

# Stability of (Pyrrolidone-5-hydroxamato)iron(III) Chelates

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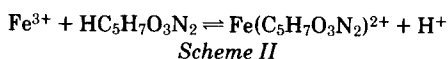
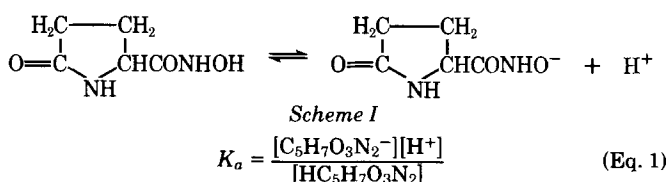
**Abstract** □ Overall stability constants of mono-, bis-, and tris(pyrrolidone-5-hydroxamato)iron(III) chelates were determined in aqueous solutions at 25° as  $\log \beta_I = 1.49$ ,  $\log \beta_{II} = 1.55$ , and  $\log \beta_{III} = 0.21$ , respectively, where  $\beta_I = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}][\text{H}^+]/[\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$ ,  $\beta_{II} = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+][\text{H}^+]^2/[\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^2$ , and  $\beta_{III} = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3][\text{H}^+]^3/[\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^3$ . Stability constants of all three chelates were determined potentiometrically in the 3.00–3.23 pH region. The stability constant of the mono chelate also was determined spectrophotometrically at 25° as  $\log \beta_I = 1.52$  by measuring absorbance at 500 nm (absorbance maximum), where the molar absorptivity was  $\epsilon = 1124$  liters/(mole cm). Biological implications of hydroxamic acid-containing compounds are discussed.

**Keyphrases** □ (Pyrrolidone-5-hydroxamato)iron(III)—mono, bis, and tris chelates, stability constants determined in aqueous solutions □ Iron(III) chelates—mono, bis, and tris, with pyrrolidone-5-hydroxamic acid, stability constants in aqueous solutions □ Chelates—pyrrolidone-5-hydroxamic acid with iron(III), mono, bis, and tris, stability constants in aqueous solutions □ Hydroxamic acid derivatives—(pyrrolidone-5-hydroxamato)iron(III) mono, bis, and tris chelates, stability constants in aqueous solutions □ Stability constants—mono-, bis-, and tris(pyrrolidone-5-hydroxamato)iron(III) chelates in aqueous solutions

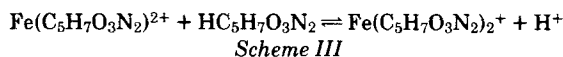
The role of the hydroxamic acid-containing compounds in living systems has become a lively issue since they are constituents of antibiotics, growth factors, tumor inhibitors, cell division factors, and pigments and are intimately associated with iron transport phenomena (1–7). A striking feature of hydroxamic acids is the ability to form coordination compounds with the pronounced preference for iron(III) (1). There is much interest in the hydroxamic acid–iron(III) chelates (6, 8–12). The paucity of data on heterocyclic aminohydroxamic acids and their iron(III) complexes necessitated the selection of pyrrolidone-5-hydroxamic acid and their iron(III) chelates as a model system for the nitrogen heteropentacyclic hydroxamic acids and their complexes.

A previous paper (12) described the coordination ability of pyrrolidone-5-hydroxamic acid to bind ferric ion, forming a mixture of mono, bis, and tris chelates from which the tris(pyrrolidone-5-hydroxamato)iron(III) complex was isolated and characterized.

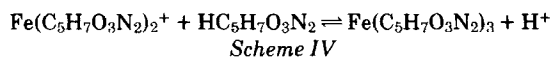
This paper reports results related to the thermodynamic stability of mono, bis, and tris chelates in aqueous solutions. In a solution containing iron(III) ions and a bidentate pyrrolidone-5-hydroxamic acid, the system at equilibrium may be described by Schemes I–IV and Eqs. 1–4, where  $K_a$  is the acidity constant and the stepwise equilibrium constants are  $K_I$ ,  $K_{II}$ , and  $K_{III}$  (12).



$$K_I = \frac{[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}][\text{H}^+]}{[\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]} \quad (\text{Eq. 2})$$



$$K_{II} = \frac{[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+][\text{H}^+]}{[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]} \quad (\text{Eq. 3})$$



$$K_{III} = \frac{[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3][\text{H}^+]}{[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]} \quad (\text{Eq. 4})$$

The overall  $\beta$  and stepwise stability constants  $K$  are related by the familiar expressions:

$$\beta_I = K_I \quad (\text{Eq. 5a})$$

$$\beta_{II} = K_I K_{II} \quad (\text{Eq. 5b})$$

$$\beta_{III} = K_I K_{II} K_{III} \quad (\text{Eq. 5c})$$

Constants  $\beta_{1,2,3}$  and  $K_{1,2,3}$  are defined by:

$$\beta_1 = \beta_I K_a^{-1} \quad (\text{Eq. 6a})$$

$$\beta_2 = \beta_{II} K_a^{-2} \quad (\text{Eq. 6b})$$

$$\beta_3 = \beta_{III} K_a^{-3} \quad (\text{Eq. 6c})$$

$$K_1 = K_I K_a^{-1} \quad (\text{Eq. 7a})$$

$$K_2 = K_{II} K_a^{-1} \quad (\text{Eq. 7b})$$

$$K_3 = K_{III} K_a^{-1} \quad (\text{Eq. 7c})$$

## EXPERIMENTAL

**Chemicals**—The preparation of pyrrolidone-5-hydroxamic acid and its iron(III) coordination compounds was described previously (12). The iron(II) solutions were prepared *in situ* by catalytic hydrogenation of iron(III) over palladium-on-carbon. All experiments involving iron(II) ions were performed under nitrogen. All other chemicals were reagent grade, and water was distilled from aqueous acidified potassium permanganate solution and stored in glass<sup>1</sup> containers.

**Physical Measurements**<sup>2</sup>—The potentiometric method (13, 14) involving nucleophilic potentiometric titration of iron(III) chelates was initially applied to the determination of the stability constants. The method was abandoned because reasonable results could not be obtained; the examined chelates were too stable for this method. The stability constants of mono, bis, and tris chelates were obtained by the potentiometric method in addition to the spectrophotometric method, which could be used only for mono chelate. In spectrophotometric and potentiometric experiments, the pH was adjusted to a constant value by hydrochloric acid or sodium hydroxide solution.

Redox potential measurements of iron(II)–iron(III) solutions were used to calculate the concentration of the free iron(III) ions at low pH, since iron(II)–hydroxamate complexes cannot be formed below pH 6 and the interfering base hydrolysis takes place above pH 8 (15, 16). In a typical redox potential measurement, the iron(II)–iron(III) solution was added to a pyrrolidone-5-hydroxamic acid solution, giving 50 ml of  $2.064 \times 10^{-4}$  M  $\text{Fe}^{3+}$  and  $2.064 \times 10^{-4}$  M  $\text{Fe}^{2+}$ . The total concentration of the pyrrolidone-5-hydroxamic acid was  $1.6 \times 10^{-3}$  M. Ionic strength was maintained at 0.1 by potassium chloride.

<sup>1</sup> Pyrex.

<sup>2</sup> The pH and potentiometric determinations were performed with a MA 5701 ISKRA pH meter and a Radiometer SRB2c/ABU 1b/TTA3 titrigrph. Visible spectra were obtained with a Opton PMQ II single-beam spectrophotometer and a Cary model 16K recording spectrophotometer equipped with quartz cells.

**Table I—Stability Constant of Mono(pyrrolidone-5-hydroxamato)iron(III) Chelate from Absorbance Measurements at 500 nm and Ionic Strength 0.1 (Sodium Chloride), Using 1-cm Quartz Cells<sup>a</sup>**

Experiment	pH	Absorbance, nm	[H <sup>+</sup> ] × 10 <sup>4</sup>	[Fe(C <sub>5</sub> H <sub>7</sub> O <sub>3</sub> N <sub>2</sub> ) <sub>2</sub> <sup>2+</sup> ] × 10 <sup>4</sup>	[Fe <sup>3+</sup> ] × 10 <sup>4</sup> = [HC <sub>5</sub> H <sub>7</sub> O <sub>3</sub> N <sub>2</sub> ] × 10 <sup>4</sup>	K <sub>I</sub>	log K <sub>I</sub>
1	1.90	0.260	126	2.31	2.69	40.2	1.60
2	2.43	0.353	57.2	3.14	1.86	33.8	1.53
3	2.49	0.371	32.4	3.30	1.70	37.0	1.57
4	2.70	0.398	20.0	3.54	1.46	33.2	1.52
5	2.78	0.409	16.6	3.64	1.36	32.7	1.51
6	2.95	0.414	11.2	3.68	1.32	23.7	1.37
7	3.19	0.424	6.46	3.77	1.23	16.1	1.21
8	3.33	0.388	4.68	3.45	1.55	6.7	0.83

<sup>a</sup>Total concentrations of pyrrolidone-5-hydroxamic acid and of iron(III) ions were kept equal at 5 × 10<sup>-4</sup> M. K<sub>I</sub> was calculated from Eq. 2.

## RESULTS AND DISCUSSION

**Spectrophotometric Determination of Stability Constant of Mono(pyrrolidone-5-hydroxamato)iron(III) Complex**—The behavior of the iron(III)-pyrrolidone-5-hydroxamic acid solution depicted by Schemes I-IV was described recently (12). In view of the interference of bis and tris chelates, only the stability constant of the mono chelate can be determined spectrophotometrically, if the pH is sufficiently low and there is an excess of iron(III) over hydroxamic acid. The visible spectrum of the mono(pyrrolidone-5-hydroxamato)iron(III) complex shows the maximum absorption at 500 nm with a molar absorptivity of ε<sub>max</sub> 500 nm = 1124 liters/(mole cm). The quantitative conversion of pyrrolidone-5-hydroxamic acid to the mono chelate is illustrated in Fig. 1. The necessary data for the calculation of K<sub>I</sub> (Eqs. 2 and 5a-5c) are given in Table I. The concentration of Fe(C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>)<sub>2</sub><sup>2+</sup> was determined spectrophotometrically, the concentration of H<sup>+</sup> was obtained from the measured pH, and the concentrations of iron(III) and pyrrolidone-5-hydroxamic acid were calculated from:

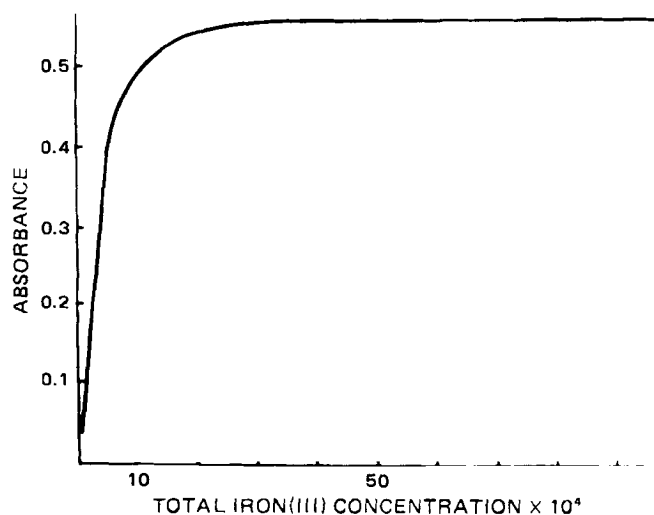
$$[\text{iron(III)}] = [\text{iron(III)}]_{\text{total}} - [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}] \quad (\text{Eq. 8})$$

and:

$$[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] = [\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]_{\text{total}} - [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}] \quad (\text{Eq. 9})$$

respectively. It was assumed that there is no appreciable amount of the C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sup>-</sup> anion below pH 3 due to the low acidity of pyrrolidone-5-hydroxamic acid [pK<sub>a</sub> = 8.65 (12)]. Both good constancy and reproducibility of K<sub>I</sub> values in the pH 1.90-2.95 region were observed, and K<sub>I</sub> = 33.4 (or log K<sub>I</sub> = 1.52) was calculated as the arithmetic mean of the six values. At the higher pH value of 2.95, there was a decrease in K<sub>I</sub> due to interfering reactions, such as base hydrolysis of iron(III) ions.

**Potentiometric Determination of Stability Constants of Mono-, Bis-, and Tris(pyrrolidone-5-hydroxamato)iron(III) Complexes**—The procedure for the determination of the stability constants of mono, bis, and tris iron(III) chelates was essentially the Schwarzenbach method



**Figure 1—Spectrophotometric titration of 5 × 10<sup>-4</sup> M pyrrolidone-5-hydroxamic acid by iron(III) solution at 500 nm, pH 2.0, and ionic strength 0.1 (sodium chloride), using 1-cm quartz cells.**

(16). According to Schemes II-IV, the total concentration of iron(III), Fe<sub>T</sub>, in solutions containing pyrrolidone-5-hydroxamic acid and iron(III) ions at low pH is:

$$\text{Fe}_T = [\text{Fe}^{3+}] + [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}] + [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+] + [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3] \quad (\text{Eq. 10})$$

Equation 10 can be rearranged to Eq. 11:

$$\text{Fe}_T/[\text{Fe}^{3+}] = \alpha = 1 + \beta_1'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] + \beta_{II}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^2 + \beta_{III}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^3 \quad (\text{Eq. 11})$$

where:

$$\beta_1' = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}]/([\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]) \quad (\text{Eq. 12a})$$

$$\beta_{II}' = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+]/([\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^2) \quad (\text{Eq. 12b})$$

$$\beta_{III}' = [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3]/([\text{Fe}^{3+}][\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^3) \quad (\text{Eq. 12c})$$

The function F<sub>1</sub> is defined by:

$$F_1 = (\alpha - 1)/[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] = \beta_1' + \beta_{II}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] + \beta_{III}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]^2 \quad (\text{Eq. 13})$$

Equation 14 can be similarly obtained for the total concentration of the pyrrolidone-5-hydroxamic acid moiety C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sub>T</sub>:

$$\text{C}_5\text{H}_7\text{O}_3\text{N}_2_T = [\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] + [\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)^{2+}] + 2[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+] + 3[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3] \quad (\text{Eq. 14})$$

The concentration of the free C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sup>-</sup> anion can be neglected because of low K<sub>a</sub> and pH values. Furthermore, a good starting approximation can be made, [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>] = C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sub>T</sub>, in Eq. 14 if there is a sufficient excess of C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sub>T</sub> over Fe<sub>T</sub> as in these experiments.

By plotting F<sub>1</sub> versus [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>], one obtains, according to Eq. 13, β<sub>1</sub>' as an intercept, since F<sub>1</sub> = β<sub>1</sub>' when [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>] = 0.

Another approximation, β<sub>III</sub>'[HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>]<sup>2</sup> = 0, can be made for very small values of [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>]. Under this condition, quadratic Eq. 13 is reduced to:

$$F_1 = \beta_1' + \beta_{II}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] \quad (\text{Eq. 15})$$

with the slope being equal to β<sub>II</sub>'.

Another rearrangement of Eq. 13 leads to:

$$F_2 = (F_1 - \beta_1')/[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] = \beta_{II}' + \beta_{III}'[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] \quad (\text{Eq. 16})$$

with β<sub>II</sub>' as an intercept and β<sub>III</sub>' as a slope.

Similarly, the third function, F<sub>3</sub>, can be obtained from:

$$F_3 = (F_2 - \beta_{II}')/[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] = \beta_{III}' = \text{constant} \quad (\text{Eq. 17})$$

In this procedure, one starts by plotting F<sub>1</sub> versus [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>], which gives diagrams such as those shown in Figs. 2 and 3. The F<sub>1</sub> in Eq. 13 is easily calculated from the known starting concentrations of Fe<sub>T</sub> and C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub><sub>T</sub> and the concentration of the free Fe<sup>3+</sup> ions determined from the redox potential. Depending on the experimental conditions, such as pH, three types of diagrams are possible (Figs. 2 and 3). Figure 2 shows that F<sub>1</sub> at pH 1.59 does not depend on [HC<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>], indicating the reduction of Eq. 13 to the form F<sub>1</sub> = β<sub>1</sub>' and, therefore, β<sub>II</sub>' = 0 and β<sub>III</sub>' = 0. It follows from Eqs. 12b and 12c that [Fe(C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>)<sub>2</sub><sup>2+</sup>] and [Fe(C<sub>5</sub>H<sub>7</sub>O<sub>3</sub>N<sub>2</sub>)<sub>3</sub>] are also zero under these conditions and that only the mono chelate is formed at pH 1.59.

Table II—Overall Stability Constants,  $\beta$

pH	$\beta_1^* \times 10^{-3}$	$\beta_1 \times 10^{-1}$	$\log \beta_1$	$\beta_{II}^*$	$\beta_{II} \times 10^{-1}$	$\log \beta_{II}$	$\beta_{III}^*$	$\beta_{III}$	$\log \beta_{III}$
1.59	1.3	3.34	1.52	—	—	—	—	—	—
1.83	2.3	3.40	1.53	$2.0 \times 10^5$	4.37	1.64	—	—	—
2.21	5.3	3.26	1.51	$9.1 \times 10^5$	3.46	1.54	—	—	—
3.00	25.0	2.50	1.40	$2.2 \times 10^7$	2.20	1.34	$1.0 \times 10^9$	1.00	0.00
3.23	50.0	2.94	1.47	$1.2 \times 10^9$	4.15	1.62	$2.05 \times 10^{10}$	4.18	0.62
Mean	—	3.09	—	—	3.55	—	—	2.59	—
Log	—	1.49	—	—	1.55	—	—	0.41	—

Linear dependence of  $F_1$  on  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$  is found at pH 1.83 and 2.21 (Fig. 2), indicating reduction of Eq. 13 to Eq. 15. Therefore, in this case,  $\beta_{III} = 0$  and, from Eq. 12c,  $[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3] = 0$ . This result is explained by the presence of both mono and bis, but not tris, chelates at pH 1.83 and 2.21. The  $\beta_1^*$  is always easily obtained from the intercept of  $F_1$ , and function  $F_2$  may be calculated from Eq. 16. Figure 2 illustrates no dependence of  $F_2$  on  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$ , because Eq. 16 is reduced to the form  $F_2 = \beta_{II}^*$ , indicating an absence of the tris chelate.

The formation of all three chelates requires the nonlinear dependence of  $F_1$  on  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$ ; furthermore, Eq. 13 must hold as is seen in Fig. 3 at pH 3.00. The nonlinear dependence is not pronounced in Fig. 3 at pH 3.23, probably due to the insufficiently high concentration of pyrrolidone-5-hydroxamic acid. In Fig. 3,  $\beta_1^*$  at pH 3.00 and 3.23 is obtained from the intercept of  $F_1$ , where  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2] = 0$ , thus enabling the plotting of  $F_2$  versus  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$  according to Eq. 16. The resulting straight line is used to obtain  $\beta_{II}^*$  from the intercept as well as to plot  $F_3$  versus  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$  to get  $\beta_{III}^*$  from the intercept at pH 3.00 and 3.23. Equation 17 requires no dependence of  $F_3$  on pyrrolidone-5-hydroxamic acid when all three chelates are present in solution (illustrated in Fig. 3 for pH 3.00). Deviation of  $F_3$  at pH 3.23 from the expected horizontal line is negligible and due to experimental error.

This procedure yields only approximate values of  $\beta_1^*$ ,  $\beta_{II}^*$ , and  $\beta_{III}^*$ . Therefore, only approximate values of  $[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^{2+}]$ ,  $[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_2^+]$ , and  $[\text{Fe}(\text{C}_5\text{H}_7\text{O}_3\text{N}_2)_3]$  can be obtained from Eqs. 12a, 12b, and 12c, respectively, and inserted in Eq. 14. However, Eq. 14 yields a more accurate value for  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$ , which is again introduced in Eqs. 13, 16, and 17, giving more accurate values of  $\beta_1^*$ ,  $\beta_{II}^*$ , and  $\beta_{III}^*$ . The procedure is to be repeated until the starting Eq. 10 is satisfied.

By following this procedure, the data for  $\beta_1^*$ ,  $\beta_{II}^*$ , and  $\beta_{III}^*$  were obtained (Tables II and III). Figures 2 and 3 represent the corresponding diagrams. The overall stability constants,  $\beta$ , are calculated from  $\beta^*$  by the simple relations  $\beta_1 = \beta_1^*[\text{H}^+]$ ,  $\beta_{II} = \beta_{II}^*[\text{H}^+]^2$ , and  $\beta_{III} = \beta_{III}^*[\text{H}^+]^3$ . All other constants such as  $K_1$ ,  $K_{II}$ ,  $K_{III}$ ,  $K_1$ ,  $K_2$ ,  $K_3$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are easily calculated from Eqs. 5a-5c, 6a-6c, and 7a-7c. They are listed in Table III to show the great influence of the hydrogen ion on various forms of the stability constants of chelates because of the rather high pKa value.

In this procedure, the best accuracy is expected in the determination of  $\beta_1^*$ ; less accuracy is expected for  $\beta_{II}^*$ , and the least accuracy is expected for  $\beta_{III}^*$ . The value of  $\beta_1^* = 1.3 \times 10^3$  obtained from Fig. 2 at pH 1.59 is substantiated by the excellent agreement between the calculated value of  $\text{Fe}_T = 2.066 \times 10^{-4} M$  from Eq. 10 as an average of six determinations and the used analytical concentration of  $\text{Fe}_T = 2.064 \times 10^{-4} M$ . The lower accuracy of  $\beta_{II}^*$  and  $\beta_{III}^*$  in the system at pH 3.23 in Fig. 3 results in less agreement between analytical and calculated values of  $\text{Fe}_T$ ,  $2.064 \times 10^{-4}$

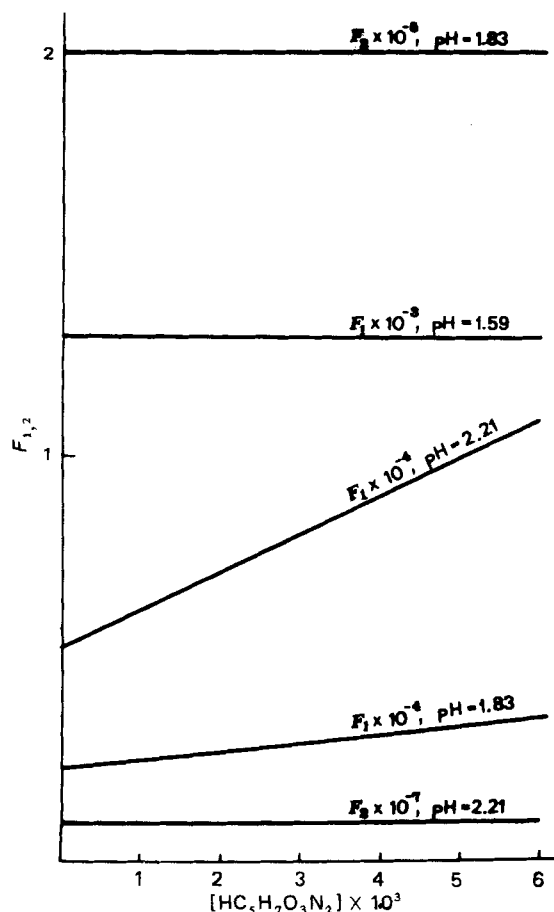


Figure 2—Dependence of  $F_1$  and  $F_2$  on  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$  at pH 1.59, 1.83, and 2.21. Intercepts of  $F_1$  at pH 1.59, 1.83, and 2.21 give  $\beta_1^*$  of  $1.3 \times 10^3$ ,  $0.23 \times 10^4$ , and  $0.53 \times 10^4$ , respectively. Intercepts of  $F_2$  at pH 1.83 and 2.21 give  $\beta_{II}^*$  of  $2.0 \times 10^5$  and  $0.091 \times 10^7$ , respectively. For details, see text.

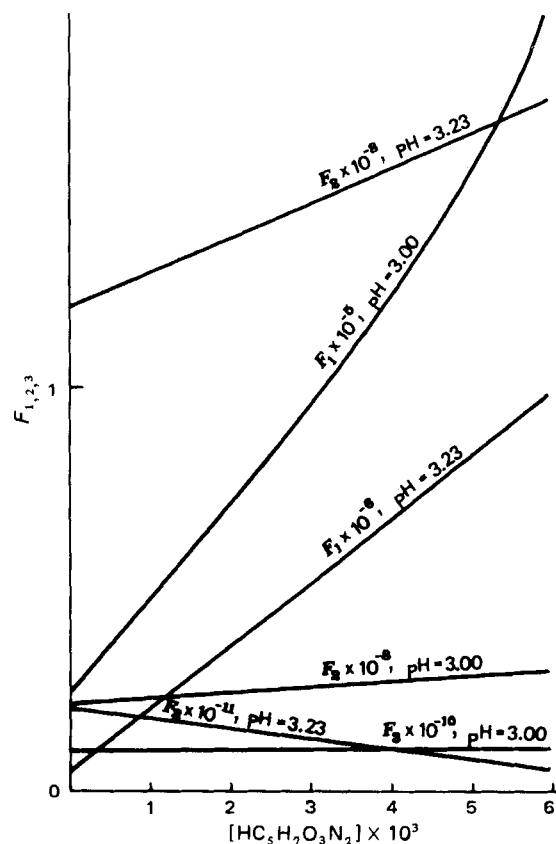


Figure 3—Dependence of  $F_1$ ,  $F_2$ , and  $F_3$  on  $[\text{HC}_5\text{H}_7\text{O}_3\text{N}_2]$  at pH 3.00 and 3.23. Intercepts of  $F_1$  at pH 3.00 and 3.23 give  $\beta_1^*$  of  $0.25 \times 10^5$  and  $0.05 \times 10^6$ , respectively. Intercepts of  $F_2$  at pH 3.00 and 3.23 give  $\beta_{II}^*$  of  $0.22 \times 10^8$  and  $1.2 \times 10^8$ , respectively. Intercepts of  $F_3$  at pH 3.00 and 3.23 give  $\beta_{III}^*$  of  $0.1 \times 10^{10}$  and  $0.205 \times 10^{11}$ , respectively. For details, see text.

**Table III—Stability Constants of (Pyrrolidone-5-hydroxamato)iron(III)<sup>a</sup>**

Index	K	log K	$\beta$	log $\beta$
I	$3.09 \times 10^1$	1.49	$3.09 \times 10^1$	1.49
II	$1.15 \times 10^0$	0.06	$3.55 \times 10^1$	1.55
III	$4.57 \times 10^{-2}$	-1.34	$1.62 \times 10^0$	0.41
1	$1.38 \times 10^{10}$	10.14	$1.38 \times 10^{10}$	10.14
2	$5.13 \times 10^8$	8.71	$7.08 \times 10^{18}$	18.85
3	$2.04 \times 10^7$	7.31	$1.44 \times 10^{26}$	26.16

<sup>a</sup>Chelates were calculated from Eqs. 5a-5c, 6a-6c, 7a-7c, and Table II and are expressed as  $K_I$ ,  $K_{II}$ ,  $K_{III}$ ,  $K_1$ ,  $K_2$ ,  $K_3$ ,  $\beta_I$ ,  $\beta_{II}$ ,  $\beta_{III}$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ .

M and  $2.168 \times 10^{-4}$  M, respectively. The agreement is significantly improved by rejecting one extreme value of  $Fe_T$ , which gives the calculated average value of  $2.075 \times 10^{-4}$  M. The precision decreases in the order  $\beta_I > \beta_{II} > \beta_{III}$  (Table II). The value of  $\log \beta_I (= \log K_I) = 1.49$  is in excellent agreement with the spectrophotometrically obtained value of  $\log \beta_I = 1.52$ .

Overall stability constants of related iron(III) hydroxamates are listed in Table IV. The decrease in stability when going from benzohydroxamic to pyrrolidone-5-hydroxamic acid chelates of iron(III) is larger than may be expected from the increase in acidity in the same direction. Some other factors must be involved such as the interference by chelating iron(III) *via* carbonyl and imine groups of the pyrrolidone ring.

The stability of (pyrrolidone-5-hydroxamato)iron(III) chelates is important for the behavior of these complexes in living systems. There is an indication that hydroxamic acid-containing compounds are constituents of siderophilin, the iron-binding  $\beta$ -pseudoglobulin of human plasma (17), and of ferritin (18). It is obvious that the higher the stability of hydroxamic acid-iron(III) chelates, the easier is iron transport and the better is iron resorption at various biological pH's. Iron-containing hydroxamic acids have been patented as metalotherapeutics (5). (Pyrrolidone-5-hydroxamato)iron(III) chelates may be considered as possible

**Table IV—Comparison of Stabilities of Iron(III) Hydroxamines**

Acid	pKa	log $\beta_3$
Acetohydroxamic	9.37 <sup>a</sup>	28.30 <sup>a</sup>
Benzohydroxamic	8.79 <sup>a</sup>	27.83 <sup>a</sup>
Pyrrolidone-5-hydroxamic	8.65 <sup>b</sup>	26.36 <sup>c</sup>

<sup>a</sup> From Ref. 16. <sup>b</sup> From Ref. 12. <sup>c</sup> This work.

metalotherapeutics in view of their stability and the presence of the pyrrolidone ring, which, like povidone, is used as a substitute for blood plasma (7) and also shows a mitostimulating effect (19).

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## ACKNOWLEDGMENTS AND ADDRESSES

Received July 9, 1975, from the Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Yugoslavia.

Accepted for publication April 23, 1976.

Supported by the Republic Foundation for Scientific Work of the Croatia.

The authors are grateful to the Alexander von Humboldt Foundation for the Opton PMQ II spectrophotometer.

M. Pečar was the recipient of a fellowship from the Foundation at the Max Planck Institute for Medical Research, Heidelberg.

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# Methods for Evaluating Hermetic Closures for Screw-Capped Bottles

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**Abstract** □ With the described method, a large number of sealed bottles can be tested to determine their effectiveness, integrity, and reliability in preserving an inert atmosphere. The method consists of placing milligram quantities of dry ice into the bottles, capping them, and periodically weighing them on an analytical balance; leaky bottles are detected by a loss in weight. This method can be applied to test a large number of bottles with a minimum of effort and manpower and without complicated instrumentation. The data presented are highly reproducible and cor-

relate well with data from other methods and with the physical defects of the bottles.

**Keyphrases** □ Seals, hermetic—testing method developed for large numbers of screw-capped bottles □ Bottles, screw capped—method of testing hermetic seals developed for large numbers □ Technology, pharmaceutical—method of testing hermetic seals of large numbers of screw-capped bottles developed

By definition, hermetic containers are impervious to air and any other gas under ordinary conditions of handling. For the primary container of a drug to qualify as hermetic,

it must be leakproof; *i.e.*, no gases or vapors must be capable of entering it or escaping from it.

A product is hermetically sealed to protect it from con-