XCIV. THE DISSOCIATION OF CALCIUM CITRATE.

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It is often stated that the addition of a citrate to milk prevents clotting by rennin because an un-ionised calcium citrate is formed, thus removing the calcium ions from solution, these ions being considered essential for the clotting process. The prevention of the clotting of blood by citrates is explained in the same way.

It is not the purpose of this paper to discuss the clotting phenomenon, but to record some measurements of the dissociation of calcium citrate. However, it must be pointed out that there is some confusion on the subject. A typical analysis of milk-serum [Van Slyke and Bosworth, 1915] shows a concentration of 0.465 g. of calcium per litre and 2.37 g. of citric acid. The citrate is present in a larger proportion than is necessary for equivalence with the calcium, so if un-ionised tricalcium citrate were formed there would be no calcium ions present. Analyses in this laboratory have shown the above figures to be typical of milks which clot normally with rennin. If the theory mentioned in the first paragraph were quite correct, most milks would not clot, for there is normally enough citrate present to prevent this.

Shear and Kramer [1928] and Shear, Kramer and Resnikoff [1929] give results of conductivity titrations of calcium salts with sodium citrate and claim that the results give "direct evidence for the binding of calcium ions by sodium citrate in some kind of soluble complex." They obtained a minimum conductivity when the added sodium citrate was approximately equivalent to the calcium present. As they adjusted the p_H of their solutions with uncertain proportions of acid and alkali, it is impossible to make an estimate of the conductivity of the solution due to any calcium ions present. The only safe deduction from their experiments seems to be that calcium citrate is less ionised than calcium chloride or sodium citrate at the same dilution. Stewart and Percival [1928] state that they have found by conductivity measurements that calcium citrate is almost undissociated in solution, but they give no particulars. It was, therefore, decided to publish the following measurements of the conductivity of simple calcium citrate solutions.

Method and apparatus.

Conductivities were measured by the usual method, using a metre bridge and post-office box with a small induction coil as source of current. The con-

ductivity cell was of 120 cc. capacity and contained electrodes of about 1 cm.² area placed 1 cm. apart. The cell constant was determined by the measurement of the conductivity of *N*/10 potassium chloride at 18° [Reilly, Rae and Wheeler, 1926].

Conductivity water was obtained by redistilling water obtained from a still of the form described by Golding [1906], rejecting the first and last portions of the distillate. It was allowed to come to equilibrium with the atmosphere and usually had a conductivity of about 2×10^{-6} mhos. This was deducted from the values obtained for the conductivity of the solutions measured.

Calcium citrate solution.

This was prepared by mixing concentrated solutions of A.R. calcium chloride and sodium citrate in equivalent proportions. Calcium citrate rapidly precipitated in the high concentration used and was washed repeatedly with distilled water by decantation until 500 cc. of the wash water, when concentrated to 20 cc., gave no reaction for chlorides. The precipitate was then washed a further ten times with conductivity water and finally allowed to stand 2 days in contact with conductivity water in order to prepare the solution for conductivity measurements. This solution was obtained by careful decantation from the solid calcium citrate. All this manipulation was done in one Pyrex flask and the solution stored in Pyrex glass. No attempt was made to obtain a saturated solution of calcium citrate but the difficulties encountered by Shear and Kramer in dissolving calcium citrate were avoided by this procedure.

150 cc. of the solution evaporated to dryness and dried to constant weight at 150° gave 0.1050 g. of total solids containing 0.0251 g. Ca; $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ requires 0.0253 g.

Table I gives results of the measurements of conductivity of different dilutions of this solution at 18°.

Table I.

Temperature 18°.

Normality of $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ solution	Specific conductivity (mhos.)	Equivalent conductivity	Degree of dissociation, α
0.00846	0.000176	20.8	0.188
0.00508	0.000118	23.2	0.209
0.00305	0.0000758	24.8	0.224
0.00183	0.0000498	27.2	0.245
0.00110	0.0000336	30.5	0.275

The degree of dissociation, α , was calculated by taking the values for the mobilities of the Ca^{++} and citrate $\equiv$ ions at 18° as 51 and 60 respectively.

On the assumption that we are simply dealing with the dissociation of $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ it is seen that the dissociation at these high dilutions is small, but by no means negligible (cf. the slight dissociation found by Riley and Fisher [1929] for some metal malonates).

Titration of citric acid with calcium hydroxide.

In order to confirm the above figures and to obtain data for more concentrated solutions, conductivity titrations of citric acid solutions with calcium hydroxide solution were made. As there were no foreign ions present in these solutions it was possible to calculate the dissociation of the calcium citrate formed in the titration.

The citric acid was recrystallised from A.R. grade citric acid, and the calcium hydroxide solution was prepared from lime obtained by heating Kahlbaum's chemically pure calcium carbonate. All solutions were made up using conductivity water.

The results of a titration of 100 cc. of 0.0104 *N* citric acid with 0.0455 *N* calcium hydroxide at 18° are shown in Fig. 1. An electrometric titration of the same solutions is also graphed. This was made by using the quinhydrone

Fig. 1.

• — • Sp. conductivity. × — × p_H .

electrode for p_H measurements. There is a sharp break in the conductivity curve when the calcium hydroxide is present in the proportion necessary for the formation of tricalcium citrate and this end-point is confirmed by the electrometric titration. The volume of calcium hydroxide solution added at the end-point and the conductivity of the resultant calcium citrate solution are readily obtained from the curve, and so the normality of the solution with respect to calcium citrate, and the dissociation, may be calculated.

Similar titrations were made of various concentrations of citric acid. The most concentrated solution used was 0.0414 *N* at the end-point. This is comparable to the usual concentration of "citric acid" in milk (about 2 g. per litre). At this concentration the solution is super-saturated. It was found that the conductivity obtained immediately after adding the equivalent proportion of calcium hydroxide did not vary over a period of 5 hours. After standing for about a week calcium citrate had precipitated but the change in conductivity over this period was not investigated.

Table II gives the results of these titrations performed at different dilutions.

Table II.

Temperature 18°.

Normality of $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ at end-point	Specific conductivity at end-point (mhos.)	Equivalent conductivity	Degree of dissociation, α
0.0414	0.000672	16.3	0.147
0.0293	0.000496	16.9	0.152
0.0134	0.000248	18.5	0.166
0.00844	0.000170	20.2	0.183
0.00425	0.000100	23.5	0.212
0.00214	0.000055	25.7	0.232

The values obtained for the degree of dissociation by the two methods are shown in Fig. 2, where they are plotted against normality of the solution.

Fig. 2.

There is close agreement between the values. Of course the numerical values obtained for the degree of dissociation could not be transferred directly to such solutions as milk and blood. The effect of ionic concentration on the dissociation of such a substance as calcium citrate is uncertain. However, the figures give some idea of the order of magnitude of the dissociation.

SUMMARY.

The dissociation of tricalcium citrate in aqueous solution at 18° has been studied, and values for the degree of dissociation are recorded.

REFERENCES.

- Golding (1906). *J. Soc. Chem. Ind.* **25**, 678.
 Reilly, Rae and Wheeler (1926). *Physico-chemical methods*.
 Riley and Fisher (1929). *J. Chem. Soc.* 2006.
 Shear and Kramer (1928). *J. Biol. Chem.* **79**, 161.
 ——— and Resnikoff (1929). *J. Biol. Chem.* **83**, 721.
 Stewart and Percival (1928). *Biochem. J.* **22**, 559.
 Van Slyke and Bosworth (1915). *J. Biol. Chem.* **20**, 135.