

737. *The Interaction of Transition-metal Ions with Oxaloacetic Acid. Part II.*¹ *Thermodynamics of Chelation.*

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The association of the bivalent ions of calcium, manganese, cobalt, nickel, copper, and zinc with oxaloacetic acid in aqueous solution at 25° has been studied potentiometrically. Thermodynamic equilibrium constants have been derived for reactions of the type $M^{2+} + A^{2-} \rightleftharpoons MA$, and evidence has also been obtained for the association $M^{2+} + MA \rightleftharpoons M_2A^{2+}$.

THE stabilities of the chelate compounds formed by transition-metal ions with oxaloacetic acid are of interest in connection with the catalysis of decarboxylation by these ions. Studies on the nature of the chelate compounds, and the mechanism of the decarboxylation catalysed by metal ions are described in the preceding paper.¹ A value for the association constant of copper oxaloacetate has already been derived indirectly from kinetic measurements.² Equilibrium constants for the association of rare-earth ions with oxaloacetic acid were obtained directly from potentiometric measurements,³ and this method has now been applied to the ions of the first transition series.

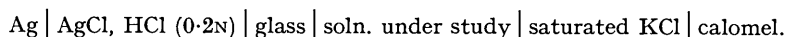
¹ Part I, preceding paper.

² Pedersen, *Acta Chem. Scand.*, 1952, **6**, 285.

³ Gelles and Nancollas, *Trans. Faraday Soc.*, 1956, **52**, 98.

EXPERIMENTAL

The pH of solutions of transition-metal oxaloacetates was measured with a glass electrode in the cell:



The glass electrode was made from Corning 015 glass and had a resistance of 20 megohms. Potentials were measured on a Tinsley potentiometer reading to 0.05 mv, a valve voltmeter being used as null-point indicator. The electrode system was standardised with standard buffer solutions: 0.01M-hydrochloric acid, 0.09M-potassium chloride, pH = 2.078; 0.05M-potassium hydrogen phthalate, pH = 4.005; and B.D.H. tabloid phosphate buffer, pH = 6.99 at 25°. The factor for converting e.m.f. into pH was constant over the whole buffer range. The calomel electrode and a beaker, containing the glass electrode and fitted with a stirrer, were mounted in a water bath kept at 25° ± 0.1°.

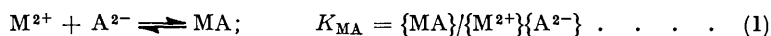
A weighed quantity of oxaloacetic acid was dissolved in water containing a known amount of hydrochloric acid or sodium hydroxide, and the solution (50 ml.) introduced into the cell. After 2 min. metal chloride solution (1–4 ml.) was added, and the e.m.f. was noted at 1 min. intervals for about 10 min. The decarboxylation of oxaloacetic acid in the presence of transition-metal ions produced a slow drift in e.m.f., and values were extrapolated to the time of mixing with an accuracy of about ±0.2 mv. Chelation is known to be very fast.¹

The purity of oxaloacetic acid was estimated by titration against alkali (Found: *M*, 132.6. Calc. for C₄H₄O₅: *M*, 132.1). Standard solutions of metal chlorides were prepared from "AnalaR" chloride or oxide and hydrochloric acid, and the strength was always checked gravimetrically.

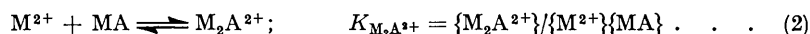
RESULTS

(The symbolism used here is the same as in Part I, preceding paper.)

Potentiometric measurements were carried out over a range of pH and metal-ion concentration. Within this range the measurements with copper oxaloacetate could be interpreted quantitatively in terms of one association equilibrium:



For other transition-metal ions the values of K_{MA} , calculated on the assumption of only one association equilibrium, varied with concentration of metal ion in such a way as to indicate that there was a small association of a second cation:



On the basis of equilibria (1) and (2) we have, for the total concentration of metal ion

$$c_{\text{m}} = [\text{M}^{2+}] + [\text{MA}] + 2[\text{M}_2\text{A}^{2+}] \quad . \quad . \quad . \quad . \quad (3)$$

for the total concentration of oxaloacetic acid

$$c_{\text{a}} = [\text{H}_2\text{A}] + [\text{HA}^-] + [\text{A}^{2-}] + [\text{MA}] + [\text{M}_2\text{A}^{2+}] \quad . \quad . \quad . \quad . \quad (4)$$

and for electroneutrality ($[\text{OH}^-] \ll [\text{H}^+]$)

$$[\text{H}^+] + 2[\text{M}^{2+}] + 2[\text{M}_2\text{A}^{2+}] + [\text{Na}^+] = 2[\text{A}^{2-}] + [\text{HA}^-] + [\text{Cl}^-] \quad . \quad . \quad (5)$$

The first and the second ionisation constant of oxaloacetic acid at 25° are ⁴

$$K_1 = \frac{\{\text{HA}^-\}\{\text{H}^+\}}{\{\text{H}_2\text{A}\}} = 2.79 \times 10^{-3} \quad . \quad . \quad . \quad . \quad (6)$$

$$K_2 = \frac{\{\text{A}^{2-}\}\{\text{H}^+\}}{\{\text{HA}^-\}} = 4.27 \times 10^{-5} \quad . \quad . \quad . \quad . \quad (7)$$

The ionic strength I is given by the equation;

$$I = \frac{1}{2} \left[[\text{Na}^+] + [\text{Cl}^-] + [\text{H}^+] + [\text{HA}^-] + 4[\text{A}^{2-}] + 4c_{\text{m}} - 4v \right] \quad . \quad . \quad (8)$$

where

$$v = c_{\text{a}} - [\text{H}_2\text{A}] - [\text{HA}^-] - [\text{A}^{2-}] \quad . \quad . \quad . \quad . \quad (9)$$

Assuming a value for the ionic strength, we can calculate the ionic activity coefficient f_1 ; a value for $[\text{H}^+]$ can then be obtained from the measured pH.

⁴ Pedersen, *Acta Chem. Scand.*, 1952, **6**, 243.

From eqns. (3)—(6) we have

$$[H_2A] = u/[2 + K_1/\{H^+\}f_1] \quad \dots \quad (10)$$

where

$$u = [Cl^-] + 2c_a - 2c_m - [Na^+] - [H^+] \quad \dots \quad (11)$$

$[HA^-]$, $[A^{2-}]$, and $\{A^{2-}\}$ can be calculated from $[H_2A]$ and eqns. (6) and (7).

The ionic strength, I , and hence the activity coefficients, are obtained by successive approximations, two such approximations usually being sufficient.

The unknown association constants K_{MA} and $K_{M_2A^{2+}}$ are then obtained from the series of pH measurements by a graphical method

$$A = (B + CK_{M_2A^{2+}}) K_{MA} - K_{MA}^2 \quad \dots \quad (12)$$

where

$$A = v / \left((c_m - v)[A^{2-}]^2 f_2^4 \right) \quad \dots \quad (13)$$

$$B = c_m / \left((c_m - v)[A^{2-}] f_2^2 \right) \quad \dots \quad (14)$$

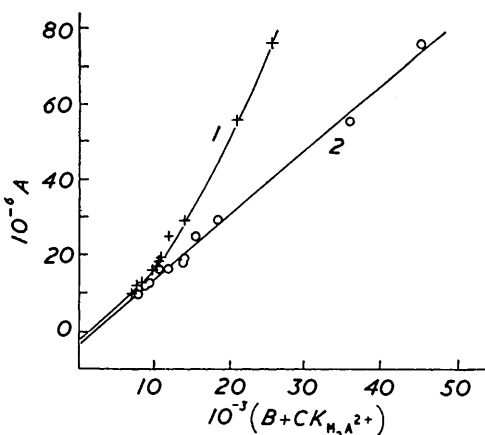
and

$$C = (c_m - 2v)^2 / \left((c_m - v)[A^{2-}] f_2^2 \right) \quad \dots \quad (15)$$

A , B , and C can be calculated for each experiment from the known initial concentrations and the values of $[H_2A]$, $[HA^-]$, and $[A^{2-}]$. When the correct value of $K_{M_2A^{2+}}$ is chosen, a plot of A against $(B + CK_{M_2A^{2+}})$ will give a straight line of slope K_{MA} and intercept $-K_{MA}^2$.

FIG. 1. Zinc oxaloacetate.

- (1) $K_{M_2A^{2+}} = 0$.
- (2) $K_{M_2A^{2+}} = 2 \times 10^2$.



Details of the pH measurements and the calculated parameters A , B , and C are given in Table 1.

Fig. 1 shows plots of A against $(B + CK_{M_2A^{2+}})$ for the experiments on zinc oxaloacetate. If only one complex of the type MA were formed, $K_{M_2A^{2+}}$ would be zero and a plot of A against B should give a straight line of slope K_{MA} and intercept $-K_{MA}^2$. In fact, when $K_{M_2A^{2+}}$ is taken as zero, a curve is obtained. Plots for assumed values of $K_{M_2A^{2+}} = 0$ and 2×10^2 are illustrated. Table 2 gives the values of K_{MA} obtained for different assumed values of $K_{M_2A^{2+}}$ and a comparison of $-K_{MA}^2$ and the intercept. It may be seen that $K_{M_2A^{2+}} = 2 \times 10^2$ fits the experimental results, giving a value of $K_{MA} = 1.7 \times 10^3$.

A plot of A against B for the experiments on copper oxaloacetate gives a straight line of slope $K_{MA} = 7.5 \times 10^4$ and intercept $-5.6 \times 10^9 = -K_{MA}^2$. The results can therefore be accounted for quantitatively in terms of one association equilibrium. The value of K_{MA} at 37° has also been obtained indirectly from kinetic data on the decarboxylation catalysed by copper ions.² From experiments at ionic strengths 0.05 and 0.100 a value of 9.7×10^4 at 37° was derived; with a reasonable estimate of the entropy change in the association reaction this value is reduced to about 7.5×10^4 at 25° .

For nickel and cobalt ions, the two association constants fitting the results were determined similarly. There is a greater scatter of the experimental points in the experiments with manganese and calcium ions. With these the second association, if it occurs at all, has an association constant $K_{M_2A^{2+}}$ less than 10^2 , but this cannot be more accurately defined, and there is therefore also a greater uncertainty in the values of K_{MA} .

TABLE I. pH measurements at 25°.

$10^3 c_m$	$10^3 c_a$	$10^3 [Na^+]$	$10^3 [Cl^-]$	I	pH	$10^{-8} A$	$10^{-8} B$	$10^{-8} C$
<i>Zinc oxaloacetate</i>								
1.94	7.35	6.72	4.12	0.0122	3.179	9.89	7.08	0.0029
1.94	7.43	6.16	4.12	0.0120	3.081	12.55	8.42	0.0048
1.94	7.18	5.32	4.12	0.0115	2.993	16.29	10.30	0.0077
3.81	7.31	7.69	8.08	0.0172	3.216	12.07	7.78	0.0060
3.81	7.13	6.59	8.08	0.0169	3.084	16.39	9.87	0.0036
3.81	7.24	6.32	8.08	0.0169	3.034	18.29	10.75	0.0150
3.81	7.24	6.32	8.08	0.0169	3.031	19.03	10.89	0.0150
3.81	7.16	6.04	8.08	0.0166	2.993	24.87	12.23	0.0164
3.81	7.17	5.50	8.08	0.0165	2.926	29.11	14.05	0.0220
7.34	6.99	5.82	15.55	0.0264	2.856	55.80	20.85	0.0746
7.34	6.78	5.03	15.55	0.0261	2.788	76.08	25.43	0.0990
<i>Copper oxaloacetate</i>								
1.96	7.32	2.80	4.12	0.0079	2.533	2817	114	0.030
1.96	7.34	—	4.12	0.0079	2.362	3552	119	0.000
1.96	7.27	—	5.52	0.0090	2.279	6831	166	0.001
1.96	7.28	—	6.92	0.0104	2.218	7283	176	0.021
3.85	7.14	—	8.08	0.0125	2.294	6717	167	0.022
3.85	7.24	—	9.45	0.0141	2.234	8160	189	0.006
3.85	7.13	—	10.83	0.0152	2.166	13,200	241	0.086
7.41	6.87	—	16.88	0.0240	2.183	15,130	279	0.254
7.41	6.86	—	18.20	0.0254	2.132	18,660	318	0.614
<i>Nickel oxaloacetate</i>								
1.92	7.22	6.72	4.04	0.0111	3.137	29.89	10.98	0.0002
1.92	7.35	6.16	4.04	0.0111	3.044	33.44	11.91	0.0013
1.92	7.31	5.60	4.04	0.0110	2.983	33.45	12.49	0.0034
1.92	7.32	5.04	4.04	0.0110	2.926	28.94	12.78	0.0072
3.77	7.27	6.59	7.92	0.0157	2.998	47.13	14.71	0.0071
3.77	7.20	6.04	7.92	0.0157	2.948	47.94	15.48	0.0117
3.77	7.21	5.49	7.92	0.0157	2.888	55.50	17.33	0.0170
3.77	7.09	4.95	7.92	0.0157	2.846	58.98	18.79	0.0235
7.26	6.93	5.82	15.26	0.0253	2.824	102.9	25.58	0.0689
7.26	6.93	5.82	15.26	0.0253	2.780	110.0	27.62	0.0849
7.26	6.87	4.76	15.26	0.0252	2.730	141.4	31.88	0.1028
7.26	6.93	4.23	15.26	0.0252	2.678	169.6	35.91	0.1237
<i>Cobalt oxaloacetate</i>								
1.96	7.40	6.72	4.12	0.0126	3.201	6.01	6.06	0.0041
1.96	7.32	5.32	4.12	0.0116	2.984	14.98	10.16	0.0084
3.85	7.15	7.69	8.09	0.0175	3.276	8.50	6.68	0.0061
3.85	7.22	6.59	8.09	0.0174	3.112	10.26	8.48	0.0140
3.85	7.16	6.04	8.09	0.0170	3.027	15.92	10.59	0.0177
3.85	7.13	5.22	8.09	0.0166	2.915	25.61	14.00	0.0257
7.42	6.93	5.82	15.58	0.0266	2.883	46.88	19.38	0.0721
7.42	6.91	5.03	15.58	0.0265	2.806	57.20	23.02	0.0972
<i>Manganese oxaloacetate</i>								
1.96	7.35	7.56	4.12	0.0137	3.398	1.97	3.72	0.0031
1.96	7.36	6.72	4.12	0.0132	3.249	2.21	4.76	0.0057
3.85	7.26	7.42	8.08	0.0183	3.277	4.07	5.49	0.0097
3.85	7.30	6.59	8.08	0.0181	3.148	4.67	6.90	0.0161
3.85	7.20	6.04	8.08	0.0177	3.072	6.44	8.37	0.0203
7.41	6.94	7.14	15.56	0.0285	3.180	5.43	7.92	0.0384
7.41	6.90	7.14	15.56	0.0281	3.160	7.56	8.70	0.0387
7.41	6.97	5.82	15.56	0.0283	3.002	9.14	11.36	0.0669
<i>Calcium oxaloacetate</i>								
2.01	7.42	9.80	4.21	0.0165	3.847	0.619	1.81	0.0009
2.01	7.41	8.96	4.21	0.0152	3.667	1.077	2.41	0.0012
2.01	7.31	7.56	4.21	0.0142	3.427	1.129	3.27	0.0038
3.93	7.25	9.62	8.25	0.0212	3.761	0.712	2.20	0.0036
3.93	7.25	8.79	8.25	0.0205	3.599	0.927	2.76	0.0056
3.93	7.14	8.24	8.25	0.0203	3.527	0.849	3.02	0.0094
3.93	7.28	7.42	8.25	0.0195	3.329	1.563	4.42	0.0118
7.58	6.87	8.46	15.90	0.0298	3.442	2.32	4.72	0.0208
7.58	6.89	7.94	15.90	0.0299	3.364	2.23	5.23	0.0267
7.58	6.89	7.14	15.90	0.0297	3.234	2.65	6.64	0.0382
7.58	6.99	6.61	15.90	0.0293	3.121	4.24	8.60	0.0502

TABLE 2. *Solution for K_{MA} and $K_{M_2A^{2+}}$: zinc oxaloacetate.*

$10^{-2}K_{M_2A^{2+}}$ (assumed)	0	1	1.5	2	3
Slope of $10^{-3}K_{MA}$	curve	2.24	1.88	1.70	1.15
$-10^{-6}K_{MA}^2$	—	-5.0	-3.5	-2.9	-1.3
Intercept	—	-7.5	-4.5	-3.0	+2.5

With calcium and manganese ions the uncertainty in K_{MA} may be of the order of 20%, while the association constants for the other metal ions are accurate to about $\pm 10\%$. Only orders of magnitude could be determined for the association constants $K_{M_2A^{2+}}$.

It has been assumed that activity coefficients of the chelate compounds are governed by the net charge. For example, those of type MA are taken as having an activity coefficient of one, although there is certainly some charge separation. The metal oxaloacetates were all studied over the same range of ionic strength. The association constant for copper oxaloacetate calculated on the basis of only one association equilibrium shows no trend with ionic strength. A comparison of the results with those for zinc, cobalt, and nickel oxaloacetate shows that the association of the second metal ion is a specific effect, which cannot be accommodated by making different assumptions about activity coefficients. The relative values of the association constants will not be greatly affected by use of different expressions for the variation of activity coefficients with ionic strength.³ The association constants are listed in Table 3.

TABLE 3. *Thermodynamic association constants at 25°.*

	Ca ²⁺	Mn ²⁺	Co ²⁺	Zn ²⁺	Ni ²⁺	Cu ²⁺
$10^{-3}K_{MA}$	0.4	0.7	1.4	1.7	3.2	75
$10^{-2}K_{M_2A^{2+}}$	<1	<1	2	2	1.5	—

DISCUSSION

The upper limit to the range of concentration of metal ion and pH studied was set by the rate of decarboxylation. Above this limit the drift of e.m.f. with time was too rapid to permit accurate back-extrapolation.

Even at the lowest pH used no evidence for the species MAH⁺ was obtained. This species can make a contribution to the rate of decarboxylation at a lower pH,⁵ but the thermodynamic tendency for its formation, $M^{2+} + HA^- \rightleftharpoons MAH^+$, is clearly much smaller than for the reaction $M^{2+} + A^{2-} \rightleftharpoons MA$, mainly because the latter association involves a much greater increase of entropy. The concentration of MAH⁺ must be very small under the present experimental conditions.

In the pH range of the potentiometric measurements the chelate compounds are predominantly of structure A.

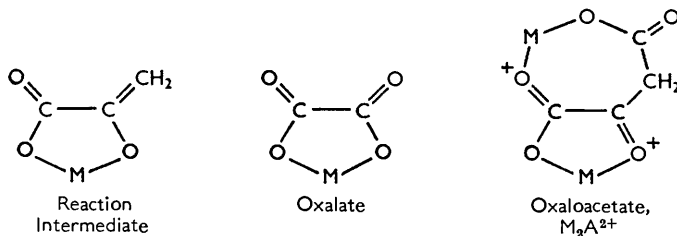


Evidence for this is discussed in the preceding paper. The proportion of enolic oxaloacetate complex B has been determined,¹ and does not appear to vary with pH in the low pH range studied. The association constants listed in Table 3 refer to any structure of type MA, either the ketonic complex A or the enolic complex B. The proportion of enolic complex is greatest for copper oxaloacetate (40%). The association constant for the ketonic complex should therefore be about 40% less than the overall association constant of 7.5×10^4 given in Table 3. A value of 4.5×10^4 would be close to that found for the association constant of copper $\alpha\alpha$ -dimethyloxaloacetate.¹

The catalytic effect of metal ions in the decarboxylation of oxaloacetic acid is related to the degree of interaction of the metal ions with oxaloacetate in the initial state and in

⁵ Gelles and Salama, following paper.

the transition state of decarboxylation.⁵ The interaction in the transition state may resemble that in metal oxalates, and the association constants of oxaloacetates and oxalates are therefore compared.^{6,7}



α -Oxocarboxylate complexes should have lower association constants than dicarboxylates of the same ring size. This is borne out by the data in Table 4. There is a general parallelism between the association constants for oxaloacetates and oxalates,

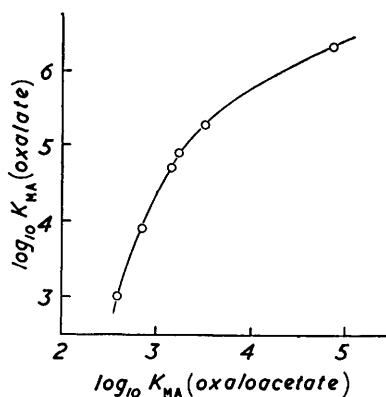


FIG. 2. Association constants for oxaloacetates and oxalates.

but Fig. 2 shows that association constants for nickel and copper oxalates are low compared with those for the oxaloacetates. This would still be true if the comparison were made with the reduced association constants for the ketonic oxaloacetate complexes.

TABLE 4. $\log_{10} K_{MA}$ for oxaloacetates, oxalates, and succinates.

	Ca ²⁺	Mn ²⁺	Co ²⁺	Zn ²⁺	Ni ²⁺	Cu ²⁺
Oxaloacetate	2.6	2.8	3.1	3.2	3.5	4.9
Oxalate	3.0	3.9	4.7	4.9	5.3	6.3
Succinate	1.9	—	—	2.5	—	3.3

The lowering of association constant with increasing ring size is illustrated by data on some succinates.⁸ Association constants for the oxaloacetate complexes M₂A²⁺ are given in Table 3. No evidence for Cu₂A²⁺ was found in the concentration range examined, but this could be due to a greater difference between the values of K_{MA} and $K_{M_2A^{2+}}$. The complexes M₂A²⁺ have association constants of the same order of magnitude as those for seven-membered ring succinates. The most likely structure for the species M₂A²⁺ is one involving a seven-membered ring compound, in which the two metal ions are competing for electrons. This could give rise to the observed variation in the values of $K_{M_2A^{2+}}$ as the complexing power of the metal ion increases from Ca²⁺ to Cu²⁺. Oxalate complexes of type M₂A²⁺ have been studied.⁹

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⁶ Money and Davies, *Trans. Faraday Soc.*, 1932, **28**, 609.

⁷ Ives, *J.*, 1933, 1360.

⁸ Irving, Williams, Ferrett, and Williams, *J.*, 1954, 3494.

⁹ Vosburgh and Beckman, *J. Amer. Chem. Soc.*, 1940, **62**, 1028.