[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Iron–Cysteinate Complexes

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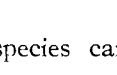
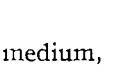
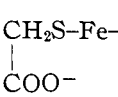
In solutions of a *p*H between 10 and 11.2, two complexes composed of iron(II) and cysteine are found: $Fe(OH)(RS)^-$ and $Fe(RS)_2^=$, in which RS denotes $-OOC\cdot CH(NH_2)\cdot CH_2S-$. The dissociation constants, $K_{Fe(OH)(RS)^-} = (a_{Fe^{++}} \times a_{OH^-} \times a_{RS^=})/a_{FeOHRS^-}$ and $K_{Fe(RS)_2} = a_{Fe^{++}} \times a^2_{RS^=}/a_{Fe(RS)_2^=}$ were estimated from solubility data to be equal to 1.7×10^{-13} and 1.7×10^{-12}, respectively, at 25°. In weakly acid medium (*p*H 5-7) a slightly soluble compound, FeRS, is formed with a solubility product, $S_{FeRS} (= a_{Fe^{++}} \times a_{RS^=})$, of 2.6×10^{-11} at 25°. Ferric iron catalyzes the air-oxidation of cysteine to cystine. From solubility data of ferric hydroxide in ammoniacal cysteinate solutions and from spectrophotometric measurements in ammoniacal Versenate–cysteinate solutions, both extrapolated to zero time when no reduction of ferric cysteinate has occurred, it was postulated that two complexes, $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$, exist at *p*H 10 to 11, with the dissociation constants $K_{FeOH(RS)_2^{2-}} (= (a_{Fe^{+++}} \times a_{OH^-} \times a^2_{RR^=})/a_{FeOH(RS)_2^{2-}})$ of 5×10^{-34}, and $K_{Fe(RS)_3^{3-}} (= a_{Fe^{+++}} \times a^3_{RS^=}/a_{Fe(RS)_3^{3-}})$ of 8×10^{-33}, at 25°.

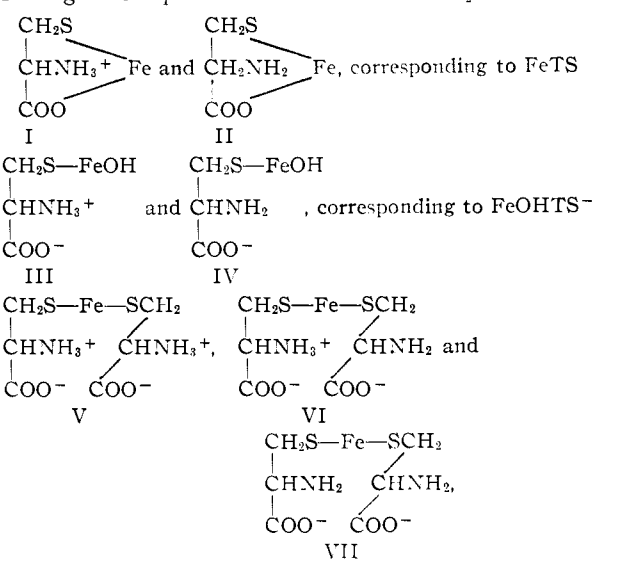
Ferric iron when added to an acid solution of cysteine hydrochloride produces a deep indigo blue color which disappears in a short time and cannot be restored by oxygen.[2,3] Harris[4] states that the violet coloration obtained when ammonia is added to a cysteine solution is due to the presence of traces of metal ions (generally ferric iron). He also mentions that ferrous iron yields no colored complex with cysteine. On the other hand, Schubert[5] reports that ferrous iron forms two kinds of compounds with cysteine, one of which is formed in acid medium (*p*H 4–6) and has the formula $Fe(OOCCHNH_2CH_2S)\cdot 1.5H_2O$. Upon mixing two moles of cysteine hydrochloride, one mole of ferrous salt and six moles of potassium hydroxide, he observed an orange color indicating the formation of a soluble ferrous cysteinate complex.[6] This complex may have the formula $Fe(SCH_2NH_2CHCOO)_2^=$, corresponding to the formula of ferrous complex with thioglycolic acid formed in alkaline medium.[5,7]

In alkaline medium, the ferrous complex is easily oxidized to a ferric complex by molecular oxygen.[3] According to Schubert[5] a violet color which develops when a trace of ferric iron is added to an oxygen-containing solution of cysteine of *p*H 8 to 9 fades and disappears when the dissolved oxygen is used up. The cysteine is oxidized to cystine. When cysteine is left in the solution, the color is regenerated by shaking with air. In order to explain the catalytic oxidation of cysteine Schubert postulated that the violet ferric complex which is formed on oxidation of the ferrous complex is reduced to the ferrous complex with the formation of the disulfide and ferrous hydroxide.

$$Fe(RS)_2 \xrightarrow{\text{Oxidation}} \{FeOH(RS)_2\}_2 \longrightarrow Fe(RS)_2 + RSSR + Fe(OH)_2$$

In the present paper we report the results of a quantitative study of the composition and the stability of complexes between cysteine with ferrous and ferric iron. The formulas used refer to composition and do not reveal structural information.

Ferrous–Cysteinate Complexes.—In the ferrous iron–thioglycolic acid system, the following three species can exist[7]:  (FeTS) in acid medium, and  (FeOHTS$^-$) and  ($Fe(TS)_2^=$) in alkaline medium. Considering the dipolar nature of cysteine the following seven species with ferrous iron may exist



corresponding to $Fe(TS)_2^=$. The species I, II, III, IV, V, VI and VII will be denoted in this paper as $FeRS^+$, FeRS, $FeOHRS^\pm$, $FeOHRS^-$, $Fe(RS)_2^{\pm\pm}$, $Fe(RS)^{+=}$ and $Fe(RS)^-$, respectively. For the formation of one mole of the above species from uncharged cysteine and ferrous ion the following number of moles of sodium hydroxide is required: for (I) 1 mole, for (II), (III) and (V) 2 moles, for (IV) and (VI) 3 moles and for (VII) 4 moles. Evidently, the system is very complex. Experimental conditions were selected which allowed reasonable conclusions regarding the kind of complexes present in the solutions.

Experimental

Cysteine hydrochloride (C.P. grade by Pfanstiel Chemical Co.) was used. Stock solutions of 0.5 *M* ferrous chloride and of sodium hydroxide were prepared in the same way as described in previous papers.[7,8]

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(2) E. Baumann, *Z. physiol. Chem.*, **8**, 279 (1883–1884).
(3) (a) A. P. Mathews and S. Walkers, *J. Biol. Chem.*, **6**, 21 (1906); (b) L. Michaelis and E. S. G. Barron, *ibid.*, **83**, 191 (1928).
(4) L. J. Harris, *Biochem. J.*, **16**, 739 (1922).
(5) M. Schubert, THIS JOURNAL, **54**, 4077 (1932).
(6) M. Schubert, *ibid.*, **55**, 4563 (1933).
(7) D. L. Leussing and I. M. Kolthoff, *ibid.*, **75**, 3904 (1953).

TABLE I

ANALYSES OF MIXTURES CONTAINING FERROUS CHLORIDE, CYSTEINE AND SODIUM HYDROXIDE. SOLUBILITY PRODUCT OF FERROUS CYSTEINATE (FeRS)

Volume of mixture 100 ml. if not stated otherwise.

Initial concn. in mixture Fe, mmoles	Cysteine, mmoles	Supernatant soln. pH	Fe, $M \times 10^3$	Cysteine, $M \times 10^3$	Precipitate Fe, mmoles	Cysteine, mmoles	Solubility product of ferrous cysteinate (FeRS)
10.04	5.11	5.83	79.3	29.4	2.11	2.17	2.4×10^{-11}
5.02	10.00	5.84	28.0	77.2	2.22	2.28	2.6×10^{-11}
5.00[b]	4.99	6.06	25.7	24.9	2.18	2.25	2.4×10^{-11}
5.00[b]	9.99	6.45	2.94	49.0	4.67	4.60	3.1×10^{-11}
5.00	5.00	7.40	2.20	1.6	4.78	4.84	$(4.8 \times 10^{-11}$[d])
7.53	5.03	8.02	45.3	34.6	3.00	1.57	
5.02	5.04	8.90	47.8	(51.1)[a]	0.24	(−0.07)[a]	
5.00[c]	10.03	9.80		No precipitate			
5.02	5.00	10.05	27.1	50.0	2.31	.....	
5.00	4.94	10.07	26.9	(49.2)[a]	2.31	(0.02)[a]	
5.00	7.46	10.18	42.3	74.6	0.77	.....	
5.02	7.53	10.38	41.6	75.3	0.86	.....	
5.02	5.00	10.69	23.0	50.0	2.72	.....	
5.02	5.02	11.13	21.3	50.2	2.89	.....	
5.02[b]	7.55	11.15	30.4	(69 5)[a]	1.68	(−0.10)[a]	
5.02	4.95	11.20	20.1	49.5	3.01	.....	

[a] Figures in brackets have not been made use of in numerical calculations. [b] Volume of mixture 110 ml. [c] Volume of mixture 98 ml. [d] Corrected for the formation of $FeOHRS^-$ and $Fe(RS)_2^=$.

The ferrous iron–cysteine system was studied in air-free media in which the amounts of iron, cysteine and sodium hydroxide were varied. For the preparation of each mixture, the same procedure was used as in the studies of ferrous–thioglycolate complexes.[7] All bottles were rotated for 5 days or longer and centrifuged when a precipitate had formed. Samples of the supernatant liquid were withdrawn by means of a syringe for the determination of *p*H, iron and cysteine.

The determination of *p*H was carried out in a nitrogen atmosphere using the Beckman Model H 2 *p*H meter. A glass electrode 1190-80 ("for general purpose") was used. The total iron analysis of the supernatant liquid was carried out by the same procedure as described previously.[7] Total cysteine was determined polarographically by measuring the anodic diffusion current at −0.48 v. *vs.* S.C.E. in oxygen-free 0.1 *M* sodium hydroxide solution.[9] In blank experiments with sodium hydroxide solutions, 0.001 *M* in cysteine and 0.0005 to 0.002 *M* in ferrous iron, it was established that the ferrous iron does not interfere with the polarographic determination.

A summary of the details and the results of the experiments is given in Table I. In the lower *p*H region a slightly colored (brownish-yellow) solution was obtained. Upon standing a brownish precipitate appeared within 24 hours, while the solution became almost colorless. From the difference in the iron and cysteine contents present initially and from those found in the supernatant solution the ratio of ferrous iron and cysteine in the precipitate is calculated to 1:1. This compound can therefore be presented by the formulas FeRS as reported by Schubert.[5]

At a *p*H of about 8 a brownish-yellow colored solution was obtained. On standing a greenish-white precipitate separated, which appeared to be a mixture of ferrous cysteinate (FeRS) and ferrous hydroxide. The brownish color in the equilibrium solution indicates the presence of a ferrous–cysteinate complex.

In the higher *p*H region (9–11.2) complexes were clearly present in the solutions. The iron concentration in the equilibrium solutions was much greater than that calculated from the solubility product of ferrous hydroxide.[8] The precipitate was found to consist only of ferrous hydroxide. The color was white at first, then gradually turned to greenish-white.

Calculation and Discussion

Almost all equilibrium mixtures of *p*H 9 or greater contained a precipitate of ferrous hydroxide and therefore the ferrous ion activity in these solutions could be calculated. Although five different iron–cysteine complexes might exist in this *p*H range stoichiometric considerations indicate that the species $FeOHRS^-$ and $Fe(RS)_2^=$ prevail. Combinations of other possible complexes were considered and sets of equations were developed. None of them satisfied the experimental results.

The following equations can be set up.

$$[Fe]_t = [Fe^{++}] + [FeOH^+] + [FeOHRS^-] + [Fe(RS)_2^=] \quad (1)$$

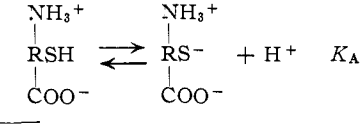
$$[RSH]_t = [NH_3{}^+RSHCOO^-] + [NH_2RSHCOO^-] + [NH_3{}^+RS^-COO^-] + [NH_2RS^-COO^-] + [FeOHRS^-] + 2[Fe(RS)_2^=] \quad (2)$$

$$[OH]_t = [OH^-] + [FeOH^+] + [NH_2RSHCOO^-] + [NH_3{}^+RS^-COO^-] + 2[NH_2RS^-COO^-] + 3[FeOHRS^-] + 4[Fe(RS)_2^=] \quad (3)$$

where the quantities in brackets on the right-hand side are the equilibrium concentrations while $[Fe]_t$, $[RSH]_t$ and $[OH]_t$ are the total concentrations of iron, cysteine and sodium hydroxide in the supernatant liquid. $[Fe]_t$ and $[RSH]_t$ were determined analytically and $[OH]_t$ was obtained by the equation:

$$[OH]_t = \frac{(\text{mmole of NaOH added}) - 2 \times (\text{mmole of ferrous hydroxide})}{\text{ml. of total vol. of supernatant liq.}} \text{ (mole/l.)}$$

At *p*H higher than 10 $[Fe^{++}]$, $[FeOH^+]$ and $[RSH^{\pm}]$ are negligibly small. Introducing the dissociation constants of the various charge species of cysteine, K_A, K_B, K_C and K_D (*cf.* ref. 10)



(8) D. L. Leussing and I. M. Kolthoff, THIS JOURNAL, **75**, 2476 (1953).

(9) I. M. Kolthoff and C. Barnum, *ibid.*, **63**, 3061 (1940).

(10) W. Stricks and I. M. Kolthoff, *ibid.*, **73**, 4569 (1951).

$$\begin{array}{lll} \mathrm{NH_3^+} & \mathrm{NH_2} & \\ \mathrm{RSH} & \rightleftharpoons \mathrm{RSH} + \mathrm{H^+} & K_B \\ \mathrm{COO^-} & \mathrm{COO^-} & \\ \mathrm{NH_3^+} & \mathrm{NH_2} & \\ \mathrm{RS^-} & \rightleftharpoons \mathrm{RS^-} + \mathrm{H^+} & K_C \\ \mathrm{COO^-} & \mathrm{COO^-} & \\ \mathrm{NH_2} & \mathrm{NH_2} & \\ \mathrm{RSH} & \rightleftharpoons \mathrm{RS^-} + \mathrm{H^+} & K_D \\ \mathrm{COO^-} & \mathrm{COO^-} & \end{array}$$

and using the simplified symbols $RSH^{\pm}$, $RS^{=}$, RSH^{-} and $RS^{\equiv}$ for the various charge species of cysteine, rearrangement of equations 1, 2 and 3 yields

$$[RS^{=}] = [OH]_t - 2[Fe]_t - [RSH]_t - [OH^-] \quad (4)$$

$$[FeOHRS^-] = 2[Fe]_t - [RSH]_t + \left\{1 + \frac{a_{H^+}\gamma_{RS^=}}{K_C\gamma_{RS^\pm}} + \frac{a_{H^+}\gamma_{RS^=}}{K_D\gamma_{RSH^-}}\right\} \times \{[OH]_t - 2[Fe]_t - [RSH]_t - [OH^-]\} \quad (5)$$

$$[Fe(RS)_2^=] = [RSH]_t - [Fe]_t - \left\{1 + \frac{a_{H^+}\gamma_{RS^=}}{K_C\gamma_{RS^\pm}} + \frac{a_{H^+}\gamma_{RS^=}}{K_D\gamma_{RSH^-}}\right\} \times \{[OH]_t - 2[Fe]_t - [RSH]_t - [OH^-]\} \quad (6)$$

The dissociation constants of the two ferrous cysteinate complexes are

$$FeOHRS^- \rightleftharpoons Fe^{++} + OH^- + RS^=; \quad K_{FeOHRS^-} = \frac{a_{Fe^{++}} \times a_{OH^-} \times a_{RS^=}}{a_{FeOHRS^-}} \quad (7)$$

$$Fe(RS)_2^= \rightleftharpoons Fe^{++} + 2RS^=; \quad K_{Fe(RS)_2^=} = \frac{a_{Fe^{++}} \times a^2_{RS^=}}{a_{Fe(RS)_2^=}} \quad (8)$$

Since the solutions are in equilibrium with solid ferrous hydroxide equations 7 and 8 can be written

$$K_{FeOHRS^-} = \frac{S_{Fe(OH)_2} \times a_{RS^=} \times a_{H^+}}{K_w \times a_{FeOHRS^-}}$$

and

$$K_{Fe(RS)_2^=} = \frac{S_{Fe(OH)_2} \times a^2_{RS^=} \times a^2_{H^+}}{K_w^2 \times a_{Fe(RS)_2^=}} \quad (8')$$

where $S_{Fe(OH)_2}$ is the solubility product of ferrous hydroxide.

In calculating the constants, values of activity coefficients for mono- and divalent ions at an ionic strength less than 0.1 were estimated from Kielland's tables.[11] At ionic strengths greater than 0.1 mean activity coefficients were used from other data.[12,13]

Concentrations of various cysteinate anions were calculated from the constants[10] at 25°, using values of pK_A, pK_B, pK_C and pK_D of 8.66, 8.60, 10.45 and 10.51, respectively. A value of 3.5×10^{-16} was taken for the solubility product of "green" ferrous hydroxide.[7,8]

The expressions for $[RS^=]$, $[FeOHRS^-]$ and $[Fe(RS)_2^=]$ in eq. 4, 5 and 6 involve the term, $\{[OH]_t - 2[Fe]_t - [RSH]_t - [OH^-]\}$. These equations cannot be applied when this term yields a negative value or a value close to zero. The results of the calculations are given in Table II.

(11) J. Kielland, THIS JOURNAL, **59**, 1675 (1937).

(12) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publ. Corp., New York, N. Y., 1950.

(13) As for the procedure of evaluation: *cf.* H. Borsook, E. L. Ellis and H. M. Huffman, *J. Biol. Chem.*, **117**, 281 (1937).

TABLE II

CALCULATED CONCENTRATIONS OF SPECIES AND DISSOCIATION CONSTANTS OF THE FERROUS-CYSTEINE COMPLEXES PRESENT IN ALKALINE SOLUTIONS (pH 10.69 TO 11.20)

$[RS^-]$, $M \times 10^3$	$[Fe\text{-}OHRS^-]$, $M \times 10^3$	$[Fe\text{-}(RS)_2^=]$, $M \times 10^3$	K_{FeOHRS^-}	$K_{Fe(RS)_2^=}$
8.9	9.5	13.5	2.8×10^{-13}	2.3×10^{-12}
17.3	12.9	8.4	1.4×10^{-13}	1.9×10^{-12}
19.4	15.2	15.2	1.3×10^{-13}	1.1×10^{-12}
18.4	13.1	8.0	1.4×10^{-13}	1.6×10^{-12}

av. values of the constants are:

$K_{FeOHRS^-} = 1.7 \times 10^{-13}$ (4.2×10^{-13})

$K_{Fe(RS)_2^=} = 1.7 \times 10^{-12}$ (1.1×10^{-11})

The values between parentheses are the corresponding dissociation constants of ferrous–thioglycolate complexes which have been recalculated from data given by Leussing and Kolthoff.[7] The stabilities of ferrous–cysteinate and of ferrous–thioglycolate complexes are of the same order of magnitude, indicating that the uncharged amino group has little effect on the stability of the iron cysteine complex.

In calculation of dissociation constants the results of experiments with pH 8.9 to 10.38 were not used. However, if the complexes $FeOHRS^-$ and $Fe(RS)_2^=$ are the predominant species in these systems, the total iron concentration calculated from the above dissociation constants (obtained from experiments with pH 10.69 to 11.2) must agree with the total analytical iron concentration. In a test of this relation equation 2 was used. Considering equations 7′ and 8′, equation 2 can be rearranged as

$$[RS^=]^2 + \frac{\left(\frac{a_{H^+}}{K_C}\frac{\gamma_2}{\gamma_1} + \frac{a_{H^+}}{K_D}\frac{\gamma_2}{\gamma_1} + 1 + \alpha\right)}{2\beta}[RS^=] - \frac{[RSH]_t}{2\beta} = 0 \quad (9)$$

where γ_1 and γ_2 represent the activity coefficients of mono- and di-valent ions, respectively, and

$$\alpha = \frac{\gamma_{RS^=} \times a_{H^+} \times S_{Fe(OH)_2}}{\gamma_{FeOHRS^-} \times K_w \times K_{FeOHRS^-}}$$

$$\beta = \frac{\gamma^2_{RS^=} \times a^2_{H^+} \times S_{Fe(OH)_2}}{\gamma_{Fe(RS^-)_2} \times K^2_w \times K_{Fe(RS)_2^=}}$$

Solving the equations, the total iron concentration $[Fe]_t$ can be obtained as

$$[Fe]_t = [FeOHRS^-] + [Fe(RS)_2^=] = \alpha[RS^=] + \beta[RS^=]^2 \quad (10)$$

Total iron concentrations calculated from four experiments are given in Table III, and compared with the experimental values.

TABLE III

OBSERVED AND CALCULATED VALUES OF TOTAL IRON CONCENTRATION IN SUPERNATANT LIQUID

	Observed		Calculated		
pH	$[RSH]_t \times 10^3$, M	$[Fe]_t \times 10^3$, M	$[Fe\text{-}OHRS^-] \times 10^3$, M	$[Fe\text{-}(RS)^=_2] \times 10^3$, M	$[Fe]_t \times 10^3$, M
10.05	50.0	27.1	14.0	14.8	28.8
10.07	49.5	26.9	13.7	14.2	27.9
10.18	74.6	42.3	18.1	24.2	42.3
10.38	75.3	41.6	17.8	23.6	41.4

The calculated values of total iron concentrations are in good agreement with the observed ones, indicating that in the *p*H region from 10.0 to 10.4 two species, $FeOHRS^-$ and $Fe(RS)_2^=$, are predominant.

However, when the same procedure was applied to expts. with *p*H 8.02 and 8.90, the calculated values of $[Fe]_t$ and $[OH]_t$ were greatly different from the experimental ones, indicating that under the particular experimental conditions another complex must be present in addition to $FeOHRS^-$ and $Fe(RS)_2^=$.

In the lower *p*H range (5.8 to 7.4) a precipitate is present composed of FeRS with a solubility product

$$S_{FeRS(s)} = a_{Fe^{++}} \times a_{RS^=}$$

The last column of Table I gives the values of the solubility product calculated from experimental data, assuming the activity coefficient of $RSH^{\pm}$ to be unity. A set of involved equations was derived which allowed by a series of approximations the calculation of concentrations of $FeOHRS^-$ and $Fe(RS)_2^=$. These concentrations increase with increasing *p*H but are negligibly small in the calculation of the solubility product up to a *p*H of 7.4. Even at this *p*H the value of the constant corrected for these concentrations hardly differs from the uncorrected value (4.8×10^{-11} as compared to 5.4×10^{-11}). From the data of experiments with *p*H 5.83 to 6.45 an average value of $S_{FeRS(s)}$ of 2.6×10^{-11} is calculated. From the data of Leussing and Kolthoff[7] a solubility product of ferrous thioglycolate of 1.6×10^{-10} is calculated.

At the intermediate *p*H of 8.02 (see Table I) the solution is in equilibrium with the two solids, ferrous hydroxide and ferrous cysteinate. From the following equilibria

$$Fe(OH)_2 \rightleftharpoons Fe^{++} + 2OH^-$$

$$FeRS \rightleftharpoons Fe^{++} + RS^=$$

it is seen that $a_{OH^-}^2/a_{RS^=}$ must be constant. From the values of $S_{FeRS(s)}$ and of $S_{Fe(OH)_2(s)}$ for "green" ferrous hydroxide,[8] $a_{OH^-}^2/a_{RS^=}$ is calculated to be 1.35×10^{-5}.[14]

Ferric–Cysteinate Complexes. Rate of Reduction of the Ferric–Cysteinate Complex.—Ferric–cysteinate is formed upon addition of ferric iron to either acid or alkaline cysteinate solutions, the complex being reduced to the ferrous complex with formation of cystine. The rate of reduction of the ferric–cysteinate complex was investigated spectrophotometrically by following the rate of disappearance of the violet color of the complex. A Beckman Spectrophotometer Model DU was used with absorption cells designed to keep the solution free from oxygen during the measurement.[7] Length of the light path was calibrated for each cell.

The absorption curve cannot be measured accurately because the violet color of the ferric–cysteinate complex disappears rapidly in air-free solutions. In air-saturated solutions the absorption maximum was found at approximately 580 mμ. All measurements were performed at that wave length.

(14) When ferrous hydroxide alone is in equilibrium with the solution $a_{OH^-}^2/a_{RS^=}$ must be greater than 1.35×10^{-5}, while, when ferrous cysteinate (FeRS) alone is in equilibrium with the solution, the ratio must be smaller than 1.35×10^{-5}.

Measurements were made in ammoniacal buffers of various *p*H and in the presence of a large excess of cysteine. Suitable amounts of ammonium hydroxide, ammonium nitrate and cysteine hydrochloride solutions were placed in the absorption cell and oxygen was removed by bubbling for 40 to 60 minutes with nitrogen which previously had been washed with a buffer of the same concentration of ammonia and ammonium nitrate as used in the experiment. The cell was immersed in a thermostat of 25° and an air-free ferric ammonium sulfate solution was injected with the aid of a syringe. The absorption of the solution was measured as a function of time. A cell filled with distilled water was used as reference. The *p*H of the solutions was calculated from the activities of ammonia and ammonium ion and determined experimentally with a Beckman Model H 2 *p*H meter after the spectrophotometric measurement.

Reciprocals of optical density plotted against time yielded straight lines as shown in Fig. 1, which indicates that the disappearance of ferric cysteinate is a second-order reaction. Under identical conditions solutions of different concentrations in ferric ion gave approximately the same rate of disappearance. The rate at a given concentration of cysteine became smaller with increasing *p*H and at constant *p*H with increasing concentration of cysteine. The experimental data and the results of calculations are summarized in Table IV. It is interesting to note that the product of the rate constant and the concentration of uncombined $NH_2RS^-COO^-$ (last column of Table IV) is practically constant. This indicates that the rate determining step in the oxidation of cysteine by ferric iron is not the direct

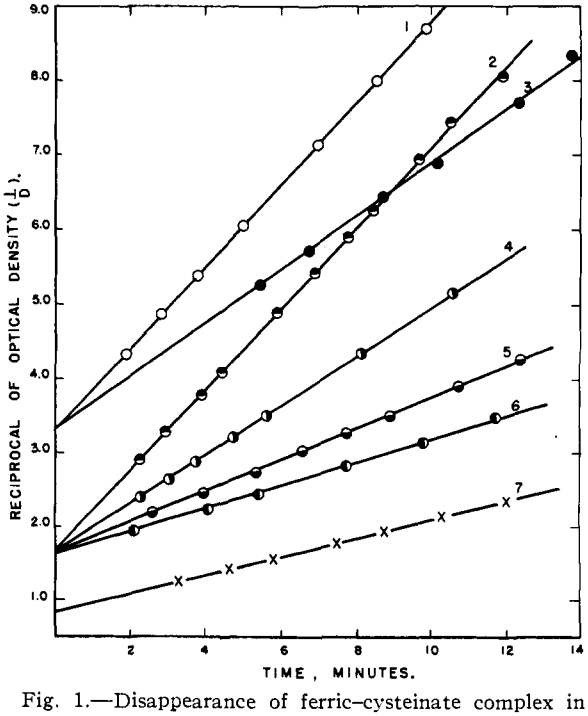


Fig. 1.—Disappearance of ferric–cysteinate complex in air-free ammoniacal buffers in the presence of a large excess of cysteine. (See Table IV for experimental conditions; the numbers in the figure correspond to the sequence of the experiments listed in Table IV.)

TABLE IV

SPECTROPHOTOMETRIC MEASUREMENT OF RATE OF DISAPPEARANCE OF FERRIC-CYSTEINATE IN AIR-FREE AMMONIA BUFFERS

($[NH_3]$ = 1.01 to 0.91 M, $[NH_4^+]$ = 0.22 to 0.12 M in final solution).

Initial concn. RSH·HCl, M	Initial concn. $Fe(NH_4)(SO_4)_2$[a] $M \times 10^4$	μ	pH	Optical density at $t = 0$	Molar ext. coeff. at $t = 0$, mole^{-1} cm.$^{-1}$	Rate of decrease in concn. of ferric–cysteinate complex: k, mole^{-1} sec.$^{-1}$	$[RS^-]_{uncomb.}$ $M \times 10^3$	$k \times [RS^-]_{uncomb.}$
0.0100	1.00	0.13	10.33	0.305	3.05×10^3	28.0	4.26	0.12
.0100	2.00	.13	10.35	.600	3.00×10^3	27.5	4.18	.12
.0200	1.00	.156	10.21	.305	3.05×10^3	18.4	7.95	.15
.0200	2.00	.156	10.22	.606	3.03×10^3	16.8	7.88	.13
.0500	2.00	.233	10.03	.617	3.08×10^3	10.9	16.3	.18
.0500	2.00	.143	10.31	.617	3.08×10^3	7.94	22.3	.18
.0500	4.00	.143	10.30	1.220	3.05×10^3	6.46	22.1	.14

[a] Concentration of $Fe(NH_4)(SO_4)_2$ added corresponds to the concentration of ferric–cysteinate at $t = 0$ ($[Fe(III)\ complex]_0$).

interaction between ferric ion and the amino acid. The molar extinction coefficient of the ferric–cysteinate complex was found by extrapolation to zero time and estimated to be 3.05×10^3 mole^{-1} cm.$^{-1}$ at 580 mμ (see Table IV).

Solubility of Ferric Iron in Ammoniacal Cysteinate Solutions.—When ferric iron is added in excess to an ammoniacal cysteinate solution a brown precipitate is obtained. Although unlikely, the formation of an insoluble ferric cysteinate complex cannot be excluded.[15] Under our experimental conditions the precipitate appeared to be composed of hydrous ferric oxide which was free of cysteine. The anodic diffusion current of air-free 10^{-3} M cysteine solutions was measured in ammonia buffers before and after addition of enough air-free ferric ammonium sulfate solutions to give a precipitate. Extrapolated to zero time the diffusion current was not affected by the addition of iron. The anodic current decreased on standing while a cathodic current of cystine appeared, the decrease of the cysteine current being almost equal to the diffusion current of the cystine formed.

The solubility of ferric hydroxide was determined in air-saturated and air-free buffers containing a relatively large concentration of cysteine. In air-saturated solutions the iron remained in the form of ferric because of the rapid reoxidation of the ferrous complexes by oxygen. In air-free solutions, the ferrous complexes formed were not reoxidized. The rate of reduction of the ferric complex is so great that solubility can be found only by extrapolation of the ferric concentration to zero time in both series of experiments.

The procedure was to add a slight excess of a ferric ammonium sulfate solution (so that the solubility of $Fe(OH)_3$ would be exceeded) to a solution of a known concentration of cysteine in a known volume of an ammonia–ammonium nitrate buffer. The solution was then analyzed for iron as a function of time by suitable means.

Air-saturated Solutions.—As the cysteine is oxdized, the solubility of iron decreases and more ferric hydroxide precipitates.

Cysteine hydrochloride was weighed into dry bottles of 250-ml. or 500-ml. capacity. Known volumes of water and of standard solutions of ammonia and ammonium nitrate were then placed into the bottles. Air, previously washed with a buffer of the same concentration of ammonia and ammonium nitrate as used in the experiment, was bubbled vigorously through the solution and finally the desired volume of standardized 0.1 M ferric ammonium sulfate in 0.05 M sulfuric acid was added rapidly. This is taken as zero time. Samples of the solutions were removed at various periods of time and filtered through glass wool contained in a syringe of 20- or 50-ml. capacity.[7] Known volumes of filtrate were analyzed volumetrically for iron.[7] At pH less than 10.6 the iron concentration *versus* time plot gave a straight line yielding the iron concentration at zero time.

Experimental data and values of the calculated constants are listed in Table V. From Fig. 2 (air-saturated solutions) it is seen that the iron concentration decreased linearly with time in solutions of pH 10 to 10.5. This is at variance with results obtained in the thioglycolic acid–ferric iron system[7] which gave straight line plots of the reciprocal of the iron concentration *versus* time in the pH range 9–10. The lines (Fig. 2) presenting experiments 1 to 3 (at various initial cysteine concentrations) are parallel, thus indicating that the rate of disappearance of iron in air-saturated solutions is independent of the cysteine concentration.

Air-free Solutions.—Difficulties were encountered in analyzing the solutions. The spectrophotometric method cannot be used in the presence of a precipitate of ferric hydroxide. The polarographic method, applied in an analogous study of thioglycolate,[7] gave results which were not easily interpreted when dealing with the cysteine system. The reason is that the ferrous iron formed by reduction of the ferric complex catalyzes the polarographic reduction of cystine at a potential where the ferric complex yields a diffusion current while cystine alone (absence of Fe(II)) is not reduced. This peculiar catalytic polarographic effect will be discussed in a subsequent paper.

It was decided to extrapolate the total diffusion current measured at -1.35 v. (at lower pH) or -1.40 v. (at higher pH) to a reaction time of zero. The measured diffusion current is the sum of that of the ferric–cysteinate complex and that of the cystine formed on autodecomposition of the complex. The value extrapolated to zero time should be that of the ferric complex. In separate experiments with a large excess of cysteine and in the absence of a precipitate of ferric hydroxide it was found by extrapolation to zero time that the diffusion current of ferric cysteinate is 5% smaller

(15) J. V. Dubsky and V. Sindelar, *Mikrochim. Acta*, **3**, 258 (1938).

TABLE V

DISSOCIATION CONSTANTS OF FERRIC–CYSTEINATE COMPLEXES IN AIR-SATURATED AND AIR-FREE SOLUTIONS

Exp. no.	RSH·HCl[a] added, $M \times 10^3$	$[Fe^{+++}]$, $M \times 10^3$	Composition of mixture at $t = 0$ $[NH_3]$, M	$[NH_4^+]$, M	pH calcd.	μ	Dissociation[b] constant of $Fe(OH)_m(RS)_n^{(m+2n-3)-}$ $m = 0$, $n = 2$ $K_{0,2}$ $\times 10^{30}$	$m = 1$, $n = 2$ $K_{1,2}$ $\times 10^{34}$	$m = 2$, $n = 1$ $K_{2,1}$ $\times 10^{34}$	$m = 0$, $n = 3$ $K_{0,3}$ $\times 10^{33}$
				In air-saturated solutions						
1	50.06	16.52	0.93	0.30	10.00	0.40	8.4	2.0	1.4	0.0058
2	40.25	12.22	0.94	.28	10.03	.37	9.0	2.3	1.6	1.1
3	30.15	8.50	0.94	.26	10.05	.35	8.4	2.3	1.7	3.4
4	40.08	12.10	1.44	.28	10.21	.37	4.5	1.8	1.5	3.0
5	40.06	11.60	1.90	.28	10.33	.37	3.2	1.7	1.3	0.84
6	40.13	10.77	1.96	.18	10.53	.27	1.7	1.4	1.1	3.0
				In air-free solutions						
7	12.50	2.75	0.97	0.21	10.10	0.24	6.6	2.0	2.4	7.3
8	12.50	2.30	.97	.06	10.63	.08	1.4	1.2	1.8	4.4
9	12.50	2.92	.97	.06	10.63	.08	1.8	1.5	2.1	7.5
10	6.25	0.56	.98	.04	10.75	.07	1.5	1.5	3.7	7.0
11	6.25	0.55	.98	.04	10.75	.07	1.6	1.6	3.8	7.3
12	12.50	2.70	.96	.16	10.18	.18	5.5	1.9	2.3	7.2
13	9.38	1.87	.96	.16	10.19	.18	4.9	1.7	2.5	6.4
14	7.81	1.43	.96	.16	10.19	.18	4.9	1.7	2.8	6.8
15	6.25	1.02	.97	.15	10.21	.18	4.6	1.7	3.2	6.7
16	3.13	0.29	.97	.14	10.25	.17	5.2	2.1	5.9	7.6

[a] Initial concentrations of Fe(III) were 20.06×10^{-3} M in exp. 1–3, 20.02×10^{-3} M in exp. 4–5, 19.95×10^{-3} M in exp. 6, 6.25×10^{-3} M in exp. 7–15 and 3.13×10^{-3} M in exp. 16. [b] $K_{m,n} = \dfrac{a_{Fe^{+++}} \times a^m_{OH^-} \times a^n_{RS^-}}{a_{Fe(OH)_mRS_n^{(m+2-3)-}}}$.

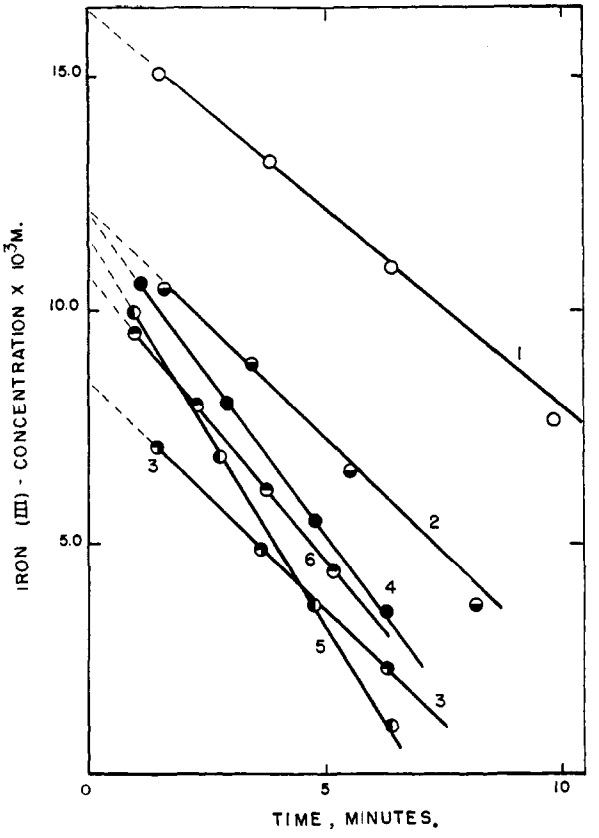


Fig. 2.—Decrease of iron(III)-concentration in air-saturated ammoniacal cysteinate solutions. (See Table V for experimental conditions.)

than that of an equivalent solution of cystine. In Fig. 3 some examples of the extrapolation to zero time are given. It is gratifying that the values of the constants calculated in Table VIII on the basis of these results are in close agreement with those obtained in air-saturated solutions.

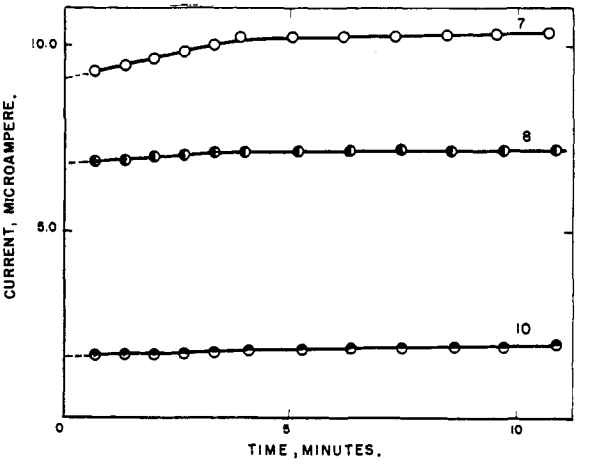


Fig. 3.—Current-time curves measured at (7), −1.35 volt *vs.* S.C.E.; (8) and (10), −1.40 volt, in contact with ferric hydroxide. (See Table V for experimental details.)

Dissociation Constants of Complexes.—The dissociation constant of the ferric–cysteinate complex

$$\{Fe(OH)_m(RS)_n\}^{(m+2n-3)-} \quad K_{Fe(OH)_m(RS)_n^{(m+2n-3)-}} = \frac{a_{Fe^{+++}} \times a^m_{OH^-} \times a^n_{RS^-}}{a_{Fe(OH)_m(RS)_n}} \quad (11)$$

corresponding to the equilibrium

$$Fe(OH)_m(RS)_n^{(m+2n-3)-} \rightleftharpoons Fe^{+++} + mOH^- + nRS^-$$

was calculated, using different values of m and n (see Table V). Introducing the solubility product

TABLE VI

SPECTROPHOTOMETRIC MEASUREMENTS OF FERRIC-CYSTEINATE IN AIR-FREE AMMONIACAL SOLUTIONS WHICH WERE INITIALLY 0.97 M IN NH_4OH, 0.10 M IN NH_4NO_3, 2×10^{-3} M IN VERSENATE (Na_2H_2Y), 10^{-3} M IN Fe(III) AND OF VARYING CYSTEINE CONCENTRATIONS. CALCULATION OF THE DISSOCIATION CONSTANT $K_{Fe(RS)_3^{3-}}$

RSH·HCl added, $M \times 10^3$	pH	μ	Optical density at 580 mμ at $t = 0$ (cor.)	[FeY-$(OH)_2^{3-}$], $M \times 10^5$	[$Fe(RS)_3^{3-}$], $M \times 10^5$	[Y^{4-}], $M \times 10^3$	[Fe^{+++}], $M \times 10^{29}$	[RS^-], $M \times 10^3$	$K_{Fe(RS)_3^{3-}} \times 10^{33}$
11.92	10.21	0.15	0.204	0.93	6.69	0.505	5.8	4.30	2.2
9.93	10.24	.14	.128	.96	4.20	.507	5.2	3.78	2.2
7.94	10.26	.14	.072	.98	2.36	.510	4.8	3.12	2.0
6.68	10.24	.14	.0516	.98	1.69	.497	5.4	2.56	(1.8)
3.34	10.29	.13	.0160	.99	0.525	.517	4.2	1.36	(0.7)

of ferric hydroxide,[16] $S_{Fe(OH)_3(s)}$ of 6×10^{-38}, when the solution is in equilibrium with solid ferric hydroxide, gives

$$K_{Fe(OH)_m(RS)_n} = \frac{S_{Fe(OH)_3(s)} \times a^n_{RS^-} \times a_{H^+}{}^{(3-m)}}{a_{Fe(OH)_mRS_n} \times K_w{}^{(3-m)}} \quad (12)$$

Introducing the concentration of total uncombined cysteine ([RSH]$_{uncomb.}$), the ionization constants of cysteinate ions (K_C and K_D[10]) and the activity coefficients yields

$$K_{Fe(OH)_mRS_n} = \frac{S_{Fe(OH)_3(s)}}{K_w{}^{(3-m)}} \times \frac{\gamma^n_{RS^-}}{\gamma_{Fe(OH)_m(RS)_n}} \times \frac{[RSH]^n_{uncomb.}}{[Fe(OH)_m(RS)_n]} \times \frac{a_{H^+}{}^{(3-m)}}{1 + \frac{a_{H^+}\gamma_{RS^-}}{K_C\gamma_{RS^\pm}} + \frac{a_{H^+}\gamma_{RS^-}}{K_D\gamma_{RSH^-}}} \quad (13)$$

$$[RSH]_{uncomb.} = [RSH]_{total} - n[Fe(OH)_m(RS)_n{}^{(m+2n-3)-}] \quad (14)$$

where [RSH]$_{total}$ is the total initial concentration of cysteine and [$Fe(OH)_m(RS)_n{}^{(m+2n-3)-}$] is equal to the total iron concentration in the solution at zero time.

The inconsistent K values (see Table V) obtained for $m = 0$ and $n = 2$ from experiments at different pH indicate that the formation of the complex $Fe(RS)_2^-$ can be excluded. From the series of experiments 12 to 16, in which the concentration of cysteine was varied keeping other conditions practically constant, the formation of $Fe(OH)_2(RS)^-$ ($m = 2$, $n = 1$) is considered unlikely, because of the systematic change in the calculated constant with change in concentration of cysteine.

The data of Table V combined with results from experiments with Versene, described in the following section, indicate that under our experimental conditions the two species $Fe(OH)(RS)_2{}^{2-}$ ($m = 1$, $n = 2$) and $Fe(RS)_3{}^{3-}$ ($m = 0$, $n = 3$) coexist in solution according to the equilibrium

$$Fe(OH)(RS)_2{}^{2-} + RS^- \rightleftarrows Fe(RS)_3{}^{3-} + OH^-$$

Evaluation of Dissociation Constants of Ferric–Cysteinate Complexes in Ammoniacal Versenate Solutions.—It was observed that the violet color of the ferric complex can be developed in alkaline Versenate solutions. Upon addition of air-free cysteine solution to an air-free ferric Versenate solution of pH 10, a violet color develops which fades on standing. Under our experimental conditions the solution of ferric Versenate was pale yellow, its mixture with cysteine becoming colorless after the reduction of ferric–cysteinate complex was completed to form ferrous–cysteinate and ferrous–Versenate complexes.

(16) W. M. Latimer, "Oxidation Potentials," 2nd ed., Prentice-Hall, Inc., New York, N. Y., 1952, p. 224.

Since the apparent dissociation constants of ferric Versenate complexes are known,[17] it is possible to calculate the dissociation constant of the ferric–cysteinate complex if the concentration of the latter can be determined in the mixture of both complexes.

The concentration of the ferric–cysteinate complex was determined by measurement of the extinction of the violet color at 580 mμ. The values were extrapolated to zero time. The experimental details and results are given in Table VI. The optical density data at zero time in Table VI are corrected for the very small absorption of the ferric Versenate complex.

The molar extinction coefficient of the ferric cysteinate complex which forms in ammoniacal buffer of pH 10.3 in the presence of a large excess of cysteine had previously been determined to be 3.05×10^3 mole^{-1} cm.$^{-1}$ at 580 mμ. Using this value the concentration of the ferric–cysteinate complex at zero time was calculated. Since all experiments were carried out with varying concentration of cysteine keeping other conditions practically constant, the ratio of [Fe-complex]/[$NH_2RS^-COO^-$]$^n_{uncomb.}$ must be independent of the concentration of uncombined cysteine if only one species is present in the solutions. It was found, however, that using either $n = 1$, or 2 or 3, the ratio does not remain constant over the entire range of cysteine concentrations. A plot of log [Fe-complex] against log [$NH_2RS^-COO^-$]$_{uncomb.}$ indicates that $Fe(RS)_3{}^{3-}$ is predominant at higher concentrations of cysteine (see Fig. 4), while the number of cysteine molecules coördinated to iron decreases with decreasing concentration of uncombined cysteine.

In a solution of pH 10.3, $FeY(OH)_2{}^{3-}$ is the most predominant species of ferric Versenate.[17] From the apparent formation constants reported by Schwarzenbach and Heller[17] for the equilibria

$$Fe^{+++} + Y^{4-} \rightleftarrows FeY^-,$$

$$FeY^- + OH^- \rightleftarrows FeY(OH)^=$$

$$FeY(OH)^= + OH^- \rightleftarrows FeY(OH)_2{}^{3-}$$

the apparent dissociation constant

$$K_{FeY(OH)_2{}^{-3}} = \frac{[Fe^{+++}][OH^-]^2[Y^{4-}]}{[FeY(OH)_2{}^{3-}]}$$

for the equilibrium

$$FeY(OH)_2{}^{3-} \rightleftarrows Fe^{+++} + 2OH^- + Y^{4-}$$

was calculated to be 8.25×10^{-37} at 20° and 0.1 ionic strength.

This constant has been used although our ex-

(17) G. Schwarzenbach and J. Heller, *Helv. Chim. Acta*, **34**, 576 (1951).

periments have been carried out at 25° and at ionic strength of 0.14 to 0.16. The concentration of ferric Versenate, $FeY(OH)_2^{3-}$, was obtained as

$$[FeY(OH)_2^{3-}] = [Fe]^t - [Fe(III)\text{-cysteinate complex}]$$

The concentration of uncombined Versenate is

$$[Versenate]_{uncomb.} = [Versenate]_{total} - [FeY(OH)_2^{3-}]$$

Introducing the ionization constant of Versenate ion ($pK_4 = 10.26$),[18] the concentration of Y^{4-} was obtained from the concentration of uncombined Versenate, neglecting the concentrations of $H_2Y^{=}$, H_3Y^- and H_4Y.

From $[FeY(OH)_2^{3-}]$, $[Y^{4-}]$, $[OH^-]$ and $K_{FeY(OH)_2^{3-}}$, the concentration of ferric ion, $[Fe^{+++}]$, was calculated (Table VI). The concentration of $RS^=$ was calculated from the ionization constants and activity coefficients of the various species of the cysteinate ions and the concentration of uncombined cysteine assuming the formation of $Fe(RS)_3^{3-}$. Introducing the activity coefficients for Fe^{+++}, $Fe(RS)_3^{3-}$ and $RS^=$, the dissociation constant of $Fe(RS)_3^{3-}$

$$K_{Fe(RS)_3^{3-}} = \frac{a_{Fe^{+++}} \times a^3_{RS^-}}{a_{Fe(RS)_3^{3-}}}$$

for the equilibrium

$$Fe(RS)_3^{3-} \rightleftarrows Fe^{+++} + 3RS^-$$

was calculated (Table VI). Considering that $Fe(RS)_3^{3-}$ is the predominant species only at higher cysteine concentrations (see Fig. 4), the average value of the dissociation constant was found to be equal to 2.1×10^{-33} from experiments with initial RSH– concentrations of 11.92 to 7.94×10^{-3} M (see Table VI).

The predominance of a complex with two molecules of cysteine may be considered in solutions of lower concentration of cysteine (see Fig. 4). Referring to the solubility studies in ammoniacal buffers (see Table V), this complex probably is $FeOH(RS)_2^{2-}$, with a dissociation constant

$$K_{FeOH(RS)_2^{2-}} = \frac{a_{Fe^{+++}} \times a_{OH^-} \times a^2_{RS^-}}{a_{FeOH(RS)_2^{2-}}}$$

From the results of an experiment with an initial RSH concn. of 6.68×10^{-3} M (Table VI) a value of 1.4×10^{-34} is calculated for the constant of this complex.

The ratio of $K_{FeOH(RS)_2^{2-}}/K_{Fe(RS)_3^{3-}}$ ($= K_{comp.}$) corresponding to the equilibrium

$$FeOH(RS)_2^{2-} + RS^- \rightleftarrows Fe(RS)_3^{3-} + OH^-$$

yields a value of 6.5×10^{-2}.

We have concluded that under the experimental conditions listed in Table V both $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$ are present in ammoniacal cysteinate solutions. Introducing $K_{comp.}$, the ratio of $FeOH(RS)_2^{2-}/Fe(RS)_3^{3-}$ is given by

$$\frac{[FeOH(RS)_2^{2-}]}{[Fe(RS)_3^{3-}]} = \frac{1}{K_{comp.}} \times \frac{\gamma_3}{\gamma_2} \times \frac{a_{OH^-}}{a_{RS^-}}$$

Since the total concentration of ferric complexes (a), that of cysteine (b) and the hydrogen ion concentration are known, the following equation was derived

$$x^2 + x\left\{(b - 3a) + \frac{K_w}{K_{comp.}} \times \frac{\gamma_3}{\gamma_2^2} \times \frac{k}{a_{H^+}}\right\} - \frac{K_w}{K_{comp.}} \frac{\gamma_3}{\gamma_2^2} \frac{k}{a_{H^+}} a = 0 \quad (15)$$

where x denotes the concentration of $FeOH(RS)_2^{2-}$ and k, the term

$$\left(1 + \frac{a_{H^+}\gamma_2}{K_C\gamma_1} + \frac{a_{H^+}\gamma_2}{K_D\gamma_1}\right)$$

Applying eq. 15 to the experiments in Table V, the concentrations of $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$ were calculated as given in Table VII.

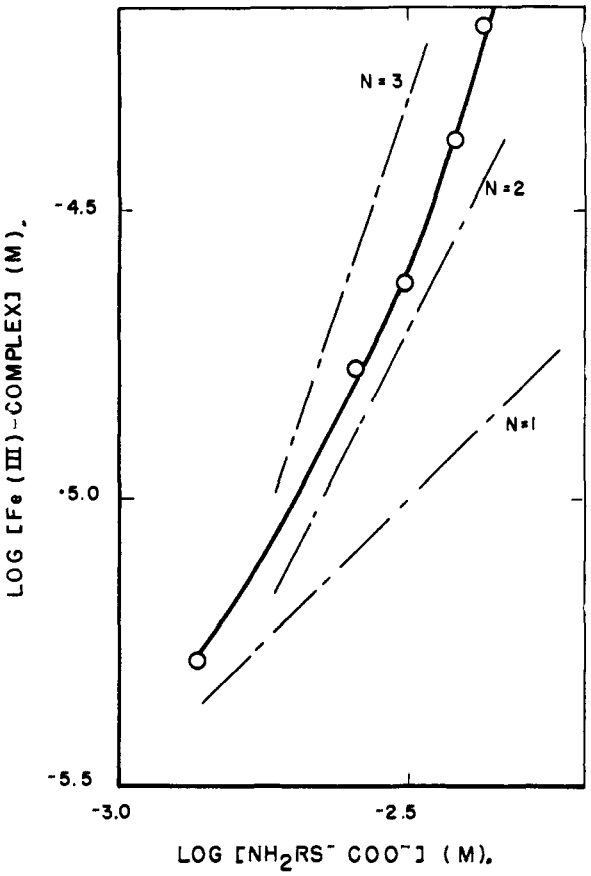


Fig. 4.—Plot of log [Fe(III)—complex] against log $[NH_2RS^-COO^-]$; -·-·-·- indicates theoretical lines for $Fe(RS)_n^{(2n-3)-}$.

At a given pH the ratio of $FeOH(RS)_2^{2-}$ to $Fe(RS)_3^{3-}$ increases with decreasing concentration of cysteine while at a given concentration of cysteine it increases with increasing pH.

The dissociation constants calculated for $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$ from these concentrations of $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$ and the corresponding concentrations of uncombined cysteine gave consistent values for both species (Table VII). This substantiates our previous conclusion that two complexes $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$ coexist in ammoniacal cysteinate solutions under our experimental conditions.

The average values for $K_{FeOH(RS)_2^{2-}}$ and $K_{Fe(RS)_3^{3-}}$ were calculated to be 1.9×10^{-33} and 2.9×10^{-32} (Table VII), respectively, compared with 1.4×10^{-34} and 2.1×10^{-33} obtained spectro-

(18) G. Schwarzenbach and H. Ackermann, *Helv. Chim. Acta*, **30**, 1798 (1947).

TABLE VII

CALCULATION OF THE CONCENTRATIONS OF $FeOH(RS)_2^{2-}$ AND $Fe(RS)_3^{3-}$ AND OF $K_{FeOH(RS)_2^{2-}}$ AND $K_{Fe(RS)_3^{3-}}$ IN AMMONIACAL CYSTEINATE SOLUTIONS OF TABLE V

All figures are listed in the sequence corresponding to the exp. No. in Table V.

Concn. of $FeOH(RS)_2^{2-}$ $\times 10^3$ M	Concn. of $Fe(RS)_3^{3-}$ $\times 10^3$, M	$K_{FeOH(RS)_2^{2-}}$ $\times 10^{33}$	$K_{Fe(RS)_3^{3-}}$ $\times 10^{22}$
7.18	9.28	1.0	1.6
4.96	7.26	1.7	2.5
3.59	4.91	2.2	3.3
5.17	6.93	1.3	2.0
4.94	6.66	1.4	2.1
4.78	5.99	1.5	2.3
1.46	1.29	2.5	3.7
1.42	0.88	1.5	2.3
1.22	0.80	2.1	3.2
0.40	0.16	2.1	3.1
0.40	0.15	2.1	3.4
1.43	1.27	2.4	3.6
1.09	0.78	2.2	3.3
0.87	0.56	2.2	3.4
0.65	0.37	2.2	3.3
0.215	0.071	2.6	3.9
	Mean	1.9	2.9

photometrically in ammoniacal Versenate solutions.

In the calculation of the latter values the dissociation constants of ferric Versenate complexes measured at 20° and 0.1 ionic strength were used instead of those at 25° and ionic strength 0.14–0.16 in the present experiments. This may be partly responsible for the fact that one set of values is about ten times greater than the other set. The main source of the difference in the two sets of values probably is the uncertainty in the solubility product of ferric hydroxide which is involved in the calculation in Table VII. The value of the solubility product depends upon the method of preparation and the age of the precipitate—using a value of 1×10^{-38} for $S_{Fe(OH)_3(s)}$ reported by Jellinek and Gordon[19] yields 3×10^{-3} [4]for the value of $K_{FeOH(RS)_2^{2-}}$ and 5×10^{-33} for that of $K_{Fe(RS)_3^{3-}}$. These are in better agreement with the constants calculated from the experiments with Versene than the constants calculated with a solubility product of 6×10^{-38}. As probable values we propose

$$K_{FeOH(RS)_2^{2-}} = 5 \times 10^{-34}$$

$$K_{Fe(RS)_3^{3-}} = 8 \times 10^{-33}$$

Schubert[5] postulated a dimer formula $\{FeOH(RS)_2\}_2^{4-}$ for the ferric–cysteinate complex. The diffusion coefficient of the ferric–cysteinate complex in solutions of ionic strength 0.12–0.31 containing no gelatin was extrapolated to be 5.7×10^{-6} cm.^{2} sec.$^{-1}$, which is of the same order as that of cystine,[20] and also of that of iron(III)–Versenate complex, $FeY(OH)^-$ (5.4×10^{-6} cm.^{2} sec.$^{-1}$).[21] This strongly suggests that the complex is a monomer.

In studies of the ferric–thioglycolate complex Leussing and Kolthoff[7] concluded that the predominant species was $FeOH(TS)_2$ similar to one of the ferric–cysteinate complexes postulated in the present study. Recalculating the dissociation constant of the thioglycolate complex[7] yields a value of 9.4×10^{-33}. This value was obtained in ammoniacal buffers and is based on a solubility product of ferric hydroxide of 6×10^{-38}. Comparison with 1.9×10^{-33} of the corresponding ferric–cysteinate complex, $FeOH(RS)_2^{2-}$, indicates that the stability of both complexes is of the same order of magnitude.

Acknowledgment.—This investigation was supported by a research grant (C-721,C$_5$) from the National Cancer Institute, U. S. Public Health Service.

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MINNEAPOLIS, MINNESOTA

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Oxidation of Ferrous–Cysteinate Complex by Cystine. Oxidation Potential of the Cystine–Cysteine System

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Upon addition of ferrous iron to an air-free ammoniacal solution containing cystine and cysteine some ferrous iron is oxidized by cystine to a violet ferric cysteinate complex. From spectrophotometric determinations of the ferric complex the equilibrium constants for the reactions $2Fe(II)(RS)_3^{3-} + {}^-RSSR^- \rightleftarrows 2Fe(III)(RS)_3^{3-}$ and $2Fe^{++} + {}^\pm RSSR^\pm + 2H^+ \rightleftarrows 2Fe^{+++} + 2RSH^\pm$ were calculated to be equal to 2.5×10^{-3} and 5.3×10^{-24}, respectively, at 25°. From the latter value the oxidation potential of the cystine–cysteine system was calculated to be +0.08 volt *vs.* N.H.E. at 25°.

In a study of ferrous- and ferric–cysteine complexes[2] we observed the development of a violet color upon addition of an air-free ferrous iron solution to an air-free ammoniacal mixture of cystine having a large concentration in cysteine. On standing the color intensity increased to a constant value. Other conditions being the same the color became more intense with increasing cystine concentration. Spectrophotometrically the color was found to be similar to that of ferric cysteinate which is formed on the addition of ferric iron to a cysteine solution.[2]

These observations indicate that under proper experimental conditions cystine can oxidize ferrous cysteinate to the ferric complex. Our observations are at variance with those of Michaelis and Barron[3] who found no indication for the oxidation

(1) On leave of absence from Tokyo University, Japan.

(2) N. Tanaka, I. M. Kolthoff and W. Stricks, THIS JOURNAL, **77**, 1996 (1955).

(3) L. Michaelis and E. S. G. Barron, *J. Biol. Chem.*, **83**, 191 (1929).