END
      SUBROUTINENIPOUT
      DIMENSIONX(23,4),A(3,5),Q(23)
      COMMONX,A,Q,NN,ZZ,PLB3,PLB2,PLB1,FN,TEST,N,SD3,SD2,SD1
      GO TO (444,500,600),NN
  600 E3=SQRT(SD3)/FN
      E3=(E3*TEST)/SQRT(FN+1.)
      E2=SQRT(SD2)/FN
      E2=(E2*TEST)/SQRT(FN+1.)
      E1=SQRT(SD1)/FN
      E1=(E1*TEST)/SQRT(FN+1.)
      PLB3=PLB3+.005
      PLB2=PLB2+.005
      PLB1=PLB1+.005
      PUNCH303,PLB3,E3
  303 FORMAT(/2X,7HLOGB3=(,F6.2,1H),2X,36H95 PER CENT CONFIDENCE LEVEL O
     UF B3=(,F6.2,1H))
      PUNCH302,PLB2,E2
  302 FORMAT(/2X,7HLOGB2=(,F6.2,1H),2X,36H95 PER CENT CONFIDENCE LEVEL O
     TF B2=(,F6.2,1H))
```

```
      PUNCH301,PLB1,E1
  301 FORMAT(/2X,7HLOGB1=(,F6.2,1H),2X,36H95 PER CENT CONFIDENCE LEVEL O
     SF B1=(,F6.2,1H))
      PUNCH821
  821 FORMAT(79X,1H-)
      RETURN
  500 E2=SQRT(SD2)/FN
      E2=(E2*TEST)/SQRT(FN+1.)
      PLB2=PLB2+.005
      PUNCH302,PLB2,E2
  444 E1=SQRT(SD1)/FN
      E1=(E1*TEST)/SQRT(FN+1.)
      PLB1=PLB1+.005
      PUNCH301,PLB1,E1
      PUNCH821
      RETURN
      END
```

TIT. OF GLYCINE HYDROXAMIC ACID AND ZN+2 RUN 1

0541109008809160721010772325000009851321 6- 915

0.2	6.883
0.5	6.926
0.8	6.976
1.1	7.032
1.4	7.089
1.7	7.164
2.0	7.234
2.3	7.330
2.6	7.428
2.9	7.544
3.2	7.666
3.5	7.805
3.8	7.960

TIT. OF GLYCINE HYDROXAMIC ACID AND ZN+2 RUN 1

ML BASE	PH	(L)	N	LOGB2	LOGB1
.200	6.883	1.1141205E-05	1.033	9.99	5.73
.500	6.926	1.2567687E-05	1.088	10.03	5.54
.800	6.976	1.4523633E-05	1.142	10.04	5.54
1.100	7.032	1.7100986E-05	1.195	10.03	5.56
1.400	7.089	2.0081147E-05	1.250	10.03	5.57
1.700	7.164	2.5107311E-05	1.301	9.99	5.63
2.000	7.234	3.0462539E-05	1.358	9.98	5.64
2.300	7.330	4.0245724E-05	1.410	9.93	5.71
2.600	7.428	5.2600862E-05	1.471	9.89	5.74
2.900	7.544	7.1673823E-05	1.538	9.86	5.77
3.200	7.666	9.6978276E-05	1.623	9.86	5.76
3.500	7.805	1.3423227E-04	1.734	9.94	5.68
3.800	7.960	1.8775104E-04	1.890	10.25	5.36

LOGB2=(10.01) 95 PER CENT CONFIDENCE LEVEL OF B2=(.01)

LOGB1=(5.60) 95 PER CENT CONFIDENCE LEVEL OF B1=(.02)

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