

# SOLUBILITY OF TRICALCIUM CITRATE IN SOLUTIONS OF VARIABLE IONIC STRENGTH AND IN MILK ULTRAFILTRATES <sup>1</sup>

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## SUMMARY

Solubility of tricalcium citrate has been determined at 21 and 95° C. by dissolution in water and by precipitation from supersaturated solutions containing various proportions of calcium to citrate and at pH 4.4 to 8.8. Solubility product in solutions at equilibrium varied with ionic strength, according to the relation  $\text{pk}_s = 17.63 - 10.84 \sqrt{\mu}$ , but was unaffected by variations in pH and temperature and by the presence of magnesium or phosphate ions. Milk ultrafiltrates were shown to be saturated with tricalcium citrate, i.e., the calculated  $\text{pk}_s$  values agreed with the solubility product in all but two of the 15 samples tested, and composition of two ultrafiltrates was unaffected by agitation with crystals of tricalcium citrate.

Few data are in the literature on the solubility of calcium citrate. It is generally believed that precipitation will not occur from moderately supersaturated solutions of calcium citrate at room temperatures and that it is necessary to heat the supersaturated solutions to about 95° C. to initiate precipitation (2, 22, 24). Crystals of tricalcium citrate obtained from concentrated solutions at 30° C. were 6-hydrate, whereas those obtained at 95° C. were 4-hydrate (2). The 6-hydrate was unstable and gradually converted to the 4-hydrate in air at room temperature (2). Solubility of the two hydrates at various temperatures, determined by agitating the crystals in water, has been reported (on anhydrous basis) as:

Partheil and Hubner (20)

4-hydrate      18° C. : 0.742 g/liter

4-hydrate      25° C. : 0.838 g/liter

Chatterjee and Dhar (2)

6-hydrate      30° C. : 2.01 g/liter

4-hydrate      30° C. : 2.20 g/liter

4-hydrate      95° C. : 1.83 g/liter

Solubility of calcium citrate has not been determined in milk ultrafiltrates or in solutions of comparable ionic strength and pH. Crystals of tricalcium citrate have been observed in evaporated milk (5), sweetened condensed milk (13), and in spray-dried skim milk powder (7). The present paper reports results of solubility measurements at 21 and 95° C. in solutions of various ionic strengths and pH values, and illustrates the effect of magnesium and phosphate on the solubility. Concentration products of tricalcium citrate in milk ultrafiltrates have been calculated from published data and from data obtained in this laboratory.

<sup>1</sup> Received for publication May 16, 1959. Issued as N.R.C. No. 5543.

## MATERIALS AND METHODS

Two methods were used to determine the solubility of calcium citrate:

- (1) Solutions containing various concentrations (10-78 mM/liter) of citric acid and calcium chloride were adjusted to pH between 4.3 and 9.0 by adding sodium hydroxide, and were seeded with crystals of tricalcium citrate and then agitated continually during crystallization. Ionic strengths of supernatants were calculated from the initial chloride and sodium concentrations and the level of residual calcium and citrate.
- (2) To obtain the solubility of the salt at low ionic strength, crystals of tricalcium citrate were agitated with distilled water.

In both methods, crystallization or dissolution at 21° C. was considered complete when the composition of supernatant remained constant for at least two days.<sup>2</sup> At 95° C., crystallization and dissolution of calcium citrate are rapid (2) and were assumed to be complete after 1 hr. with occasional agitation.

To study the effect of magnesium on the solubility of calcium citrate, 3 mM of magnesium chloride per liter was added to the supersaturated calcium chloride-trisodium citrate solutions and the pH adjusted to approximately 8.8. To study the effect of phosphate, additions of an equimolar mixture of mono- and dipotassium phosphate (1 M) were made to solutions already equilibrated at pH 6.8-7.0 with tricalcium citrate crystals. The solutions containing phosphate were agitated for a further three days to insure equilibrium under the new condition. The concentrations of phosphate chosen (3-10 mM/liter) did not induce precipitation of calcium phosphate in the calcium citrate solutions.

For analysis, supernatants were separated from crystals either by centrifugation or by filtration. Calcium was determined by a turbidimetric method (14), citrate by colorimetry (15), calcium ion with the ammonium purpurate method (27), pH with the glass electrode, magnesium by a method based on the turbidity formed by potassium erucate (16), and phosphate by the ammonium phosphomolybdate-stannous chloride method of Polley (21).

## CALCULATIONS

Solubility of tricalcium citrate was calculated as the product  $k_s = (\text{Ca}^{++})^3 \times (\text{Cit}^{\equiv})^2$ . Concentrations of  $\text{Ca}^{++}$  and  $\text{Cit}^{\equiv}$  ions are lower than the total concentrations of calcium and citrate in the supernatants because of formation of  $[\text{CaCit}]^-$  complex (8), but could be calculated (when no other complexing ions are present) from the calcium and citrate concentrations, using known dissociation constants of citric acid and calcium citrate. Two methods of calculation were used, depending on whether the pH was above or below the value for complete dissociation of citric acid (*ca.* pH 7). At pH above 7, the following known equations (8) were used:

$$\text{Ca}^{++} = \frac{-(K_4 + \text{Cit} - \text{Ca}) + \sqrt{(K_4 + \text{Cit} - \text{Ca})^2 + 4 K_4 \text{Ca}}}{2} \quad (1)$$

$$\begin{aligned} \text{since } [\text{CaCit}]^- &= \text{Ca} - \text{Ca}^{++}, \text{ and } \text{Cit}^{\equiv} = \text{Cit} - [\text{CaCit}]^- \\ \text{then } \text{Cit}^{\equiv} &= \text{Cit} - \text{Ca} + \text{Ca}^{++} \end{aligned} \quad (2)$$

<sup>2</sup> In this work, a 1% v/v addition of chloroform was added, to prevent bacterial destruction of citrate.

where Ca and Cit represent the total concentrations of calcium and citrate in moles per liter,  $K_4$  is the dissociation constant of  $[\text{CaCit}]^-$ , and brackets  $[\ ]$  indicate a complex ion.

At pH's below 7, several types of acid and salt dissociations occur (4), the most important quantitatively being:

$$\text{i) } \frac{[\text{H}_2\text{Cit}]^- \times \text{H}^+}{[\text{H}_3\text{Cit}]^\circ} = K_1 \quad \frac{[\text{HCit}]^\ominus \times \text{H}^+}{[\text{H}_2\text{Cit}]^-} = K_2 \quad \frac{\text{Cit}^\ominus \times \text{H}^+}{[\text{HCit}]^\ominus} = K_3$$

and:

$$\text{ii) } \frac{\text{Ca}^{++} \times \text{Cit}^\ominus}{[\text{CaCit}]^-} = K_4 \quad \frac{[\text{CaCit}]^- \times \text{H}^+}{[\text{CaHCit}]^\circ} = K_5$$

These were considered in the following calculations of  $\text{Ca}^{++}$  and  $\text{Cit}^\ominus$  concentrations in solutions at pH values of less than seven. The concentration of  $\text{Cit}^\ominus$  was calculated from the three dissociation constants of citric acid (11, 17):

$$\text{Cit}^\ominus = \frac{\text{Cit}_N K_1 K_2 K_3}{\text{H}^{+3} + \text{H}^{+2} K_1 + \text{H}^+ K_1 K_2 + K_1 K_2 K_3} \quad (3)$$

The net citrate was calculated as:

$$\text{Cit}_N = \text{Cit} - [\text{CaCit}]^- - [\text{CaHCit}]^\circ$$

and by substituting (ii),

$$\text{Cit}_N = \text{Cit} - \frac{\text{Ca}^{++} \times \text{Cit}^\ominus}{K_4} - \frac{\text{Ca}^{++} \times \text{Cit}^\ominus \times \text{H}^+}{K_4 K_5} \quad (4)$$

Concentration of calcium ion can be expressed from total calcium as:

$$\text{Ca} = \text{Ca}^{++} + [\text{CaCit}]^- + [\text{CaHCit}]^\circ$$

and, by substitution of (ii)

$$\text{Ca}^{++} = \frac{\text{Ca} K_4 K_5}{\text{Cit}^\ominus K_5 + \text{Cit}^\ominus \text{H}^+ + K_4 K_5} \quad (5)$$

Substituting Equation (4) and then (5) in Equation (3), and solving for trivalent citrate ions, we obtain the following equation:

$$\text{Cit}^\ominus = \frac{-b + \sqrt{b^2 + 4ZK_4 K_5 \text{Cit} (K_5 Y + \text{H}^+ Y)}}{2 (K_5 Y + \text{H}^+ Y)} \quad (6)$$

$$\text{Where } b = K_4 K_5 Y + ZK_5 \text{Ca} + Z\text{H}^+ \text{Ca} - ZK_5 \text{Cit} - Z\text{H}^+ \text{Cit},$$

$$Y = \text{H}^{+3} + \text{H}^{+2} K_1 + \text{H}^+ K_1 K_2 + K_1 K_2 K_3,$$

$$Z = K_1 K_2 K_3$$

The citric acid dissociation constants used where those reported in the literature for ionic strengths and temperatures close to those existing in the supernatants studied:  $K_1$ :  $1.19 \times 10^{-3}$ ;  $K_2$ :  $4.30 \times 10^{-5}$ ;  $K_3$ :  $1.73 \times 10^{-6}$  for  $\mu$  of 0.1

and a temperature of 18° C. (1), and  $K_5: 2 \times 10^{-5}$  for  $\mu$  of 0.08 and a temperature of 37° C. (19).  $K_5$  values were not found for ionic strengths other than 0.08 and, therefore, the study of solubility at low pH was done with solutions close to that ionic strength only.  $K_4$  was used with supernatants of various ionic strengths and its value was, therefore, calculated for each supernatant from the relationship  $pK_4 = 4.64 - 3.64 \sqrt{\mu}$ , derived from  $pk_4$  values determined by various techniques (4, 8, 9, 18, 19, 25) at temperatures around 20° C. and ionic strengths between 0 and 0.16.

Magnesium, when present, competes with calcium for the binding of citrate but, because the citrates of magnesium and calcium are about equally dissociated (8, 19), it can be shown that substituting the sum of  $Ca + Mg$  for  $Ca$  in Equations (1), (6), and (5), will give the sum of  $Ca^{++} + Mg^{++}$  in Equations (1) and (5). Since  $Ca^{++}/Mg^{++}$  will be approximately equal to  $Ca/Mg$ , the  $Ca^{++}$  and  $Mg^{++}$  concentrations can be calculated, once the sum is known.

It can be estimated (6) that, usually, about 1 mM of calcium or magnesium per liter is bound by phosphate in solutions comparable to milk serum. Although small, this binding can not be neglected, because it might reduce  $Ca^{++}$  concentration significantly. The amount of calcium (or  $Ca + Mg$ ) bound was, therefore, calculated, assuming that  $Ca^{++}$  (or  $Mg^{++}$ ) and  $HPO_4^-$  associate in the ratio of 1:1 to form  $CaHPO_4$  and that the concentration of other forms of undissociated calcium phosphate was negligible.

$$\text{Since} \quad \frac{Ca^{++} \times HPO_4^-}{CaHPO_4} = K_p$$

$$\frac{HPO_4^- \times H^+}{H_2PO_4^-} = K_A$$

$$\text{and} \quad P = H_2PO_4^- + HPO_4^- \quad (\text{between pH 5 and 8})$$

therefore, by substitution:

$$CaHPO_4 = \frac{Ca^{++} \times P \times K_A}{(K_A + H^+) \times K_p} \quad (7)$$

where  $P$  is the total phosphate concentration,  $K_A$  is the second acid dissociation constant of phosphoric acid, i.e.,  $1.68 \times 10^{-7}$  (10), and  $K_p$  is the dissociation constant of  $CaHPO_4$ , i.e.,  $1.5 \times 10^{-2}$  (6).

In solutions containing calcium, citrate, and phosphate, the calculation of  $Ca^{++}$  and  $Cit^{\equiv}$  concentrations was made as follows: A first approximation of  $Ca^{++}$  was made, using Equations (6) and (5); concentration of  $CaHPO_4$  was then calculated from Equation (7) (two approximations were made) and then subtracted from the concentration of total calcium; finally, the net calcium concentration was used in Equations (6) and (5) to calculate the second approximation of  $Ca^{++}$  and  $Cit^{\equiv}$  concentrations. A greater number of approximations did not change the results significantly. Where magnesium also was present in the solution, the sum of concentrations of  $Ca + Mg$  was used in the calculations instead of  $Ca$  alone.

## RESULTS AND DISCUSSION

*Calcium citrate solutions.* Crystals started to appear in supersaturated solutions of calcium citrate after about two days at 21° C. and continued to form for about 12 days. Seeding and agitation reduced crystallization time to three or four days over-all. However, to obtain rapid crystal growth in solutions that were only slightly supersaturated, it was found that at least 25 mg. of calcium citrate (anhydrous basis) per 100 ml. of solution had to be introduced as seed crystals. At 95° C., crystals appeared rapidly and crystallization appeared complete in less than 1 hr. The crystals obtained at both temperatures were examined microscopically and were found to have the appearance of clusters of needles. The molar calcium/citrate ratio  $1.497 \pm .014$  was that of tricalcium citrate.

The instability of water of crystallization at normal experimental temperatures prevented its accurate determination. However, no difference was found in the amount of water of crystallization, nor in the rate of dehydration, between crystals obtained at 21 and 95° C. Crystals kept for ten days in open air and then ten days over P<sub>2</sub>O<sub>5</sub> at 25° C. lost over 60% of their water of crystallization. Complete dehydration was accomplished by heating samples overnight in a vacuum oven at 100° C.; further heating at 130° C. did not change the weight. Other workers (2) have found up to 16 molecules of water of crystallization in crystals obtained at room temperature from highly supersaturated solutions, and four waters in crystals obtained at 95° C.

The relation between calcium and citrate concentrations in the supernatants at equilibrium at pH 7 or above is shown in Figure 1. The curve passed through a minimum at a point corresponding to 6 mM of calcium and 3 mM of citrate per liter. The increased concentration of calcium at higher citrate concentrations was due partly to binding of calcium by citrate, and partly to increased solubility owing to increased ionic strength.

Analysis of supernatants at equilibrium below pH 7 are given in Table 1 and show that, when ionization of citric acid was repressed by a lower pH value, greater concentrations of citrate and calcium were required to saturate the solu-

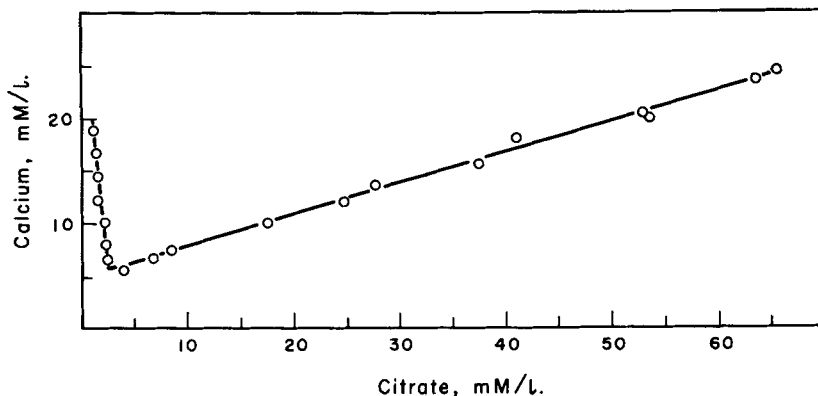


FIG. 1. Concentration of calcium and citrate at equilibrium, in solutions at or above pH 7 and at 21° C.

TABLE 1  
Composition of supernatants at equilibrium below pH 7 and at 21 and 95° C.

| Temp.<br>° C. | Initial<br>pH | At equilibrium, concentrations in mM/liter |      |      |                  |                  |       |
|---------------|---------------|--|------|------|------------------|------------------|-------|
|               |               | pH   | Ca   | Cit  | Ca <sup>++</sup> | Cit <sup>≡</sup> | $\mu$ |
|               |               | By precipitation <sup>a</sup>              |      |      |                  |                  |       |
| 21            | 4.40          | 4.05                                       | 21.7 | 10.8 | 14.8             | .027             | .094  |
| 21            | 4.90          | 4.35                                       | 14.9 | 6.55 | 10.3             | .035             | .082  |
| 21            | 6.70          | 5.50                                       | 9.56 | 2.75 | 7.01             | .066             | .069  |
| 21            | 8.20          | 5.80                                       | 8.96 | 2.45 | 6.64             | .068             | .068  |
| 95            | 6.70          | 5.92                                       | 11.0 | 3.32 | 7.89             | .095             | .070  |
|               |               | By dissolution <sup>b</sup>                |      |      |                  |                  |       |
| 21            | .....         | 6.72                                       | 4.00 | 2.87 | 1.22             | .090             | .004  |
| 95            | .....         | 6.52                                       | 3.12 | 2.02 | 1.17             | .067             | .004  |

<sup>a</sup> Initial solution contained 28.0 mM/liter calcium and 15.0 mM/liter citrate.

<sup>b</sup> Crystals of tricalcium citrate dissolved in water. Since  $K_s$  was not known at low ionic strength, calculations were made by Equation (1).

tion. However, as stated previously, the composition of the precipitate remained that of tricalcium citrate.

Solubility products  $(Ca^{++})^3 \times (Cit^{\equiv})^2$  obtained at 21° C. varied with ionic strength. The relation between  $pk_s$  ( $-\log$ . of solubility product) and  $\sqrt{\mu}$  can be expressed as:  $pk_s = (17.63 \pm 0.08) - (10.84 \pm 0.23)\sqrt{\mu}$  over the range of ionic strengths studied (Figure 2). The  $pk_s$  values for solutions at equilibrium

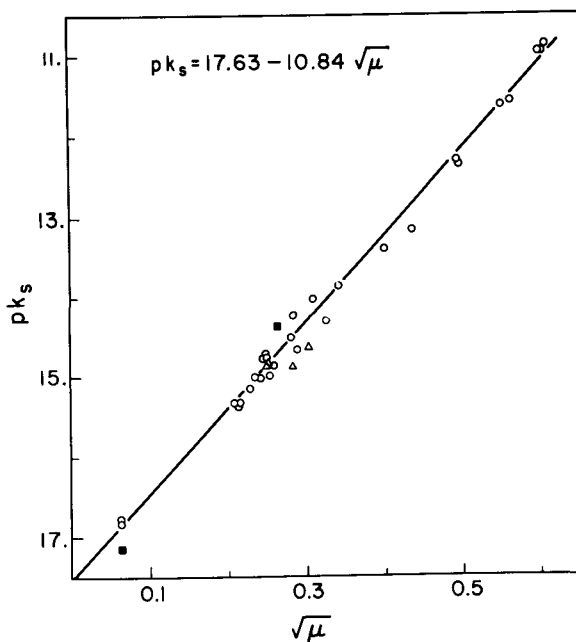


FIG. 2. Variation of  $pk_s$  value with  $\sqrt{\mu}$  ionic strength:

- pH > 7, 21° C. (data of Figure 1)
- △ pH < 7, 21° C. (data of Table 1)
- pH < 7, 95° C. (data of Table 1)

below pH 7 ( $\mu$  0.08) agreed within experimental variations with the value calculated for ionic strength 0.08. Also, solubility products calculated for supernatants obtained at 95° C. were not significantly different from those obtained at 21° C.

The presence of phosphate or magnesium in the calcium citrate solutions had no effect on the  $pk_s$  values, beyond those expected from the increased ionic strength (Tables 2 and 3). Addition of phosphate to the three saturated calcium citrate solutions did not measurably affect concentration either of total supernatant calcium and of citrate or  $Ca^{++}$  ion. The presence of magnesium, however, increased the level of  $Ca^{++}$  and decreased  $Cit^{\equiv}$  concentration in all solutions studied. In two of the solutions, these changes were just compensating and, therefore, no other changes were required to maintain saturation. However, in the solution containing an excess of calcium over citrate, saturation was maintained by an increase in concentration of total calcium and citrate.

*Milk ultrafiltrates.* Concentrations of calcium and magnesium ions were calculated for ultrafiltrates prepared in this laboratory and for ultrafiltrates obtained by other workers (19, 23, 26, 28) and compared with the determined values (Table 4). The pH was not available for ultrafiltrates prepared by Smeets (26), but was assumed to be 6.77. Small errors in pH do not introduce large errors in calculating  $Ca^{++}$  and  $Cit^{\equiv}$  concentrations. Even completely disregard-

TABLE 2  
Effect of magnesium on solubility of calcium citrate at pH above 7 and at 21° C.  
(Concentrations in mM/liter)

| Initial concentration |      |           | Concentrations at equilibrium |      |           |           |                |       |          |
|-----------------------|------|-----------|-------------------------------|------|-----------|-----------|----------------|-------|----------|
| Ca                    | Cit  | Mg. added | Ca                            | Cit  | $Ca^{++}$ | $Mg^{++}$ | $Cit^{\equiv}$ | $\mu$ | $pk_s^a$ |
| 18.5                  | 12.0 | Nil       | 5.53                          | 4.01 | 1.81      | .....     | 0.29           | .044  | 14.77    |
| 18.5                  | 12.0 | 3.0       | 7.03                          | 4.33 | 4.07      | 1.74      | 0.10           | .054  | 14.66    |
| 22.8                  | 21.8 | Nil       | 8.50                          | 12.5 | 0.38      | .....     | 4.38           | .069  | 14.80    |
| 22.8                  | 21.8 | 3.0       | 8.10                          | 12.0 | 0.79      | 0.28      | 2.07           | .075  | 14.67    |
| 30.0                  | 38.0 | Nil       | 13.4                          | 27.7 | 0.57      | .....     | 14.9           | .162  | 14.77    |
| 30.0                  | 38.0 | 3.0       | 13.4                          | 27.3 | 0.71      | 0.16      | 11.8           | .153  | 14.57    |

<sup>a</sup>  $pk_s$  expressed on the basis of ionic strength ( $\mu$ ) 0.08.

TABLE 3  
Effect of phosphate on solubility of calcium citrate at pH's 6.8-7.0 and at 21° C.  
(Concentrations in mM/liter)

| Initial concentrations <sup>b</sup> |      |         | Concentrations at equilibrium |      |           |                |       |          |  |
|-------------------------------------|------|---------|-------------------------------|------|-----------|----------------|-------|----------|--|
| Ca                                  | Cit  | P added | Ca                            | Cit  | $Ca^{++}$ | $Cit^{\equiv}$ | $\mu$ | $pk_s^a$ |  |
| 6.45                                | 3.7  | Nil     | idem                          | idem | 2.92      | .27            | .054  | 14.26    |  |
| 6.45                                | 3.7  | 3       | 6.55                          | 3.67 | 2.74      | .21            | .058  | 14.52    |  |
| 8.35                                | 12.3 | Nil     | idem                          | idem | 0.45      | 4.40           | .075  | 14.72    |  |
| 8.35                                | 12.3 | 8       | 8.73                          | 11.8 | 0.55      | 3.80           | .085  | 14.79    |  |
| 12.6                                | 26.8 | Nil     | idem                          | idem | 0.55      | 14.7           | .153  | 14.73    |  |
| 12.6                                | 26.8 | 10      | 13.0                          | 27.0 | 0.58      | 14.8           | .165  | 14.86    |  |

<sup>a</sup>  $pk_s$  expressed on the basis of ionic strength ( $\mu$ ) 0.08.

<sup>b</sup> Solutions were already equilibrated with tricalcium citrate before phosphate was added. Dilution introduced by addition of phosphate was of the order of 1%.

TABLE 4  
Composition of milk ultrafiltrates  
(Concentrations in mM/liter)

| Sample   | Determined                     |      |      |                 |      |                  | Calculated       |                  |                  |                    |
|----------|--------------------------------|------|------|-----------------|------|------------------|------------------|------------------|------------------|--------------------|
|          | pH                             | Ca   | Mg   | Cl <sup>-</sup> | P    | Ca <sup>++</sup> | Mg <sup>++</sup> | Ca <sup>++</sup> | Mg <sup>++</sup> | pk <sub>s</sub>    |
| 1        | 6.77                           | 7.77 | 2.73 | 8.34            | 14.5 | 2.7              | .....            | 1.38             | 0.48             | 14.64              |
| 1 seeded | 6.74                           | 7.80 | 2.73 | 8.35            | 14.5 | .....            | .....            | 1.41             | 0.50             | 14.63              |
| 2        | 6.88                           | 6.80 | 2.65 | 7.80            | 14.1 | 2.6              | .....            | 1.16             | 0.45             | 14.78              |
| 2 seeded | 6.72                           | 6.44 | 2.84 | 7.77            | 14.1 | .....            | .....            | 1.18             | 0.52             | 14.81              |
| 3        | 6.87                           | 9.74 | 2.86 | 8.72            | 9.81 | .....            | .....            | 2.49             | 0.72             | 14.22              |
|          | White and Davies (28)          |      |      |                 |      | 4.25             | .....            | 3.53             | 1.32             | 14.16 <sup>a</sup> |
|          | White and Davies (28)          |      |      |                 |      | 3.78             | .....            | 2.76             | 0.78             | 14.36              |
|          | White and Davies (28)          |      |      |                 |      | 2.90             | .....            | 2.78             | 0.97             | 14.34              |
|          | Smeets (26)                    |      |      |                 |      | 3.3              | .....            | 2.58             | 0.80             | 14.18              |
|          | Smeets (26)                    |      |      |                 |      | 2.3              | .....            | 1.62             | 0.55             | 14.86 <sup>a</sup> |
|          | Smeets (26)                    |      |      |                 |      | 4.4              | .....            | 3.71             | 1.25             | 14.44              |
|          | Rose and Tessier (23)          |      |      |                 |      | 2.8              | .....            | 1.82             | 0.63             | 14.47              |
|          | Nordbo (19)                    |      |      |                 |      | 1.77             | 0.46             | 1.62             | 0.48             | 14.51              |
|          | Nordbo (19)                    |      |      |                 |      | 2.06             | 0.48             | 1.62             | 0.47             | 14.41              |
|          | Nordbo (19)                    |      |      |                 |      | 1.66             | 0.47             | 2.20             | 0.60             | 14.24              |
|          | Kreveld and van Minnen (12)    |      |      |                 |      | 1.8              | 0.65             | .....            | .....            | .....              |
|          |                                |      |      |                 |      | to               | to               | .....            | .....            | .....              |
|          |                                |      |      |                 |      | 3.0              | 1.10             | .....            | .....            | .....              |
|          | Christiansen <i>et al.</i> (3) |      |      |                 |      | 1.7              | 0.74             | .....            | .....            | .....              |
|          |                                |      |      |                 |      | to               | to               | .....            | .....            | .....              |
|          |                                |      |      |                 |      | 2.1              | 0.82             | .....            | .....            | .....              |

<sup>a</sup> Differed significantly from statistically determined value for ionic strength 0.08. Seeded samples were agitated 67 hr. at 21° C., after seeding.



ing acid dissociations for an ultrafiltrate at pH 6.53 (i.e., assuming pH 7.0 or above), resulted in a  $\text{Cit}^{\equiv}$  value that was only 3.8% high, a  $\text{Ca}^{++}$  that was 2% low, and a  $\text{Mg}^{++}$  that was 2.4% low but caused no change in  $\text{pk}_s$  value. The calculated  $\text{Mg}^{++}$  concentration varied with different ultrafiltrates and covered about the same range of values as the published data (3, 12, 19) summarized in Table 4. Calculated  $\text{Ca}^{++}$  concentrations were generally lower than the determined values, but the difference was not constant, indicating that the determined  $\text{Ca}^{++}$  concentration did not always follow changes in concentration of the complexing ions.

The ion product ( $\text{pk}_s$ ) for the ultrafiltrates was obtained from the calculated  $\text{Ca}^{++}$  and  $\text{Cit}^{\equiv}$  concentrations (Table 4) and it agreed in most cases with the values obtained with solutions of calcium citrate at  $\mu$  0.08 (Table 2). Also, agitation of two different milk ultrafiltrates with crystals of tricalcium citrate at 21° C. for 67 hr. did not change their composition (1 seeded and 2 seeded, Table 4), indicating that these ultrafiltrates were already saturated with the salt. The two significant deviations may have been caused by differences in ionic strength or by the presence of unknown complexing agent(s). Actually, relatively small variations in ionic strength (from 0.11 to 0.07) could explain these variations.

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