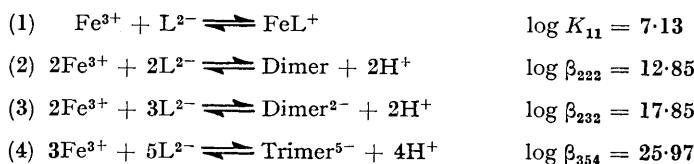


975. *Iron-Malate and Iron-Citrate Complexes.*

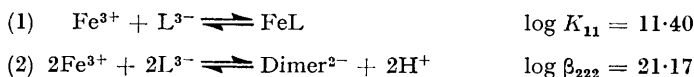
By C. F. TIMBERLAKE.

Complex-formation between ferric ions ( $\text{Fe}^{3+}$ ) and excess malic acid ( $\text{H}_2\text{L}$ ), and between ferric ions and excess citric acid ( $\text{H}_3\text{L}$ ), has been studied in acid solutions at  $20^\circ$ , with  $I = 0.1\text{M}$  ( $\text{NaClO}_4$ ), by means of potentiometric, oxidation-reduction potential, and spectrophotometric measurements. Results were consistent with the following reactions, for which equilibrium constants were calculated.

## (a) Ferric-malate



## (b) Ferric-citrate



Stability constants of ferrous malate and citrate complexes were also determined. The results are compared with those found previously for iron tartrates.

IRON-MALATE complexes appear to have been little investigated since the work of Pickering,<sup>1</sup> who suggested the formation of  $\text{Fe}_2\text{L}_3$  and  $\text{Fe}_2\text{L}_3, \text{Fe}_2\text{O}_3$ , where malic acid is represented by  $\text{H}_2\text{L}$ . Iron-citrate complexes have been studied more fully, but, despite previous findings<sup>2</sup> that polynuclear complexes may be formed, the results of recent investigations<sup>3-6</sup> have been expressed solely in terms of mononuclear complex formation. The recent development of methods of investigating polynuclear iron complexes using oxidation-reduction potential measurements<sup>7,8</sup> has prompted the reinvestigation of complex-formation between iron and malic and citric acids. Results of a similar study of iron-tartrate complexes have been reported.<sup>9</sup>

## EXPERIMENTAL

The experimental technique and nomenclature are as described previously.<sup>9</sup> (-)-Malic acid (L. Light & Co.), recrystallised from ethyl acetate and light petroleum,<sup>10</sup> had m. p.  $105-107^\circ$ , higher than that reported previously,<sup>11</sup> possibly owing to the presence of a small amount of the optically inactive form which should, however, not have exceeded 2-3%, since the rotation in the presence of molybdate,  $[\alpha]_D^{21} + 695^\circ$ , was very similar to previous values.<sup>11,12</sup> Analytical grade citric acid (B.D.H.) was used without further purification. Solutions of the ferric complexes were kept in the dark to avoid photoreduction. Most measurements were

<sup>1</sup> Pickering, *J.*, 1913, **103**, 1358.

<sup>2</sup> Bobtelsky and Jordan, *J. Amer. Chem. Soc.*, 1947, **69**, 2286.

<sup>3</sup> Lanford and Quinan, *J. Amer. Chem. Soc.*, 1948, **70**, 2900.

<sup>4</sup> Bertin-Batsch, *Ann. Chim. (France)*, 1952, **7**, 481.

<sup>5</sup> Warner and Weber, *J. Amer. Chem. Soc.*, 1953, **75**, 5086.

<sup>6</sup> Hamm, Schull, and Grant, *J. Amer. Chem. Soc.*, 1954, **76**, 2111.

<sup>7</sup> Perrin, *J.*, 1959, 1710.

<sup>8</sup> Anderegg, *Helv. Chim. Acta*, 1960, **43**, 1530.

<sup>9</sup> Timberlake, *J.*, 1964, 1229.

<sup>10</sup> Brummond and Burris, *J. Biol. Chem.*, 1954, **209**, 755.

<sup>11</sup> Whiting and Coggins, *J. Sci. Food Agric.*, 1960, **11**, 337.

<sup>12</sup> Krebs and Eggleston, *Biochem. J.*, 1943, **37**, 334.

made with solutions containing a large excess of the organic acid, so that the ligand concentration,  $[L]$ , could be obtained as a first approximation from  $[L] = ([L]_t - [Fe^{III}]_t)/\alpha$ , where  $\alpha$  has the usual meaning.

*Ionisation Constants.*—The acid ionisation constants of (–)-malic acid were  $pK_{c1} = 3.28 \pm 0.02$ ,  $pK_{c2} = 4.72 \pm 0.02$ , and those of citric acid were  $pK_{c1} = 2.96 \pm 0.03$ ,  $pK_{c2} = 4.38 \pm 0.02$ , and  $pK_{c3} = 5.68 \pm 0.02$ .

*Ferrous-Malate.*—The pH depression which occurred on titration of ferrous perchlorate ( $10^{-3}M$ ) and malic acid ( $10^{-3}M$ ) with alkali was slightly greater than that which occurred with tartaric acid,<sup>9</sup> and allowed calculation of  $\log K(Fe^{2+}L) = 2.49 \pm 0.14$  (mean of 7 readings). The ion-exchange method, as for tartrate,<sup>9</sup> gave  $\log K(Fe^{2+}L) = 2.68 \pm 0.08$  (mean of 4 readings). The overall mean was taken as  $\log K(Fe^{2+}L) = 2.60_{-0.25}^{+0.16}$ .

*Ferric-Malate Complexes.*—*Oxidation-reduction potential measurements.* Measurements were possible up to pH 5.6, above which attainment of equilibrium was sluggish. As described previously,<sup>9</sup> potential measurements were made at constant pH and nearly constant  $[L]_t$  and  $[Fe^{II}]_t$  but with varying  $[Fe^{III}]_t$ . The results, similar to those obtained with tartrate, indicated a transition from mononuclear complexes at low pH to trinuclear complexes near pH 5. However, when  $[L]_t$  was varied at constant pH and nearly constant  $[Fe^{III}]_t$  and  $[Fe^{II}]_t$ , the product  $[Fe^{3+}][L]$  was not constant, unlike the tartrate findings. The variation in  $[Fe^{3+}][L]$ , although small at low pH (2.40), increased with increasing pH and suggested the formation of complexes containing an excess of malate over iron. At three pH values within the range 4.5–5.5, plots of  $\log [Fe^{III}]_t - 3 \log [Fe^{3+}]$  against  $\log [L]$  were linear and gave the average number of ligand ions ( $n$ ) in the complexes between 4.4 and 5.0, suggesting that the main complex present was  $Fe_3L_5$ , with possibly a small amount of a dimer. Spectrophotometric measurements, which will be described later, also indicated that mainly one complex existed within this pH range. Best constancy was obtained for complex  $Fe_3L_5(-H)_4$ , as indicated by the following results.

Method	Vary pH	Vary $[Fe^{III}]_t$		Vary $[L]_t$		
				4.52	4.88	5.43
pH	4.45–5.36	4.75	5.58	44.06	45.53	48.03
$\log K_{35}$	44.05–47.63	44.96	48.32	26.00	26.01	26.31
$\log \beta_{354}$	26.25–26.19	25.98	25.99			

The mean value of  $\log \beta_{354}$  was  $26.06_{-0.08}^{+0.14}$ , for experiments within pH range 4.5–5.0. Consideration of this value together with the results obtained at lower pH values indicated that complex  $Fe_3L_5(-H)_4$  would not be formed in appreciable amounts below pH 3.1 under our experimental conditions. The experimental data at lower pH values were then evaluated without interference from  $Fe_3L_5(-H)_4$ .

Complexes  $FeL$ ,  $Fe_2L_2(-H)_2$ , and  $Fe_2L_3(-H)_2$ . Experiments were made varying  $[Fe^{III}]_t$  at constant pH and nearly constant  $[L]_t$  and  $[Fe^{II}]_t$ . For the general case, assuming complexes  $FeL$ ,  $FeL_2 \dots$ ,  $Fe_2L_2$ ,  $Fe_2L_3 \dots$ , to be present,

$$([Fe^{III}]_t - [Fe^{3+}])/[Fe^{3+}][L] = (K_{11} + K_{12}[L] + \dots) + 2[Fe^{3+}][L](K_{22} + K_{23}[L] + \dots)$$

Plots of  $([Fe^{III}]_t - [Fe^{3+}])/[Fe^{3+}][L]$  against  $[Fe^{3+}][L]$  were linear, with the following results:

pH	$\log (K_{11} + K_{12}[L] + \dots)$	$\log (K_{22} + K_{23}[L] + \dots)$	$\log (K_{22} + K_{23}[L] + \dots)[H]^2$
2.20	7.15	17.15	12.74
2.51	7.17	18.17	13.16
2.84	(7)	19.31	13.62

The constancy of  $\log (K_{11} + K_{12}[L] + \dots)$  indicated that  $FeL$  was the only monomeric complex present under these conditions. Unlike the results of similar experiments carried out with tartrate,  $\log (K_{22} + K_{23}[L] + \dots)[H]^2$  was not constant. That the inconstancy was due to the formation of the higher  $Fe_2L_3$  complex rather than a different degree of protonation was shown by varying  $[L]_t$  at constant pH and nearly constant  $[Fe^{III}]_t$  and  $[Fe^{II}]_t$ . Plots of  $\{([Fe^{III}]_t - [Fe^{3+}]) - K_{11}[Fe^{3+}][L]\}/[Fe^{3+}]^2[L]^2$  against  $[L]$  were linear, the intercepts giving  $2K_{22}$  and the slopes  $2K_{23}$ , as follows:

pH	$\log K_{22}$	$\log K_{22}[H]^2$	$\log K_{23}$	$\log K_{23}[H]$	$\log K_{23}[H]^2$	$\log K_{23}[H]^3$
2.40	17.62	12.82	(22.80)	(20.40)	(18.00)	(15.60)
2.92	18.70	12.87	23.62	20.70	17.78	14.86
3.06	19.00	12.87	23.89	20.83	17.77	14.71

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where the mean value of  $\log K_{11}$  was taken as 7.13. The results suggested the presence of complex  $\text{Fe}_2\text{L}_2(-\text{H})_2$  and probably  $\text{Fe}_2\text{L}_3(-\text{H})_2$ . The mean value of  $\log K_{22}[\text{H}]^2 = 12.85$  was then used to evaluate  $\log K_{23}$  from the previously describe experiments in which  $[\text{Fe}^{\text{III}}]_t$  was varied, except that at pH 2.20 where  $[\text{Fe}_2\text{L}_3]$  would be very small, with the following results:

pH	$\log K_{23}$	$\log K_{23}[\text{H}]$	$\log K_{23}[\text{H}]^2$	$\log K_{23}[\text{H}]^3$
2.51	22.99	20.50	17.99	15.48
2.84	23.73	20.87	18.03	15.19

Best constancy was shown by  $\log K_{23}[\text{H}]^2$  (overall mean  $17.90 \pm 0.13$ ), followed by  $\log K_{23}[\text{H}]$  (overall mean  $20.75 \pm 0.12$ ).

When the pH was varied between 2.02 and 2.89 a plot of  $([\text{Fe}^{\text{III}}]_t - [\text{Fe}^{3+}])/[\text{Fe}^{3+}][\text{L}]$  against  $2[\text{Fe}^{3+}][\text{L}]/[\text{H}]^2$  [*i.e.*, assuming only complexes  $\text{FeL}$  and  $\text{Fe}_2\text{L}_2(-\text{H})_2$ ] gave a curve intercepting the ordinate at a value giving  $\log K_{11} = 7.05$ . Assuming that the deviation from linearity was due to an  $\text{Fe}_2\text{L}_3$  complex, its amount was calculated over the pH range 2.47—2.89 (9 readings) from

$$[\text{Fe}_2\text{L}_3] = \{([\text{Fe}^{\text{III}}]_t - [\text{Fe}^{3+}]) - K_{11}[\text{Fe}^{3+}][\text{L}] - 2(\beta_{222}/[\text{H}]^2)[\text{Fe}^{3+}]^2[\text{L}]^2\}/2$$

using  $\log K_{11} = 7.05$  and  $\log \beta_{222} = 12.85$ , and gave  $\log \beta_{231} = 20.40 \pm 0.15$  and  $\log \beta_{232} = 17.70 \pm 0.03$ ; the better constancy of the latter suggested that the complex present was  $\text{Fe}_2\text{L}_3(-\text{H})_2$ . In a further experiment,  $[\text{Fe}^{\text{III}}]_t$  was varied at constant pH 3.24, and when correction was made for the small amounts (15%) of the other complexes  $\text{Fe}_2\text{L}_2(-\text{H})_2$  and  $\text{Fe}_3\text{L}_5(-\text{H})_4$  present,  $\log \beta_{232}$  was calculated to be  $17.81 \pm 0.11$  (5 readings). The overall mean value of  $\log \beta_{232}$  was then  $17.87 \pm 0.16$  (mean of 6 means).

Complexes  $\text{Fe}_2\text{L}_3(-\text{H})_2$  and  $\text{Fe}_3\text{L}_5(-\text{H})_4$ . The results of experiments carried out in the pH range 3.40—5.23, where  $\text{FeL}$  and  $\text{Fe}_2\text{L}_2(-\text{H})_2$  are negligible, are summarised in Table 1.

TABLE 1.

Evaluation of  $\beta_{232}$  and  $\beta_{354}$  for ferric-malate complexes  $\text{Fe}_2\text{L}_3(-\text{H})_2$  and  $\text{Fe}_3\text{L}_5(-\text{H})_4$ .

Expt.	Method	Readings	pH	$\log \beta_{232}$	$\log \beta_{354}$	$\log \beta_{354}^*$
1	Vary pH	14	3.40—5.23	17.90	26.13	—
2	Vary $[\text{Fe}^{\text{III}}]_t$	7	3.75	$17.87 \pm 0.08$ $-0.13$	$26.06 \pm 0.08$ $-0.13$	—
3	„	5	4.24	$17.76 \pm 0.02$ $-0.05$	$25.98 \pm 0.03$ $-0.04$	—
4	Vary $[\text{L}]_t$	7	3.75	$17.77 \pm 0.03$ $-0.07$	$25.94 \pm 0.06$ $-0.05$	—
5	„	6	4.52	—	$26.00 \pm 0.06$ $-0.07$	$25.83 \pm 0.07$ $-0.08$
6	„	7	4.88	—	$26.01 \pm 0.05$ $-0.07$	$25.90 \pm 0.04$ $-0.05$
7	Vary $[\text{Fe}^{\text{III}}]_t$	5	4.75	—	$25.98 \pm 0.05$	$25.90 \pm 0.06$ $-0.11$

\* Corrected for the presence of small amounts of  $\text{Fe}_2\text{L}_3(-\text{H})_2$ .

Experiment 1. Assuming  $[\text{Fe}^{\text{III}}]_t = 2[\text{Fe}_2\text{L}_3(-\text{H})_2] + 3[\text{Fe}_3\text{L}_5(-\text{H})_4]$ , a plot of  $[\text{Fe}^{\text{III}}]_t[\text{H}]^2/[\text{Fe}^{3+}]^2[\text{L}]^3$  against  $[\text{Fe}^{3+}][\text{L}]^2/[\text{H}]^2$  should be linear, the intercept giving  $2\beta_{232}$  and the slope giving  $3\beta_{354}$ . A linear plot was found with  $\log \beta_{232} = 17.90$  and  $\log \beta_{354} = 26.13$ .

Experiments 2—4. Satisfactory linear plots were not easily obtained because of somewhat larger variation in measurements. Values of  $\beta_{232}$  and  $\beta_{354}$  were therefore calculated by proportionality using equations

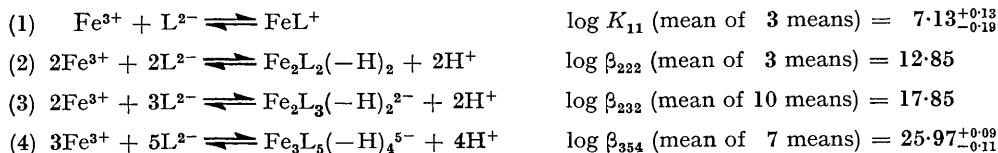
$$[\text{Fe}_2\text{L}_3(-\text{H})_2] = [\text{Fe}^{\text{III}}]_t / \{2 + (3\beta_{354}[\text{Fe}^{3+}][\text{L}]^2/[\text{H}]^2\beta_{232})\}$$

$$[\text{Fe}_3\text{L}_5(-\text{H})_4] = [\text{Fe}^{\text{III}}]_t / \{3 + (2\beta_{232}[\text{H}]^2/\beta_{354}[\text{Fe}^{3+}][\text{L}]^2)\},$$

and the mean values  $\log \beta_{232} = 17.87$  and  $\log \beta_{354} = 26.06$  already found.

Experiments 5—7. The magnitude of  $\log \beta_{232}$  indicated that complex  $\text{Fe}_2\text{L}_3(-\text{H})_2$  was still present in small amounts under the conditions of these experiments. Corrected values of  $\log \beta_{354}$  are shown in the last column of Table 1, which, together with the result of Experiment 1, gave a mean value  $\log \beta_{354} = 25.96 \pm 0.17$ . This value, if used instead of  $\log \beta_{354} = 26.06$  to evaluate the results of Experiments 2—4 would tend to make  $\beta_{232}$  slightly larger and  $\beta_{354}$  slightly smaller. However, the differences would probably lie within experimental error. The mean value of  $\log \beta_{232} = 17.83$  obtained from Experiments 1—4 is in excellent agreement with the mean value  $\log \beta_{232} = 17.87$  found independently below pH 3.1.

The following is a summary of the reactions and constants.



The tolerances given for  $K_{11}$  and  $\beta_{354}$  are 95% confidence limits to the mean of the number of mean observations.

*Potentiometric titration.* When solutions containing a large excess of disodium malate ( $10^{-2}\text{M}$ ) over ferric perchlorate ( $2 \times 10^{-4}\text{M}$  and  $5 \times 10^{-4}\text{M}$ ) were titrated with 0.1N-sodium hydroxide, a small pH inflection was found (pH 7.7) at a ratio  $[\text{NaOH}]/[\text{Fe}^{\text{III}}]_t$  of 2.0, corresponding to the liberation of  $2\text{H}^+$  per iron atom. At smaller ratios of disodium malate to iron (3:1, 2:1, and 1:1) pH values became unsteady as they appeared to approach an inflection near  $[\text{NaOH}]/[\text{Fe}^{\text{III}}]_t$  values of 2. The results indicate that above pH 5, complex  $\text{Fe}_3\text{L}_5(-\text{H})_4$  either ionises

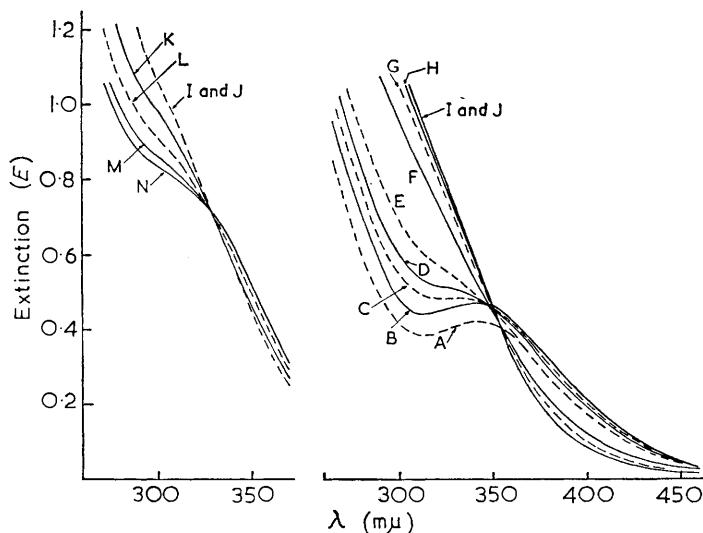


FIG. 1. Absorption spectra of ferric-malate complexes.

$[\text{L}]_t = 0.01\text{M}$ ;  $[\text{Fe}^{\text{III}}]_t = 3.18 \times 10^{-4}\text{M}$ ; 1-cm. silica cells.

pH A, 2.03; B, 2.17; C, 2.30; D, 2.37; E, 2.52; F, 3.04; G, 3.51; H, 4.02; I, 4.50; J, 5.00; K, 6.11; L, 6.68; M, 7.2; N, 8.2.

with liberation of two protons or is transformed into a further complex with overall liberation of  $2\text{H}^+$  per iron atom. For this reason, values of  $\beta_{354}$  were not calculated above pH 5.2 in the oxidation-reduction potential measurements.

*Spectrophotometric measurements.* As with ferric tartrate,<sup>9</sup> the existence of a 1:1 ferric-malate complex at pH 1.5 was confirmed by Job's method of continuous variations. Similarly, using Agren's<sup>13</sup> method, with a large excess of malate ( $10^{-2}\text{M}$ ) over ferric perchlorate ( $4.24 \times 10^{-4}\text{M}$ ), linear plots were obtained at 350, 370, and 400  $\text{m}\mu$ , giving  $\log K_{11} = 7.09 \pm 0.03$ , in good agreement with the value obtained from the oxidation-reduction potential measurements. Under the conditions used  $\text{Fe}_2\text{L}_2(-\text{H})_2$  was negligible. Values of  $\epsilon$  for  $\text{FeL}$  at the three wavelengths were 1540, 1270, and 690, respectively. Results obtained with equal malate and iron concentrations were more variable, because of the low degree of complex-formation (only 5–15%  $\text{FeL}$ ).

The absorption spectra of the ferric-malate system at different pH values are shown in Fig. 1.

<sup>13</sup> Agren, *Acta Chem. Scand.*, 1954, **8**, 266.

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Complex FeL (curve A) shows a peak near 341 m $\mu$ . The absorption at 280—340 m $\mu$  increased rapidly with increasing pH, corresponding to the formation of the dimers and trimer. Within the pH range 4.5—5.0, however, the absorption was practically constant, suggesting that mainly a single complex existed, which the oxidation-reduction potential measurements indicated to be Fe<sub>3</sub>L<sub>5</sub>(-H)<sub>4</sub>. The extinction was almost constant at 350 m $\mu$  from pH 2.2 to 4, and at 330 m $\mu$  from 4.25 to 7.2. The system therefore appeared to exhibit isosbestic points at these two wavelengths. The occurrence of an isosbestic point is usually held to signify the presence of not more than two components.<sup>14,15</sup> On the basis only of the spectrophotometric and potentiometric results, therefore, the following sequence would be expected with increasing pH: FeL  $\longrightarrow$  unknown complex  $\longrightarrow$  third complex with overall liberation of 2H<sup>+</sup> per iron atom. But the oxidation-reduction potential measurements could only be explained satisfactorily by the system of complexes outlined. It must be concluded that FeL, Fe<sub>2</sub>L<sub>2</sub>(-H)<sub>2</sub> and Fe<sub>2</sub>L<sub>3</sub>(-H)<sub>2</sub>, which are present in similar amounts about pH 2.6 (Fig. 1), exhibit almost identical absorption at 350 m $\mu$  by virtue of some common feature. Similarly, a common feature of Fe<sub>3</sub>L<sub>5</sub>(-H)<sub>4</sub> and further complexes must give rise to the isosbestic point at 330 m $\mu$ .

*Ferrous-Citrate.*—A solution (100 ml.) of citric acid (10<sup>-3</sup>M, H<sub>3</sub>L) and ferrous perchlorate (10<sup>-3</sup>M) was titrated with 0.1N-sodium hydroxide in the presence of hydrogen and palladium-charcoal. The stability constant of Fe<sup>II</sup>L, calculated from the pH data, fell with increasing pH, suggesting that complex Fe<sup>II</sup>H<sub>2</sub>L<sup>+</sup> was also present.<sup>6</sup> On the assumption that only these two complexes were present, constant values of [L] were obtained by successive approximations, and mean values of the stability constants K<sub>1</sub> and K<sub>2</sub> were found from the linear plot of the equation

$$([\text{Na}^+] + [\text{H}^+] - \beta[\text{L}])/\alpha[\text{L}]^2 = (2K_1/K_{c3})[\text{H}] + 3K_2,$$

where

$$\alpha = 1 + [\text{H}]/K_{c3} + [\text{H}]^2/K_{c2}K_{c3} + [\text{H}]^3/K_{c1}K_{c2}K_{c3},$$

and

$$\beta = 3 + 2[\text{H}]/K_{c3} + [\text{H}]^2/K_{c2}K_{c3}.$$

From the slope and intercept, log K<sub>1</sub>(Fe<sup>II</sup>H<sub>2</sub>L) = 2.65, log K<sub>2</sub>(Fe<sup>II</sup>L) = 4.4. Hamm *et al.*<sup>6</sup> found log K<sub>1</sub> = 2.12 and log K<sub>2</sub> = 3.08 in m-sodium perchlorate at 25°.

*Ferric-Citrate Complexes.*—*Oxidation-reduction potential measurements.* Above pH 3.1, attainment of equilibrium was sluggish. The results, which were therefore confined to those obtained from measurements below this pH indicated the formation of a monomer FeL and a

TABLE 2.

Evaluation of K<sub>11</sub> and  $\beta_{222}$  for ferric-citrate complexes FeL and Fe<sub>2</sub>L<sub>2</sub>(-H)<sub>2</sub>.

Expt.	Method	Readings	pH	log K <sub>11</sub>	log K <sub>22</sub>	log K <sub>22</sub> [H] <sup>2</sup> = log $\beta_{222}$
1	Vary [Fe <sup>III</sup> ] <sub>t</sub>	6	1.97	11.42	25.18	(21.24)
2	"	7	2.35	11.38	25.75	21.05
3	"	6	2.80	11.40	26.72	21.13
4	"	8	3.08	(11.33)	27.54	21.38
5	Vary pH	24	2.14—3.00	11.41 <sup>+0.12</sup> <sub>-0.17</sub>		21.30 <sup>+0.09</sup> <sub>-0.11</sub>
6	Vary [L] <sub>t</sub>	9	2.39	11.39 ± 0.03		21.18 <sup>+0.05</sup> <sub>-0.04</sub>
7	"	6	2.86	11.37 <sup>+0.11</sup> <sub>-0.07</sub>		21.16 <sup>+0.10</sup> <sub>-0.03</sub>

dimer Fe<sub>2</sub>L<sub>2</sub>(-H)<sub>2</sub> (Table 2). The method of evaluation was similar to that described previously for ferric tartrate and malate complexes.

Experiments 1—4. K<sub>11</sub> and K<sub>22</sub> were obtained from the linear plots of

$$([\text{Fe}^{\text{III}}]_t - [\text{Fe}^{3+}])/[\text{Fe}^{3+}][\text{L}] \text{ against } 2[\text{Fe}^{3+}][\text{L}].$$

Experiment 5. K<sub>11</sub> and  $\beta_{222}$  were obtained from the linear plot of  $([\text{Fe}^{\text{III}}]_t - [\text{Fe}^{3+}])/[\text{Fe}^{3+}][\text{L}]$  against  $2[\text{Fe}^{3+}][\text{L}]/[\text{H}]^2$ , fitted by the method of least-squares.

<sup>14</sup> Charlot and Gauguin, "Methodes d'Analyse des Reactions en Solution," Masson, Paris, 1951, p. 89.

<sup>15</sup> Morton, *Nature*, 1962, **193**, 314.

Experiments 6 and 7. Values of  $\log [\text{Fe}^{3+}][\text{L}]$  were constant at constant pH, indicating that, like tartrate but unlike malate, complex-formation was in the ratio 1:1. Further values of  $K_{11}$  and  $\beta_{222}$  were then obtained by proportionality, using mean values of  $K_{11}$  and  $\beta_{222}$  from Experiments 1—3 and 5. A worked example is given in Table 3.

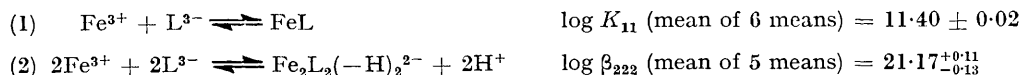
TABLE 3.

Variation of citrate concentration at constant iron concentrations and pH.

Mean volume =  $103 \pm 2$  ml.,  $[\text{Fe}^{\text{III}}]_t = [\text{Fe}^{\text{II}}]_t = 2.09 \times 10^{-4}\text{M}$ , citric acid added =  $0.502\text{M}$ ,  $I = 0.1\text{M}$  ( $\text{NaClO}_4$ ), pH = 2.39,  $\log \alpha = 5.968$ . The percentage of the total ferric iron in each form was calculated from ratio  $K_{11} : 2\beta_{222} [\text{Fe}^{3+}][\text{L}]/[\text{H}]^2$ , i.e.,  $70 \pm 1\%$  as  $\text{FeL}$  and  $30 \pm 1\%$  as  $\text{Fe}_2\text{L}_2$ .

$10^3[\text{L}]_t$	$E$ (mv)	$-\log [\text{Fe}^{3+}]$	$-\log [\text{L}]$	$-\log [\text{Fe}^{3+}][\text{L}]$	$\log K_{11}$	$\log K_{22}$
0.97	264.2	6.13	9.08	15.21	11.37	25.93
1.95	243.0	6.49	8.73	15.22	11.38	25.93
3.90	224.8	6.80	8.60	15.21	11.37	25.91
5.84	213.0	7.00	8.22	15.22	11.39	25.94
7.79	205.1	7.14	8.09	15.23	11.39	25.96
9.74	200.2	7.23	7.99	15.22	11.38	25.93
12.18	192.8	7.35	7.89	15.24	11.40	25.98
14.61	187.6	7.44	7.79	15.25	11.42	26.00
17.05	183.8	7.51	7.74	15.25	11.42	25.98

The reactions and constants are summarised as follows:



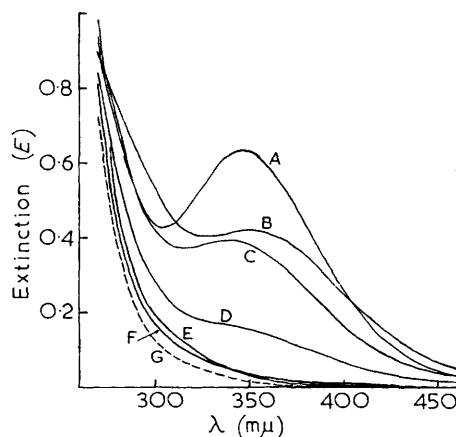
where the tolerances given are 95% confidence limits to the mean of the number of mean observations. Values at pH 3.08 were not included because of the possible influence of further complexes which may be formed at higher pH.

FIG. 2. Absorption spectra of iron-organic acid systems.

$[\text{L}]_t = 0.01\text{M}$ ,  $[\text{Fe}^{\text{III}}]_t = 4.24 \times 10^{-4}\text{M}$ ,  $[\text{H}]_t = 0.031$ ,  $I = 0.1\text{M}$  ( $\text{NaClO}_4$ ).

A, Tartaric acid; B, citric acid; C, malic acid; D, lactic acid; E, tricarballic acid; F, succinic acid; G, without organic acid.

By calculation, %  $[\text{FeL}]/[\text{Fe}^{\text{III}}]_t = 83\%$  for tartaric, 55% for malic, and 78% for citric acid.



In a previous study<sup>4</sup> the results of potential measurements in the vicinity of pH 2 in solutions containing an excess of iron over citrate were explained by the formation of the dimer  $\text{Fe}_2\text{L}$  ( $\log K_{21} = 13.9$ ) in addition to  $\text{FeL}$  ( $\log K_{11} = 11.7$ ).

**Potentiometric titration.** On titration of a 1:1 mixture of trisodium citrate ( $10^{-3}\text{M}$ ) and ferric perchlorate ( $10^{-3}\text{M}$ ) with 0.1N-sodium hydroxide, equilibrium became very slow in the region of pH 3.5. In solutions containing less iron ( $5 \times 10^{-4}\text{M}$ ,  $3.3 \times 10^{-4}\text{M}$ ), pH values were stable only up to 6.5 at  $[\text{NaOH}]/[\text{Fe}^{\text{III}}]_t$  values of 1.0—1.5.

**Spectrophotometric measurements.** In agreement with previous results in acid solutions,<sup>3,5</sup> Job's method of continuous variations showed that a 1:1 complex was present at pH 1.5. Using Agren's method<sup>13</sup> with a large excess of citrate ( $10^{-2}\text{M}$ ) over iron ( $4.24 \times 10^{-4}\text{M}$ ) from  $[\text{H}]_t$  values of 0.03 to 0.10, plots of  $[\text{Fe}^{\text{III}}]_t/E$  against  $\alpha/[\text{L}]_t$  at three wavelengths were linear only at the lowest acidities. At the highest acidity values, the plot curved towards the  $\alpha/[\text{L}]_t$

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axis, indicating that the extinction was larger than expected. An additional contribution by a further complex, probably  $\text{FeHL}^+$ , was suggested, since, when the ligand was assumed to be  $\text{HL}^{2-}$ , the corresponding plots of  $[\text{Fe}^{\text{III}}]_t/E$  against  $(K_{c3}\alpha/[\text{H}][\text{L}]_t)$  were linear. From the plots of  $[\text{Fe}^{\text{III}}]_t/E$  against  $\alpha/[\text{L}]_t$ , where these were linear,  $\log K_{11} = 11.56 \pm_{0.26}^{+0.14}$  (mean of 5 values). The slightly higher value for  $\log K_{11}$  than that obtained from the oxidation-reduction potential measurements suggested that complex  $\text{FeHL}^+$  was already present in small amounts under the acid conditions (0.02–0.05M) of the measurements. Lanford and Quinan<sup>3</sup> showed that  $\text{FeHL}^+$  was formed at high acidities (0.08–0.15M).

The effect of pH on the absorption spectra of the ferric-citrate system at different pH values is generally similar to that observed with the ferric-tartrate and ferric-malate systems. At low pH a peak is exhibited near 350 m $\mu$  and attributed to  $\text{FeL}$  (Fig. 2). This complex has been reported<sup>4</sup> to show a peak at 425 m $\mu$ .

## DISCUSSION

A common feature of complex-formation between ferric ions and tartrate, malate, and citrate is the formation of complexes designated as  $\text{FeL}$  and  $\text{Fe}_2\text{L}_2(-\text{H})_2$ . It is reasonable to assume that they have similar structures and that direct comparison of their stabilities, also the stabilities of the ferrous complexes, can be made as follows:

	$\log K(\text{FeHL})$	$\log K_{11}$	$\log \beta_{222}$
Tartrate .....	2.2	6.49	11.87
Malate .....	2.6	7.13	12.85
Citrate .....	4.4	11.40	21.17

The constants increase in the order tartrate < malate < citrate. Values reported for tartrate and malate complexes with other cations have been conflicting. Thus, malate forms weaker barium and strontium complexes but stronger magnesium and zinc complexes than tartrate.<sup>16</sup> Calcium malate has been reported as less stable than,<sup>17</sup> of equal stability to,<sup>18</sup> and more stable<sup>19,20</sup> than calcium tartrate. Feldman *et al.*<sup>21</sup> found that whereas the uranyl malate (and citrate) dimer was more stable than the uranyl tartrate dimer, the stabilities of the corresponding trimers were reversed; the findings were explained by the smaller basicity of the tartrate ion, attributed to hydrogen bonding and the inductive effect of its non-chelating hydroxyl group, compared with malate. Tridentate chelation by two carboxylate groups and one hydroxyl group had been proposed<sup>22</sup> since it was the only feature common to uranyl malate, tartrate, and citrate which could account for their similar absorption spectra. Our results with iron complexes (Fig. 2) suggest that the hydroxyl groups may be involved in complex-formation, but too firm conclusions should not be drawn because of the different degrees of complex-formation. The more pronounced peak exhibited by the tartrate monomer, compared with malate, has been attributed<sup>9</sup> to possible chelation by both hydroxyl groups; our finding that malate is the stronger complex is therefore surprising. It must be concluded that the additional measure of stability which would be expected to be conferred by co-ordination of a second hydroxyl group of tartrate must be insufficient to counteract the decrease in stability resulting from the lowered basicity of the ligand.

Although it forms weaker complexes, tartrate can be a more effective chelating agent for ferric and ferrous iron than malate under certain conditions, owing to its smaller basicity. Below pH about 3.3, tartrate reduced the concentration of ferric ions to lower values than those produced by the equivalent quantity of malate (Fig. 3). At higher pH

<sup>16</sup> Bjerrum, Schwarzenbach, and Sillén, "Stability Constants. Part I. Organic Ligands," *Chem. Soc. Special Publ.*, No. 6, 1957, Tables 64 and 65.

<sup>17</sup> Topp and Davies, *J.*, 1940, 87.

<sup>18</sup> Cannan and Kibrick, *J. Amer. Chem. Soc.*, 1938, **60**, 2314.

<sup>19</sup> Schubert and Lindenbaum, *J. Amer. Chem. Soc.*, 1952, **74**, 3529.

<sup>20</sup> Schubert, *J. Phys. Chem.*, 1952, **56**, 113.

<sup>21</sup> Feldman, North, and Hunter, *J. Phys. Chem.*, 1960, **64**, 1224.

<sup>22</sup> Feldman, Havill, and Neuman, *J. Amer. Chem. Soc.*, 1954, **76**, 4726.

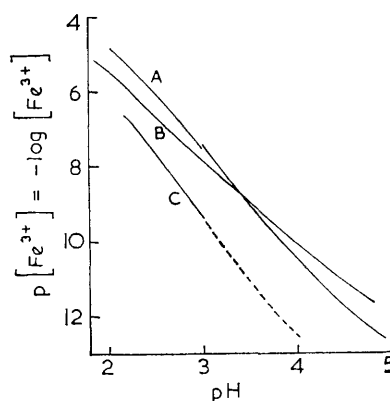
malate is the more effective chelating agent, the additional stability imparted to its complexes by virtue of their higher charge being more than sufficient to counteract the basicity effect. Citrate was more effective than either tartrate or malate (Fig. 3). The malate and citrate dimers are probably olated or oxolated structures, as suggested for the tartrate dimers,<sup>9</sup> containing internuclear hydroxyl or oxygen bridges. No further comparison can be made between malate and tartrate because of the different stoichiometry of the higher complexes. In the case of malate, these are probably non-olated and contain ionised hydroxyl groups.

The results have been interpreted on the assumption that the ligand is the species in which all the carboxyl groups are ionised. An alternative explanation, that the ligand

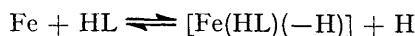
FIG. 3. Comparison of tartrate, malate, and citrate as chelating agents for ferric iron.

$[L]_t$  approx. 0.01M;  $[Fe^{III}]_t$  approx.  $2 \times 10^{-4}M$ , approx.  $5 \times 10^{-4}M$  for malate at pH > 3.

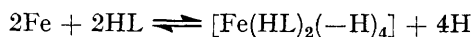
A, Malate; B, tartrate; C, citrate.



contains an undissociated carboxyl group and that the hydroxyl group ionises completely to give a five-membered ring with a carboxylate group, according to the scheme



is less likely. The equilibrium constant of the above reaction would be  $K_{11}K_{c3}$  for citrate, or  $10^{5.72}$ , and smaller than  $K_{11}$  for malate and tartrate. Similarly, the equilibrium constant for formation of the dimer according to



would be  $\beta_{222}(K_{c3})^2$ , or  $10^{9.81}$ , again smaller than  $\beta_{222}$  for malate and tartrate. Citrate would then be expected to be a less effective chelating agent than malate or tartrate, which is not in accordance with the facts. Moreover, the spectrophotometric investigation showed that consideration of  $HL^{2-}$  as the citrate ligand need be made only in very acid solutions. In this respect complex-formation between citrate and ferric iron differs from that between citrate and uranyl nitrate, where the ligand contains an undissociated carboxyl group up to pH 3.5.<sup>21,22</sup>

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