

**842.** *The Interaction of Calcium Ions with Some Phosphate and Citrate Buffers.*

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The pH of phosphate and citrate buffers is markedly reduced by the addition of calcium ions, owing to the formation of association products.

Dissociation constants in water at 25° are reported for the species  $\text{CaH}_2\text{PO}_4^+$ ,  $\text{CaHPO}_4$ ,  $\text{CaH}_2\text{Cit}^+$ , and  $\text{CaHCit}$  (Cit = citrate).

It is to be expected by analogy with other multivalent electrolytes that fairly extensive ion-pair formation will occur when calcium ions are introduced into buffer solutions containing multivalent anions (Davies, *Endeavour*, 1945, 4, 15). We have confirmed this by pH measurements. The pHs of Sorensen's hydrogen phosphate buffers are appreciably lowered by saturating them with the sparingly soluble calcium iodate; and with acid citrate buffers the effect is so great that the pH may be reduced by more than a unit. This increase in the hydrogen-ion concentration is due to the preferential association of the calcium ion with the most highly charged anions, leading to the displacement of equilibrium by reactions such as:  $\text{Ca}^{2+} + \text{H}_2\text{Cit}^- \longrightarrow \text{CaHCit} + \text{H}^+$ . A further effect of the interaction is the enhancement of the solubility of the calcium salt, and we have studied the interaction quantitatively by means of solubility measurements.

## EXPERIMENTAL

Calcium iodate was prepared, and its solubility measured, according to the methods used in our previous work (*J.*, 1951, 233). The buffer solutions were made up from "AnalaR" reagents and carbon dioxide-free water. pH measurements were made colorimetrically by

comparison with the succinic acid-borax standards of Kolthoff (*J. Biol. Chem.*, 1925, **63**, 135) in a Hellige comparator. A standard solution of bromocresol-green was used as indicator, except for some solutions with  $\text{pH} > 5$  when methyl-red replaced it.

*Phosphate Solutions.*—The range of solutions available for study was severely limited in this case by the insolubility of the calcium salt. According to Cameron and Seidell (*J. Amer. Chem. Soc.*, 1904, **26**, 1460) the solubility of calcium hydrogen phosphate at 25° is 1.00 millimole/l., and in agreement with this we found that potassium dihydrogen phosphate solutions, and a 19 : 1  $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$  buffer mixture, could be saturated with calcium iodate, but that precipitation occurred in the saturating column when the 9 : 1 buffer was used.

Our results are in Table 1. The first column gives the concentration of the buffer (molarity of phosphate radical), the second the solubility of calcium iodate, the third and fourth the

TABLE 1.

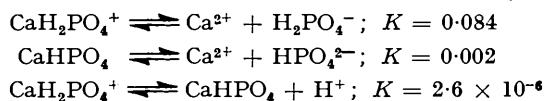
<i>M</i> *	<i>S</i> *	pH buffer	pH sat. soln. $[\text{CaH}_2\text{PO}_4^+]$	$[\text{CaHPO}_4]$	<i>I</i>	<i>K</i>
<i>A. Potassium dihydrogen phosphate solutions.</i>						
0	7.85	—	—	—	—	$(\text{CaH}_2\text{PO}_4^+)$
25.0	9.07	4.73	4.65	0.94	0.09	0.0492
50.0	9.99	4.66	4.57	1.74	0.13	0.0752
66.67	10.53	4.62	4.54	2.27	0.15	0.0924
<i>B. Mixtures of <math>\text{K}_2\text{HPO}_4</math> (0.95M) and <math>\text{Na}_2\text{HPO}_4</math> (0.05M).</i>						
						$(\text{CaHPO}_4)$
25.0	9.14	5.33	5.17	0.94	0.25	0.0497
50.0	10.11	5.30	5.10	1.68	0.44	0.0764
66.67	10.68	5.27	5.08	2.09	0.63	0.0940

\* For explanation see text.

measured pH before and after saturation with calcium iodate. In calculating the composition of the solutions allowance must be made for the species  $\text{CaIO}_3^+$ ,  $\text{KIO}_3$ ,  $\text{HPO}_4^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{CaHPO}_4$ , and  $\text{CaH}_2\text{PO}_4^+$ ;  $\text{PO}_4^{3-}$  and  $\text{HIO}_3$  are fortunately negligible at the pH's considered. A method of successive approximations was used. By starting from an assumed value for the ionic strength (*I*), the concentration of free calcium ions is obtained from the four equations (*J.*, 1951, 233; *Trans. Faraday Soc.*, 1927, **23**, 355) :

$$\begin{aligned}
 -\log_{10} f_z &= 0.5Z^2\{I^{1/2}/(1+I^{1/2}) - 0.2I\} = 0.5Z^2F(I) \\
 [\text{CaIO}_3^+] &= f_2[\text{Ca}^{2+}][\text{IO}_3^-]/0.13 \\
 [\text{KIO}_3] &= f_1^2[\text{K}^+][\text{IO}_3^-]/2.0 \\
 [\text{Ca}^{2+}] &= 7.116 \times 10^{-7}/f_2f_1^2[\text{IO}_3^-]^2
 \end{aligned}$$

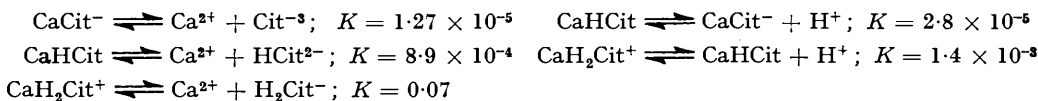
By difference,  $([\text{CaH}_2\text{PO}_4^+] + [\text{CaHPO}_4])$  is now known, and hence, again by difference,  $([\text{H}_2\text{PO}_4^-] + [\text{H}_3\text{PO}_4] + [\text{HPO}_4^{2-}])$ ; and the relative proportions of these three are fixed by the dissociation constants of phosphoric acid:  $K_1 = 7.537 \times 10^{-3}$ ,  $K_2 = 6.226 \times 10^{-8}$  (Nims, *J. Amer. Chem. Soc.*, 1933, **55**, 1946; 1934, **56**, 1110). We found it simplest to consider series *A* first on the assumption that  $[\text{CaHPO}_4]$  was negligible. This led to the extrapolated value  $K = 0.084$  for the dissociation constant of  $\text{CaH}_2\text{PO}_4^+$ , which was then used in series *B* to determine the dissociation constant of  $\text{CaHPO}_4$ . Finally this value was used in series *A* to check the dissociation constant of  $\text{CaH}_2\text{PO}_4^+$ . The concentrations calculated in these last two stages are shown in Table 1, all concentrations being in millimoles/l. The dissociation constants derived show some variation, which may be due to traces of  $\text{CaPO}_4^-$  and  $\text{KHPO}_4^-$  for which we were unable to allow; corrections for these two species would act in opposition, however, so we think the mean values 0.084 and 0.0020 are the most reliable at present available. The first acid dissociation constant of  $\text{CaH}_2\text{PO}_4^+$  is equal to  $K_{\text{HPO}_4} \times K_{\text{CaH}_2\text{PO}_4^+}/K_{\text{CaHPO}_4}$ , so we have :



This last figure is forty times greater than the dissociation constant of the  $\text{H}_2\text{PO}_4^-$  ion.

*Citrate Solutions.*—Association is very extensive in these solutions, and the solubility of calcium iodate rises much more steeply in disodium hydrogen citrate solutions than in any other salt solution yet studied. The problem of calculating the amounts of the various association products is complicated by the closeness of the three  $\text{pK}$  values of citric acid, which results in the species  $\text{H}_3\text{Cit}$ ,  $\text{H}_2\text{Cit}^-$ ,  $\text{HCit}^{2-}$ ,  $\text{Cit}^{3-}$ ,  $\text{CaH}_2\text{Cit}^+$ ,  $\text{CaHCit}$ , and  $\text{CaCit}^-$  all being present at

appreciable concentrations in the buffer solutions studied. Fortunately, the dissociation constant of  $\text{CaCit}^-$  is already known, from work in neutral citrate solutions; the value  $K = 2.3 \times 10^{-4}$ , given by Schubert and Lindenbaum (*J. Amer. Chem. Soc.*, 1952, **74**, 3529) for a solution of ionic strength 0.16, becomes, on application of the general activity coefficient expression of the previous section,  $1.27 \times 10^{-5}$  at zero ionic strength, and we have used this value throughout. The method of calculating the free calcium-ion concentration was the same as for the phosphate buffers, except that a correction for the iodate bound as  $\text{HIO}_3$  was just appreciable in the most acid buffers, and was made by using the dissociation constant  $K_{\text{HIO}_3} = 0.17$  (Davies, *J. Phys. Chem.*, 1925, **29**, 983). By subtraction, the values of ( $[\text{CaCit}^-] + [\text{CaHCit}] + [\text{CaH}_2\text{Cit}^+]$ ) and of ( $[\text{Cit}^{3-}] + [\text{HCit}^{2-}] + [\text{H}_2\text{Cit}^-] + \text{H}_3\text{Cit}$ ) are now known, and the relative proportions of these four last-named species are fixed by the dissociation constants of citric acid ( $\text{p}K_1 = 3.128$ ;  $\text{p}K_2 = 4.761$ ;  $\text{p}K_3 = 6.396$ ; Bates and Pinching, *J. Amer. Chem. Soc.*, 1949, **71**, 1274). These proportions are critically dependent on the pH; our colorimetric pH values were useful in early approximations, but the values employed in the final calculations, and shown in Table 2, were those required by the three  $\text{p}K$  values and the stoichiometric composition; they were always within 0.1 unit of the measured value. Of the three calcium ion pairs,  $[\text{CaCit}^-]$  was calculated from the equation:  $[\text{CaCit}^-] = f_2 f_3 [\text{Ca}^{2+}] [\text{Cit}^{3-}] / f_1 \cdot 1.27 \times 10^{-5}$ . An approximate value ( $9 \times 10^{-4}$ ) was calculated for  $K_{\text{CaHCit}}$  by neglecting  $[\text{CaH}_2\text{Cit}^+]$  in series *A*; this was used to obtain the values of  $K_{\text{CaH}_2\text{Cit}^+}$  shown in Table 2, series *D*; and finally the most probable value of this constant, 0.07, was used to calculate the final values of  $K_{\text{CaHCit}}$  shown in the Table. The first acid dissociation constant of  $\text{CaH}_2\text{Cit}^+$  is equal to  $K_{\text{CaH}_2\text{Cit}^+} \times K_{\text{H}_2\text{Cit}^-} / K_{\text{CaHCit}}$ , and the acid dissociation constant of  $\text{CaHCit}$  is given by  $K_{\text{CaHCit}} \times K_{\text{HCit}^{2-}} / K_{\text{CaCit}^-}$ , so the constants determined are:



The acid dissociation constant of  $\text{CaHCit}$  is 70 times that of  $\text{HCit}^{2-}$ , and that of  $\text{CaH}_2\text{Cit}^+$  is 79 times that of  $\text{H}_2\text{Cit}^-$ , so that the effect of the double positive charge is approximately the same in the two cases.

TABLE 2.

<i>M</i> *	<i>S</i> *	pH buffer	pH sat. soln.	$[\text{CaH}_2\text{Cit}^+]$	$[\text{CaHCit}]$	$[\text{CaCit}^-]$	<i>I</i>	$K \times 10^3$
<i>A. Disodium hydrogen citrate solutions.</i>								
								(CaHCit)
5.0	9.20	5.48	4.44	0.05	1.67	1.33	0.0286	0.78
10.0	10.61	5.32	4.48	0.08	2.73	2.69	0.0371	0.86
20.0	13.30	5.20	4.55	0.11	4.09	5.19	0.0576	0.91
<i>B. Na<sub>2</sub>HCit (0.9M) + HCl (0.1M).</i>								
5.0	8.98	5.18	4.34	0.05	1.48	1.00	0.0284	0.82
10.0	10.15	5.18	4.36	0.09	2.71	1.82	0.0357	0.79
20.0	12.32	5.17	4.43	0.14	4.00	3.74	0.0537	0.90
<i>C. Na<sub>2</sub>HCit (0.8M) + HCl (0.2M).</i>								
5.0	8.77	4.90	4.22	0.06	1.27	0.67	0.0282	0.86
10.0	9.66	4.84	4.24	0.11	2.23	1.29	0.0350	0.89
20.0	11.36	4.80	4.29	0.19	2.98	2.70	0.0520	1.18
<i>D. Na<sub>2</sub>HCit (0.5M) + HCl (0.5M).</i>								
								(CaH <sub>2</sub> Cit <sup>+</sup> )
10.0	8.53	3.85	3.63	0.16	0.69	0.11	0.0327	66
20.0	9.08	3.85	3.60	0.19	1.25	0.17	0.0430	104
40.0	10.04	3.77	3.56	0.56	1.99	0.27	0.0632	62

\* For explanations see text.

It may be seen from Table 2 that  $[\text{CaCit}^-]$  is an important term in all the solutions studied, so that any modification of its dissociation constant would cause a significant change in the other constants. The absence of any marked drift in the  $K_{\text{CaHCit}}$  figures is reassuring, however, and also suggests that more complex association products, such as  $\text{Ca}_2\text{Cit}^+$ , are not present at the concentrations studied.