726. *The Stability Constants of Magnesium Citrate Complexes*

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The stability constants of the magnesium complexes of the hydrogen citrate and citrate anions have been determined at 25° and $I \simeq 0{\cdot}1$ from electrometric titrations of aqueous magnesium chloride–citric acid mixtures with tetramethylammonium hydroxide solution: $\log K_1 = 1{\cdot}85$, $\log K_2 = 3{\cdot}73$.* The theory of the calculation is described. The experimental technique has been tested by determining the acid dissociation constants of citric acid and comparing them with the most reliable literature data. At 25° and $I = 0{\cdot}1$, $pK_{a1}' = 2{\cdot}88$, $pK_{a2}' = 4{\cdot}36$, and $pK_{a3}' = 5{\cdot}84$.

For detailed studies near pH 7 of magnesium-activated enzyme-catalysed reactions, such as the cleavage of citrate to oxaloacetate and acetate catalysed by citrate lyase, it is necessary to know the stoicheiometry and stability constants of the complexes of Mg^{2+} ions with both hydrogen citrate and citrate anions. The only data available are for the magnesium complex with citrate anions.[1-5] We have determined the acid dissociation constants of citric acid and the stability constants of its magnesium complexes by electrometric titration at a glass electrode with tetramethylammonium hydroxide in the presence of tetramethylammonium chloride at 25° and $I \simeq 0{\cdot}1$. Tetramethylammonium hydroxide and chloride were used because citrate anions are known to form complexes with alkali metal cations.[3,6] The measurements of the acid dissociation constants provide a valuable check of our experimental technique, since very accurate values of the constants have been obtained by e.m.f. measurements in cells without liquid junction.[7]

Acid Dissociation Constants.—These were resolved from the pH titration curve by an extension of Speakman's method.[8] For a tribasic acid H_3A we have the following equilibrium constants:

$$K_{a1}' = [H^+][H_2A^-]/[H_3A], \qquad (1)$$

$$K_{a2}' = [H^+][HA^{2-}]/[H_2A^-], \qquad (2)$$

and

$$K_{a3}' = [H^+][A^{3-}]/[HA^{2-}]. \qquad (3)$$

H_3A, H_2A^-, HA^{2-}, and A^{3-} will be used in this Paper to denote the various citrate species. Thus, Na_2HA is disodium hydrogen citrate, etc. From equations (1), (2), and (3) and $\bar{h}$, the average number of hydrogen ions removed from the acid at any given pH, we have the general expression:

$$\bar{h} = \frac{K_{a1}'/[H^+] + 2K_{a1}'K_{a2}'/[H^+]^2 + 3K_{a1}'K_{a2}'K_{a3}'/[H^+]^3}{1 + K_{a1}'/[H^+] + K_{a1}'K_{a2}'/[H^+]^2 + K_{a1}'K_{a2}'K_{a3}'/[H^+]^3}. \qquad (4)$$

Equation (4) may be rearranged as follows:

$$\bar{h}[H^+]^2/(2 - \bar{h}) = K_{a1}'[H^+](1 - \bar{h})/(2 - \bar{h}) + K_{a1}'K_{a2}' + + K_{a1}'K_{a2}'K_{a3}'(3 - \bar{h})/[H^+](2 - \bar{h}), \qquad (5)$$

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* This notation is used to avoid ambiguity in the equations below. In the generally accepted notation (see "Stability Constants," The Chemical Society, London, 1964), the constants would be K_1 (H Cit^{2-}) and K_1 (Cit^{3-}).

[1] A. B. Hastings, F. C. McLean, L. Eichelberger, J. L. Hall, and E. Da Costa, *J. Biol. Chem.*, 1934, **107**, 351.

[2] R. Nordbö, *Skand. Arch. Physiol.*, 1938, **80**, 341.

[3] M. Walser, *J. Phys. Chem.*, 1961, **65**, 159.

[4] R. J. Harvey and E. B. Collins, *J. Biol. Chem.*, 1963, **238**, 2648.

[5] S. K. Tobia and N. E. Milad, *J.*, 1964, 1915.

[6] O. Jardetzky and J. E. Wertz, *Arch. Biochem. Biophys.*, 1956, **65**, 569.

[7] R. G. Bates and G. D. Pinching, *J. Amer. Chem. Soc.*, 1949, **71**, 1274.

[8] J. C. Speakman, *J.*, 1940, 855.

which may be fitted to the values from the entire titration curve to yield the three acid dissociation constants. This method is not entirely satisfactory, since application of the method of least squares to equation (5) leads to very unwieldy numbers.

We therefore fitted the first part of the titration curve (Figure 1), from $\bar{h} \simeq 0{\cdot}4$ to $\bar{h} \simeq 1{\cdot}7$, to an equation similar to (5) for a dibasic acid:

$$\bar{h}[H^+]/(1 - \bar{h}) + K_{a1}'K_{a2}'(\bar{h} - 2)/[H^+](1 - \bar{h}) - K_{a1}' = 0, \qquad (6)$$

the assumption being made that the third acid dissociation does not interfere much in this region. $[H^+]$ was obtained from the experimental pH by adding $\log \gamma_{H^+}$ which was taken to be equal to the logarithm of the mean activity coefficient of hydrochloric acid[9] at the ionic strength used (0·1). Having obtained K_{a1}' and K_{a2}', we evaluated K_{a3}' from the equation [eqn. (5) rearranged]

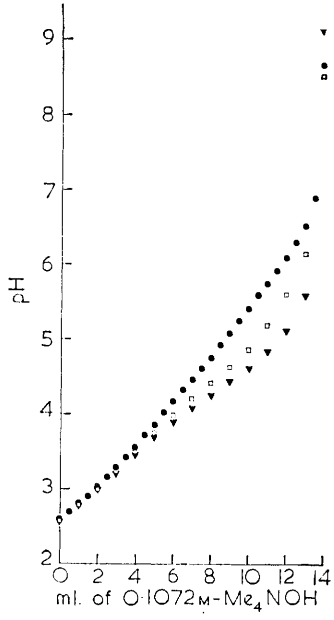

FIGURE 1

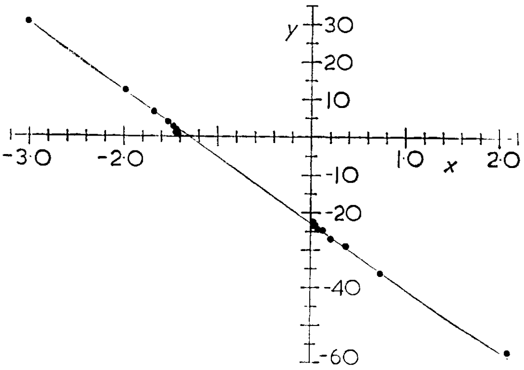

FIGURE 2

FIGURE 1. Titration curves of citric acid with and without $MgCl_2$

● 5 ml. of 0·1M-citric acid + 12·5 ml. of 0·4M-Me_4NCl + 32·5 ml. of H_2O; ionic strength kept at 0·1 by addition of water (see text). ▼ 5 ml. of 0·1M-citric acid + 5 ml. of 0·0982M-$MgCl_2$ + 8·75 ml. of 0·4M-Me_4NCl + 31·25 of ml. H_2O; citrate : magnesium = 1 : 1. ■ 5 ml. of 0·1M-citric acid + 2·5 ml. of 0·0982M-$MgCl_2$ + 10·63 ml. of 0·4M-Me_4NCl + 31·68 ml. of H_2O; citrate : magnesium = 2 : 1.

FIGURE 2. Graphical determination of the final [eqn. (8)] value of K_{a1}'

$$x = 10^3\bar{h}[H^+]/(\bar{h} - 1)$$

$$y = 10^{-3}\left[\frac{(\bar{h} - 3)K_{a3}'}{[H^+]^2(\bar{h} - 1)} + \frac{(\bar{h} - 2)}{[H^+](\bar{h} - 1)}\right]$$

$$\bar{h}[H^+]^2/(\bar{h} - 2)K_{a1}' + (\bar{h} - 1)[H^+]/(\bar{h} - 2) + K_{a2}'K_{a3}'(\bar{h} - 3)/(\bar{h} - 2)[H^+] + K_{a2}' = 0. \qquad (7)$$

$\bar{h}[H^+]^2/(\bar{h} - 2)K_{a1}' + (\bar{h} - 1)[H^+]/(\bar{h} - 2)$ was plotted against $(\bar{h} - 3)/(\bar{h} - 2)[H^+]$ using

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[9] R. G. Bates and V. E. Bower, *J. Res. Nat. Bur. Stand.*, 1954, **53**, 283.

values of $\bar{h}$ between ~1·1 and ~2·9. Having found K_{a2}' and K_{a3}', we used the latter in another form of equation (5) to find an improved value of K_{a1}':

$$K_{a3}'(\bar{h} - 3)/(\bar{h} - 1)[\mathrm{H}^+]^2 + (\bar{h} - 2)/(\bar{h} - 1)[\mathrm{H}^+] + \bar{h}[\mathrm{H}^+]/(\bar{h} - 1)K_{a1}'K_{a2}' + 1/K_{a2}' = 0. \qquad (8)$$

Then, using the new value of K_{a1}', we recalculated K_{a3}' from equation (7). Since this proved the same as before ($1{\cdot}4286 \times 10^{-6}$), we regarded the calculation as complete. The values of K_{a2}' obtained in the four iterations were $4{\cdot}87 \times 10^{-5}$, $4{\cdot}50 \times 10^{-5}$, $4{\cdot}36 \times 10^{-5}$, and $4{\cdot}39 \times 10^{-5}$, respectively. To obtain a final value of K_{a2}', we evaluated it from the expression [derived from eqn. (5)]

$$K_{a2}' = \frac{-\{\bar{h}[\mathrm{H}^+]^2/(\bar{h} - 2)K_{a1}' + (\bar{h} - 1)[\mathrm{H}^+]/(\bar{h} - 2)\}}{\{1 + K_{a3}'(\bar{h} - 3)/(\bar{h} - 2)[\mathrm{H}^+]\}}, \qquad (9)$$

for each point over the $\bar{h}$ range 1·1—1·9. The average was $4{\cdot}40_5 \times 10^{-5}$. The final plot [eqn. (8)] is presented in Figure 2. The dissociation constants are given in Table 1.

TABLE 1

Apparent dissociation constants of citric acid at 25° and $I = 0{\cdot}1$

$\mathrm{p}K_{a1}'$	$\mathrm{p}K_{a2}'$	$\mathrm{p}K_{a3}'$	Method	Ref.
2·88	4·36	5·84	*a*	This work
2·94	4·14	5·82	*a*	11
2·88	4·27	5·79	*a*	10
2·93	4·37	5·81	*b* *	7
2·94	4·41	5·83	*c*	12

a, Glass electrode titration. *b*, E.m.f. measurements in cells without liquid junction. *c*, E.m.f. measurements in cells with liquid junction

* These results were adjusted from $I = 0$ to $I = 0{\cdot}1$ by using $-\log \gamma_{\pm\mathrm{HCl}(I = 0\cdot1)} = 0{\cdot}0985$.[7]

To ensure constancy of the ionic strength during the titration, appropriate amounts of water as well as alkali were added. These were calculated as follows: the ionic strength of the solution at any point on the titration curve is given by

$$I = \tfrac{1}{2}\Sigma\{[\mathrm{Me_4N^+}] + [\mathrm{H^+}] + [\mathrm{Cl^-}] + [\mathrm{H_2A^-}] + 4[\mathrm{HA^{2-}}] + 9[\mathrm{A^{3-}}]\}. \qquad (10)$$

If V is the initial volume (ml.) of the solution, x the titre (ml.) of the tetramethylammonium hydroxide solution, and a its normality, and the concentration of tetramethylammonium chloride initially present is 0·1M, then

$$[\mathrm{Me_4N^+}] = \left(\frac{0{\cdot}1 \times V}{V + x} + \frac{xa}{V + x}\right).$$

The chloride ions are contributed by the tetramethylammonium chloride, so that

$$[\mathrm{Cl^-}] = \frac{0{\cdot}1 \times V}{V + x}.$$

From equations (1), (2), and (7) we have

$$[\mathrm{HA^{2-}}] = \left(\frac{3A_{\mathrm{T}} \times \mathrm{V}}{V + x} - \frac{xa}{V + x} - [\mathrm{H^+}]\right)\Big/\left(\frac{3[\mathrm{H^+}]^2}{K_{a1}'K_{a2}'} + \frac{2[\mathrm{H^+}]}{K_{a2}'} + 1\right).$$

Then from equations (2) and (3), we can obtain $[\mathrm{H_2A^-}]$ and $[\mathrm{A^{3-}}]$ and thus calculate the ionic strength at any value of $[\mathrm{H^+}]$.

[10] E. Heinz, *Biochem. Z.*, 1951, **321**, 314.

[11] R. C. Warner and I. Weber, *J. Amer. Chem. Soc.*, 1953, **75**, 5086.

[12] N. Bjerrum and A. Unmack, *Kgl. danske Videnskab. Selskab, Mat.-fys. Medd.*, 1929, **9**, No. 1.

From the definition of $\bar{h}$, we have

$$\bar{h} = \left(\frac{xa}{V + x} + [\text{H}^+]\right) \Big/ \left(\frac{V \times \text{A}_\text{T}}{V + x}\right) = \{xa + [\text{H}^+](V + x)\}/(V \times \text{A}_\text{T}). \qquad (11)$$

We now evaluate $[\text{H}^+]$ graphically from equation (4) for a series of values of $\bar{h}$ spanning the titration curve, and then calculate the corresponding values of x from equation (11).

In order to maintain a constant ionic strength I of 0·1 we must add an amount of water y such that

$$\frac{(V + x)I}{V + x + y} = 0{\cdot}1,$$

i.e.,

$$y = (V + x)(10I - 1). \qquad (12)$$

The values of K_{a1}', K_{a2}', and K_{a3}' required to calculate the concentrations of the various citric acid species were obtained approximately from a preliminary rough titration.

Stability Constants of the Magnesium Complexes.—The titration curves of citric acid in the presence of magnesium chloride (Figure 1) show a large drop in the region of the second and third dissociations, indicating the presence of magnesium complexes of the hydrogen citrate and citrate anions. It is difficult to determine the stoicheiometry of the complexes, since $\bar{n}$, the number of ligand species bound per metal atom cannot be evaluated in this case without making arbitrary *a priori* assumptions, unless a large number of titrations is carried out. We therefore decided to proceed initially on the simplest assumption that only 1 : 1 complexes are formed. As will be shown below, it is entirely consistent with the experimental data and there was therefore no need to invoke more complex hypotheses. We may note that a 2 : 1 hydrogen citrate : magnesium complex is in any case unlikely, by analogy with other organic doubly charged anions of similar structure, *e.g.*, tartrate,[10] which give 1 : 1 complexes only with doubly charged metal ions, such as Ca^{2+}.

Calculation of the Stability Constants.—The following equilibria for the formation of the complexes were considered:

$$\text{Mg}^{2+} + \text{HA}^{2-} \rightleftharpoons \text{MgHA};\ K_1 = \frac{[\text{MgHA}]}{[\text{Mg}^{2+}][\text{HA}^{2-}]}; \qquad (13)$$

$$\text{Mg}^{2+} + \text{A}^{3-} \rightleftharpoons \text{MgA}^-;\ K_2 = \frac{[\text{MgA}^-]}{[\text{Mg}^{2+}][\text{A}^{3-}]}; \qquad (14)$$

where HA^{2-} and A^{3-} are the hydrogen citrate and citrate anions, respectively.

At any given pH the conservation equations for total citrate and magnesium are:

$$\text{A}_\text{T} = [\text{H}_3\text{A}] + [\text{H}_2\text{A}^-] + [\text{HA}^{2-}] + [\text{A}^{3-}] + [\text{MgHA}] + [\text{MgA}^-] \qquad (15)$$

and

$$\text{Mg}_\text{T} = [\text{Mg}^{2+}] + [\text{MgHA}] + [\text{MgA}^-]. \qquad (16)$$

The equation defining the electroneutrality condition is

$$[\text{Me}_4\text{N}^+] + [\text{H}^+] + 2[\text{Mg}^{2+}] = [\text{H}_2\text{A}^-] + 2[\text{HA}^{2-}] + 3[\text{A}^{3-}] + [\text{MgA}^-] + [\text{Cl}^-] + [\text{OH}^-]. \qquad (17)$$

From equations (15) and (17) we obtain, remembering that $[\text{Cl}^-] = 2\text{Mg}_\text{T}$,

$$3\text{A}_\text{T} - [\text{Me}_4\text{N}^+] - [\text{H}^+] + [\text{OH}^-] = 3[\text{H}_3\text{A}] + 2[\text{H}_2\text{A}^-] + [\text{HA}^{2-}] + [\text{MgHA}]. \qquad (18)$$

From equations (1), (2), (3), (13), and (18), we obtain, after collecting and rearranging terms,

$$\alpha = [\text{HA}^{2-}]\{3[\text{H}^+]^2/K_{a1}'K_{a2}' + 2[\text{H}^+]/K_{a2}' + K_1[\text{Mg}^{2+}] + 1\}, \qquad (19)$$

where $\alpha = 3\text{A}_\text{T} - [\text{Me}_4\text{N}^+] - [\text{H}^+] + [\text{OH}^-]$.

Another combination of equations (15) and (17) yields

$$2A_T - [Me_4N^+] - [H^+] + [OH^-] = 2[H_3A] + [H_2A^-] - [A^{3-}] - [MgA^-], \qquad (20)$$

which on combination with equations (1), (2), (3), and (14) becomes

$$\psi = [HA^{2-}]\{2[H^+]^2/K_{a1}'K_{a2}' + [H^+]/K_{a2}' - K_2K_{a3}'[Mg^{2+}]/[H^+] - K_{a3}'/[H^+]\}, \qquad (21)$$

where $\psi = 2A_T - [Me_4N^+] - [H^+] + [OH^-]$.

Now from equations (15) and (16) we have

$$[Mg^{2+}] = [HA^{2-}]\{1 + K_{a3}'/[H^+] + [H^+]/K_{a2}' + [H^+]^2/K_{a1}'K_{a2}'\} - A_T + Mg_T. \qquad (22)$$

On substituting equation (22) for $[Mg^{2+}]$ in equations (19) and (21), we finally obtain

$$K_1\beta[HA^{2-}]^2 + \{1 + 2[H^+]/K_{a2}' + 3[H^+]^2/K_{a1}'K_{a2}' + K_1(Mg_T - A_T)\}[HA^{2-}] - \alpha = 0 \qquad (23)$$

and

$$K_2\beta(K_{a3}'/[H^+])[HA^{2-}]^2 + \{K_{a3}'/[H^+] - 2[H^+]^2/K_{a1}'K_{a2}' - [H^+]/K_{a2}' - K_2K_{a3}'(A_T - Mg_T)/[H^+]\}[HA^{2-}] + \psi = 0, \qquad (24)$$

where $\beta = 1 + [H^+]/K_{a2}' + [H^+]^2/K_{a1}'K_{a2}' + K_{a3}'/[H^+]$.

We now make use of the fact that MgHA is itself an acid with its own dissociation constant:

$$MgHA \rightleftharpoons MgA^- + H^+;\ K_c = [MgA^-][H^+]/[MgHA] = K_2K_{a3}'/K_1. \qquad (25)$$

From equations (18), (20), and (25) we obtain

$$K_c = \{[H_2A^-] - [A^{3-}] + 2[H_3A] - \psi\}[H^+]/\{\alpha - 3[H_3A] - 2[H_2A^-] - [HA^{2-}]\}. \qquad (26)$$

Using equations (1), (2), and (3), cross-multiplying, and rearranging terms, we finally get

$$[HA^{2-}] = \{\alpha K_c + \psi[H^+]\}/\{[H^+]^2/K_{a2}' - K_{a3}' + 2[H^+]^3/K_{a1}'K_{a2}' + 3[H^+]^2K_c/K_{a1}'K_{a2}' + 2[H^+]K_c/K_{a2}' + K_c\}. \qquad (27)$$

Equations (23), (24), and (27) contain the unknown variable $[HA^{2-}]$ and cannot give a closed solution for K_1 and K_2. The difficulty could be obviated by determining $[Mg^{2+}]$ and hence finding $[HA^{2-}]$ from equation (22). In the absence of a reliable experimental method, an iteration procedure was resorted to.

A series of values of K_c was assumed, and the corresponding values of $[HA^{2-}]$ [eqn. (27)] and then of K_1 and K_2 [eqn. (23) and (24)] were calculated. Incorrect values of K_c give variable values for K_1 and K_2 and correspondingly large standard deviations of the mean. K_c was then plotted against Σd^2, where d is the deviation from the mean, and the K_c corresponding to the minimum value of Σd^2 was taken as the correct value. The values of K_c obtained for the 1 : 1 and 2 : 1 titrations were $1{\cdot}062_1 \times 10^{-4}$ and $1{\cdot}110_5 \times 10^{-4}$, respectively (average $1{\cdot}086_3 \times 10^{-4}$), the corresponding values of K_2 being $5{\cdot}400 \times 10^3$ and $5{\cdot}328 \times 10^3$ (average $5{\cdot}364 \times 10^3$) and those of K_1 72·70 and 68·93 (average 70·82).

An interesting feature of the plots of K_2 against titre is that they intersect in one point (Figure 3) for all values of K_c ("isosbestic" point). However, the point is different for the two citrate : Mg ratios. Although the reason for this behaviour remains obscure, it is nevertheless evident that the plot for the true value of K_c must also pass through this point, which therefore corresponds to the true value of K_2. The respective values for the 1 : 1 and 2 : 1 ratios are $5{\cdot}40 \times 10^3$ and $5{\cdot}24 \times 10^3$, *i.e.*, the agreement with those found by minimising the variance can be regarded as satisfactory.

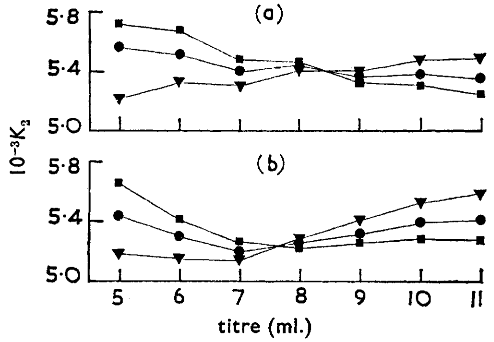

FIGURE 3. Variation of K_2 with titre for various assumed values of K_c
(a) Citrate : magnesium = 1 : 1.
(b) citrate : magnesium = 2 : 1.
■, $K_c = 1{\cdot}2 \times 10^{-4}$
●, $K_c = 1{\cdot}1 \times 10^{-4}$
▼, $K_c = 1{\cdot}0 \times 10^{-4}$.

DISCUSSION

Table 2 lists the stability constants of magnesium complexes and compares them with the values obtained by other workers, the latter being recalculated to conform with our conditions, *i.e.*, $I = 0{\cdot}1$ and 25°.

The stability constants of the magnesium complexes of hydrogen citrate and citrate anions show satisfactory constancy throughout the titration range employed in the calculations, the standard deviation of K_2 being $0{\cdot}072 \times 10^3$.

The value of log K_1 is of the order obtained for magnesium complexes of dicarboxylic acids, *e.g.*, log K_1 is 1·96 for oxaloacetic acid [13] at 25° and $I = 0{\cdot}1$ and 1·2, 1·36, and 1·55 for succinic, tartaric, and malic acids [14] at 25°, and $I = 0{\cdot}2$. No evidence has been adduced for 2 : 1 magnesium complexes of these acids.

TABLE 2

Apparent stability constants of magnesium citrate complexes at 25° and $I \simeq 0{\cdot}1$

log K_1	log K_2	Method	Ref.	log K_1	log K_2	Method	Ref.
1·85	3·73	*a*	This work	1·59	3·26	*d* †	15
—	3·11 *	*b*	5	—	3·61	*a* †	2
—	3·70	*c* †	3	—	3·61	*e* †	1

a, Glass electrode titration. *b*, Ion exchange. *c*, Spectrophotometry. *d*, Polarography. *e*, " Frog heart " method.

* Adjusted to $I = 0{\cdot}1$, by using the log K_2–I plot of Tobia and Milad [5]. † Values adjusted to $I = 0{\cdot}1$, by using the activity coefficients of Bates and Bower [9] assuming that $\log \gamma = \log \gamma_{\pm(\mathrm{HCl})}$

The stability of the magnesium complex of the citrate anion has been measured in other investigations, the most recent being that of Tobia and Milad [5] who employed an ion-exchange method and obtained log $K_2 = 3{\cdot}12$ at pH 7·1—7·2, $I = 0{\cdot}09$, and 25°. They also made measurements over a range of ionic strengths, which they extrapolated to $I = 0$ and found log $K_{2(\mathrm{I}=0)} \doteq 3{\cdot}96$. Neither of these agrees with our value (log $K_{2(\mathrm{I}=0{\cdot}1)} = 3{\cdot}73$), even when a correction for the ionic strength is applied. It is remarkable that Tobia and Milad observe virtually no variation in log K_2 at ionic strengths between about 0·045 and 0·09. The very different conditions used by Tobia and Milad, citrate : Mg ratios ranging from 40 to 100, and the fact that at pH 7·1—7·2 citrate solution contains 4—5% of the hydrogen citrate anions, cannot account for the observed discrepancy. Walser [3] determined the stability constant by a spectrophotometric method, in which he used an indicator dye. He obtained the value log $K_2 = 3{\cdot}55$ at $I = 0{\cdot}16$, which becomes 3·70 when recalculated for $I = 0{\cdot}1$, *i.e.*, is in excellent agreement with ours. Li *et al.*[15]

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[13] S. S. Tate, A. K. Grzybowski, and S. P. Datta, *J.*, 1964, 1381.
[14] R. K. Cannan and A. C. Kibrick, *J. Amer. Chem. Soc.*, 1938, **60**, 2314.
[15] N. C. Li, A. Lindenbaum, and J. M. White, *J. Inorg. Nuclear Chem.*, 1959, **12**, 122.

measured the stability constants of both hydrogen citrate and citrate anion complexes by using an indirect polarographic method. They found log $K_1 = 1{\cdot}60$ and log $K_2 = 3{\cdot}29$ at $I = 0{\cdot}09$. These are somewhat lower than our values, but their accuracy is probably low because of the approximations made, *e.g.*, the assumption that K_1/K_2 for cadmium and magnesium citrate complexes is the same. Hastings *et al.*[1] measured the constant by inhibiting the contraction of perfused frog heart by calcium citrate in the presence of varying amounts of magnesium. Nordbö[2] measured the constant by observing the pH depression (measured with a glass electrode) on addition of magnesium chloride to trisodium citrate solutions. The data of both these workers give log $K_2 = 3{\cdot}61$ when recalculated for our conditions, *i.e.*, the agreement with our values may be regarded as satisfactory. Harvey and Collins[4] recently made an electrophoretic and conductometric study of the magnesium complexes of the citrate anion. From their conductometric results they made a rough estimate of the stability constant as 10^5—10^6, which is grossly at variance with both our own and other workers' results. They found that the electrophoretic mobility of the complex was about two-thirds of that of the free citrate anion, and therefore concluded that the complex had a bi-negative charge, *i.e.*, that the hydroxyl hydrogen was ionised. Our results lend no support to this claim, there being no evidence for the ionisation of an extra proton in the presence of magnesium. Harvey and Collins apparently ignored the fact that mobility is proportional to ionic charge only at infinite dilution (for ions of the same size). The effect of ionic strength, which in their solutions appears to have been about 0·3, would tend to slow down the citrate anion much more than the singly-charged complex.

EXPERIMENTAL

Titrations.—The electrometric titrations were carried out in a cell comprising a saturated calomel electrode of the usual type and a glass electrode (EIL type GHS23). The measurements were made at 25° with a precision laboratory-built pH meter. The cell was standardised with 0·05M-potassium hydrogen phthalate (pH 4·005) and 0·01M-borax (pH 9·18).

Two titrations of citric acid alone were made. In each case a mixture of 5 ml. of 0·1M-citric acid + 12·5 ml. of 0·4M-tetramethylammonium chloride + 32·5 ml. of water (total volume 50·0 ml.) was equilibrated at 25° in a thermostatically controlled paraffin-oil bath. Tetramethylammonium hydroxide (0·1072M) was then added in 1·0 ml. portions from an automatic burette. The volume increments in the second titration were offset by 0·5 ml. and the results combined to give a single curve with experimental points at 0·5 ml. intervals. Water was added during the titration (between about 1 and 16 ml.) to keep the ionic strength constant (see above). Carbon dioxide-free nitrogen was constantly bubbled through the solution during the titration.

Two titrations of 0·5 mmole of citric acid were made in the presence of ~0·5 and ~0·25 mmole of magnesium chloride. The solutions had the following composition: (a) 5 ml. of 0·1M-citric acid + 5 ml. of 0·0982M-$MgCl_2$ + 8·75 ml. of 0·4M-Me_4NCl + 31·25 ml. of H_2O; (b) 5 ml. of 0·1M-citric acid + 2·5 ml. of 0·0982M-$MgCl_2$ + 10·63 ml. of 0·4M-Me_4NCl + 31·68 ml. of H_2O. The amounts of water added were calculated to give $I = 0{\cdot}1$ (ignoring the contribution due to citric acid). As the concentration of the alkali was about 0·1M, the ionic strength was maintained roughly at 0·1. All the constants have been calculated as true concentration constants for $I = 0{\cdot}1$.

Materials.—*Anhydrous citric acid.* The anhydrous acid was obtained by recrystallising AnalaR citric acid monohydrate from hot water, the hot solution being filtered under suction. The crystals obtained were stored in a vacuum desiccator over phosphorus pentoxide after being kept in an oven at about 55° for 3 days. A sample heated in a Stanton thermobalance was found to be completely anhydrous. The melting point was between 152 and 153°. The material was further analysed by titrating a weighed sample with a solution of carbonate-free sodium hydroxide. Its neutralisation value was 99·6.

Trisodium citrate. AnalaR trisodium citrate was recrystallised from water, the pH of the hot solution being adjusted to about 8·5 with sodium hydroxide. The crystals were dried at 110°.

Tetramethylammonium chloride. The salt (Eastman-Kodak Ltd.) was purified by precipitation from ethanol solution with anhydrous ether. The crystals were collected on a filter, washed quickly with ether, and dried in a vacuum desiccator over calcium chloride. A 0·4M-solution was prepared.

Tetramethylammonium hydroxide. An approximately 0·1M-solution of the base was prepared by diluting about 19 ml. of ~25% commercial solution (British Drug Houses Ltd.) with 500 ml. of water. Although care had been taken to exclude carbon dioxide during the dilution (constant stream of carbon dioxide-free nitrogen bubbled through water previously freed from carbon dioxide), potentiometric titration (Radiometer Automatic Titrimeter) showed an inflexion at about pH 7, indicating the presence of about 30% of carbonate. This was removed by ion-exchange chromatography on Amberlite IR400 anion-exchange resin in the hydroxide form. The initial resin in the chloride form was regenerated with 1N-sodium hydroxide until the amount of chloride in the eluate was very small. It was then washed with carbon-dioxide-free water until the pH of the effluent was 7—8. The ~0·1M-tetramethylammonium hydroxide solution was passed through the resin and titrated again. Carbonate could no longer be detected. The strength of the final alkali was 0·1072M.

Magnesium chloride. Solutions were prepared from AnalaR magnesium chloride, $MgCl_2,6H_2O$, and their magnesium content was standardised by gravimetric determination of magnesium as the pyrophosphate.

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