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The Complex between Hydroxamic Acids and Ferric Ions, and the Use of the Complex for Quantitative Determination of Hydroxamic Acids, Acylderivatives and Ferric Salts

GUNNAR AKSNES

Forsvarets forskningsinstitutt, Kjeller, Norway

The dissociation constants of the complex between ferric ions and acet- and benzhydroxamic acids have been determined in acid solution (pH = 1.4). The ratio between hydroxamic acid and ferric ions in the complex is 1:1.

When the complex is used for quantitative determination of acylderivatives the hydroxylamine, which must always be present in excess, will influence the stability of the complex.

In weakly alkaline solution the complex between iron and hydroxamic acids is probably $Fe(RCONHO)_3$. This complex can be used for quantitative determination of ferric salts.

Hydroxamic acids and ferric ions give complex compounds which absorb light in the visible region [1,2]. For the last 10 years there has been a growing interest in this reaction, because a variety of acylderivatives can be quantitatively converted into hydroxamic acids with the aid of hydroxylamine [3-6].

Hestrin [6] observed that, when using the hydroxamic acid-ferric complex method for determination of acetylcholin, the colour of the complex will fade when the pH of the solution is allowed to get below 1.2. The quantity of ferric chloride which is necessary to give complete transformation of hydroxamic acids to the complex, is very high. Hestrin therefore concluded that the dissociating tendency of the ferric-acethydroxamic acid complex must be fairly high at the pH used (1.4).

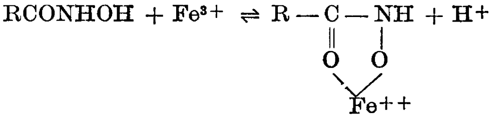
It will be shown in this work that the complex is very stable. It is further found that the dissociating tendency of the complex observed by Hestrin is partly caused by the reduction of ferric to ferrous iron by hydroxylamine.

DETERMINATION OF THE DISSOCIATION CONSTANTS OF ACET- AND BENZHYDROXAMIC ACIDS-FERRIC COMPLEXES

In order to determine the true dissociation constants for the two complexes it was necessary to work in the absence of hydroxylamine. The hydroxamic acids (acet- and benzhydroxamic acids) were therefore synthesized separately.

The absorption curves of the two complexes at pH 1.4 are shown in Fig. 1. In Fig. 2 log ε is given as a function of the ferric ion concentration with a fixed concentration of hydroxamic acid. In Fig. 3 log ε is given as a function of the acethydroxamic acid concentration with great excess of ferric chloride (lowest ratio approx. 20). The extinction of the complex follows Beer's law over a wide range of hydroxamic acid concentrations. This enables one to determine the dissociation constants from absorption measurements.

It is commonly believed that the complex between ferric ions and hydroxamic acids in acid solution is a 1:1 complex, though this has not yet been proved:



$$\mathrm{RCONHOH} + \mathrm{Fe}^{3+} \rightleftharpoons \begin{array}{c}\mathrm{R-C-NH} \\ \;\;\| \quad\; | \\ \;\;\mathrm{O} \quad \mathrm{O} \\ \diagdown\!\diagup \\ \mathrm{Fe}^{++}\end{array} + \mathrm{H}^{+}$$

When the hydrogen ion concentration is fixed, we therefore have:

$$K_{\text{complex}} = \frac{C_{\text{hydroxamic acid}} \times C_{\text{Fe}}^{3+}}{C_{\text{complex}}}$$

If the concentration of complex is measured in a mixture of known amounts of hydroxamic acid and ferric ions, the dissociation constant, K_{complex}, can be evaluated from the equation:

$$K_{\text{complex}} = \frac{(a-c)(b-c)}{c}$$

a = initial concentration of hydroxamic acid
b = " " " ferric ions
c = the measured complex concentration

Because the extinction of the complex follows Beer's law we have:
$C = fI$
I = log extinction (log ε)
f = proportionality factor

From this one obtains:

$$K_{\text{complex}} = \frac{(a - fI)\,(b - fI)}{fI}$$

The proportionality factor f has been evaluated by two different methods:

a) Determination of the complex extinction when a given quantity of hydroxamic acid is quantitatively transformed to the ferric-hydroxamic acid complex. This can be done by using ferric ions in great excess. Then we have:

$$C = a = fI_{\max}, \text{ hence } f = \frac{a}{I_{\max}}$$

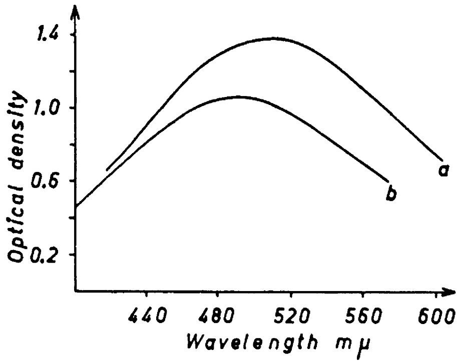


Fig. 1. (a) Absorption curves of complex between ferric ions and benzhydroxamic acid, and (b) complex between ferric ions and acethydroxamic acid. Concentration: 5 mg hydroxamic acid in 10 ml of 0.00925 M iron chloride adjusted to pH = 1.4 with hydrochloric acid.

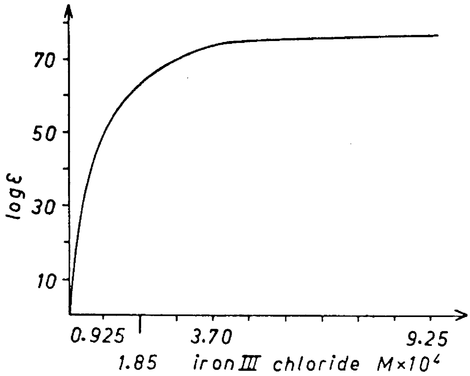


Fig. 2. Stability of ferric-acethydroxamic acid complex at constant pH (1.4). Concentration of acethydroxamic acid: 5×10^{-4} M. pH adjusted with hydrochloric acid.

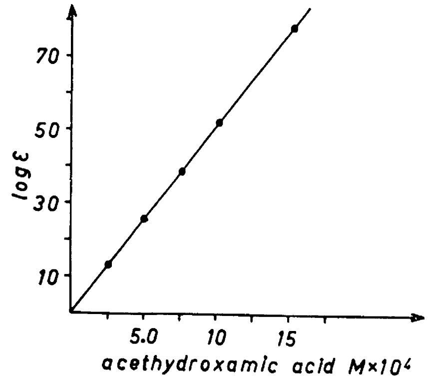


Fig. 3. log ε of ferric-acethydroxamic acid complex as function of acethydroxamic acid concentration (pH = 1.54). Concentration of ferric chloride: 0.0185 M. pH adjusted with hydrochloric acid.

b) If log ε of the complex is measured for two different sets of values for a and b, we get:

$$K_{\text{complex}} = \frac{(a_1 - fI_1)(b_1 - fI_1)}{fI_1} = \frac{(a_2 - fI_2)(b_2 - fI_2)}{fI_2}$$

f is found as the root in a second order equation.

The results are shown in Tables 1 and 2. The constancy of K_{complex} found for different values of a and b, indicates that the ratio between hydroxamic acid and ferric ions in the complex is 1:1.

Table 1. Determination of the complex constant of ferric-benzhydroxamic acid complex. Temperature: 20 °C, pH = 1.40.

$a \times 10^4$	$b \times 10^4$	I	$I_{\max}$	$f = \frac{a}{I_{\max}}$	$f_{\text{calc.}}$	K_{complex}
3.75	9.25	42.0	59.8	6.27×10^{-6}	6.20×10^{-6}	2.76×10^{-4}
2.5	9.25	29.0	39.3	6.36×10^{-6}		2.66×10^{-4}
1.25	9.25	15.0	19.8	6.31×10^{-6}		2.62×10^{-4}

Table 2. Determination of the complex constant of ferric-acethydroxamic acid complex. Temperature: 20 °C, pH = 1.40.

$a \times 10^4$	$b \times 10^4$	I	$I_{\max}$	$f = \frac{a}{I_{\max}}$	$f_{\text{calc.}}$	K_{complex}
3.75	18.5	31.4	39.5	9.49×10^{-6}	9.4×10^{-6}	4.0×10^{-4}
1.875	18.5	16.1	20.1	9.33×10^{-6}		4.2×10^{-4}
0.9375	18.5	7.9	9.9	9.45×10^{-6}		4.5×10^{-4}
3.75	9.25	23.9	39.5	9.49×10^{-6}		4.0×10^{-4}

THE INFLUENCE OF HYDROXYLAMINE ON THE STABILITY OF THE COMPLEX IN ACID SOLUTION

As mentioned in the introduction, hydroxylamine is always present in the solution when the complex is used for quantitative determination of acyl-derivatives.

The stability of the complex with and without a great excess of hydroxylamine, is shown in Fig. 4. The presence of hydroxylamine is seen to cause a rapid decrease in extinction during the first minutes; then the extinction drops more slowly. The reason for this rapid decrease in extinction is found in the ability of hydroxylamine to reduce ferric to ferrous ions according to the following equation:

$$2NH_2OH + 2Fe^{3+} \rightarrow 2Fe^{2+} + N_2O + 2H^+ + 2H_2O$$

The ferrous ions have been shown to give no complex formation with hydroxamic acids.

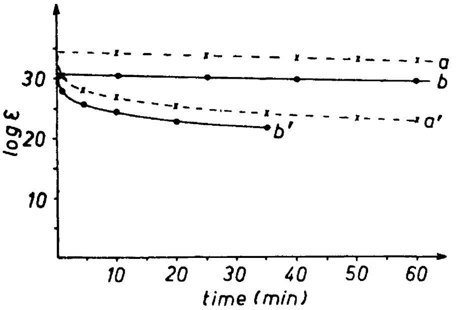


Fig. 4. Stability of ferric-hydroxamic acid complex as function of time.

a. 2.5 × 10^{-4} M benzhydroxamic acid + 1.85 × 10^{-3} M $FeCl_3$

b. 3.75 × 10^{-4} M acethydroxamic acid + 1.85 × 10^{-3} M $FeCl_3$

a'. 2.5 × 10^{-4} M benzhydroxamic acid + 1.85 × 10^{-3} M $FeCl_3$ + 0.05 M hydroxylamine

b'. 3.75 × 10^{-4} M acethydroxamic acid + 1.85 × 10^{-3} M $FeCl_3$ + 0.05 M hydroxylamine

The reaction is irreversible, but due to the formation of hydrogen ions the reaction will be greatly retarded with time in unbuffered solution (Fig. 4). The pH dependence of the reaction is shown in Fig. 5. The extinction and pH are measured 30 min after the start.

In an isolated experiment with benzhydroxamic acid and the same excess of Fe^{3+} the dissociation tendency of the complex was measured as function of pH. As seen from Fig. 5 the stability of the complex is greatly dependent on pH. The decrease in colour extinction in the presence of hydroxylamine is therefore dependent on both the reduction of the ferric concentration and the accompanying increase in hydrogen ion concentration.

Since the reduction of ferric ions by hydroxylamine will be greatly accelerated at higher pH, it is clear that when hydroxylamine is in excess, the extinction of the complex measured at fixed time intervals after mixing must show a maximum at a certain pH. On one side of the maximum (towards lower pH), the extinction of the complex will be reduced because the dissociation of the complex will increase; towards higher pH values the extinction will decrease because of rapid reduction of ferric to ferrous ions. This conclusion is clearly demonstrated in Fig. 5. The maximum is approximately pH 1.4 for both hydroxamic acids studied.

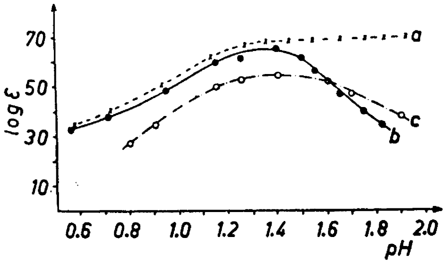


Fig. 5. pH dependence of the ferric-hydroxamic acid complex.

a. 8.41 × 10^{-3} M $FeCl_3$ + 4.57 × 10^{-4} M benzhydroxamic acid

b. 8.41 × 10^{-3} M $FeCl_3$ + 4.57 × 10^{-4} M benzhydroxamic acid + 0.182 M hydroxylamine.

c. 8.41 × 10^{-3} M $FeCl_3$ + 4.57 × 10^{-4} M acethydroxamic acid + 0.182 M hydroxylamine.

The extinction and pH were measured 30 min after start. pH was varied with hydrochloric acid.

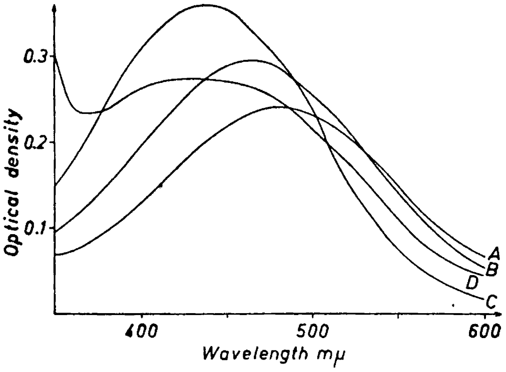


Fig. 6. Absorption curves of ferric-benzhydroxamic acid complex at different pH. A, pH = 2.59; B, pH = 3.5; C, pH = 6.8; D, pH = 10.0.

THE VARIATION OF THE STRUCTURE OF THE FERRIC-HYDROXAMIC ACID COMPLEX WITH pH, AND THE USE OF THE COMPLEX IN ALKALINE SOLUTION FOR QUANTITATIVE DETERMINATION OF FERRIC IRON

It was of interest to study the absorption spectrum of the complex as a function of pH. Because the experiments were performed in both acid and alkaline solution, the hydroxamic acid was here used in great excess to avoid formation of ferric hydroxide.

From the absorption curves at different pH (Fig. 6) it is seen that there is a successive shift of the absorption maximum towards shorter wave length with increasing pH. At the same time the extinction of the complex increases although the iron and hydroxamic acid concentrations are kept constant. A new maximum seems to be formed at 440 mμ in neutral or weakly alkaline solution. At very high pH values this maximum disappears.

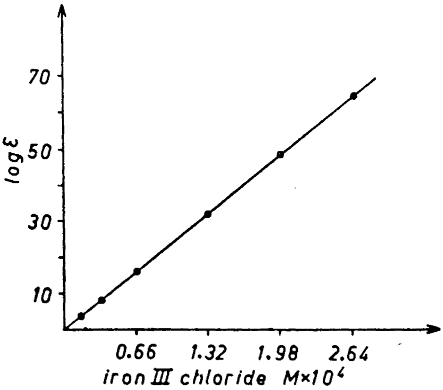


Fig. 7. Determination of ferric iron with acethydroxamic acid in alkaline solution. Concentration of acethydroxamic acid = 1.8×10^{-2} M. pH = 9.2 (*cf.* exptl. section).

The successive increase in extinction with pH suggests that the amount of hydroxamic acid in the complex increases successively with pH. This also explains the isolation of a neutral compound from alkaline alcoholic solution with an iron content corresponding to the formula $Fe(RCONHO)_3$. A possible variation of the structure of the complex with pH is the following:

$$\begin{array}{ccccccc} Fe(RCONHO)^{++} & \rightleftharpoons & Fe(RCONHO)_2^{+} & \rightleftharpoons & Fe(RCONHO)_3 & \rightleftharpoons & Fe(RCONO)_3^{---} \\ \text{(acid)} & & \text{(weakly acid)} & & \text{(neutral or weakly alkaline)} & & \text{(strongly alkaline)} \end{array}$$

It was of interest to examine whether the absorption maximum, which seems to be formed in weakly alkaline solution might be used for quantitative determination of ferric iron. The experiments were performed in a sodium carbonate-hydrogen carbonate buffer with great excess of hydroxamic acid. The results are shown in Fig. 7. It is seen that Beer's law is exactly valid over a wide range of ferric ion concentrations.

EXPERIMENTAL

Reagents. All reagents used were p.a. grade. The hydroxamic acids were synthesized according to descriptions in the literature[7].

Apparatus. The absorption curves were measured in a Beckman D.U. spectrophotometer. The colorimeter readings were taken with a Beckman electrophotometer. The hydrogen ion concentrations were measured with a Beckman pH meter, model H. 2.

Isolation of $Fe(C_6H_5CONHO)_3$. To a solution of 0.5 g ferric chloride and 3 g benzhydroxamic acid in 20 ml alcohol was added a 3 % solution of sodium alcoholate. A brownish precipitate was formed. It was filtered off and washed with alcohol and ether. (Found: Fe 12.20. Calc. for $Fe(C_6H_5CONHO)_3$ (464.22): Fe 12.46).

Determination of iron in alkaline solution. Reagents: Acethydroxamic acid 2 mg/ml. Buffer: 1.0 g sodium hydrogen carbonate + 0.5 g sodium carbonate in 100 ml water. Iron(III) chloride standard: 0.0001156–0.00185 M.

5 ml acethydroxamic acid solution was mixed with 1 ml iron chloride solution, and 1 ml buffer was thereafter added. The colorimeter readings were taken with a Beckman electrophotometer, filter 525 mμ.

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