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Original Article

Codissolution of calcium hydrogenphosphate and sodium hydrogencitrate in water. Spontaneous supersaturation of calcium citrate increasing calcium bioavailability

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ABSTRACT

The sparingly soluble calcium hydrogenphosphate dihydrate, co-dissolving in water during dissolution of freely soluble sodium hydrogencitrate sesquihydrate as caused by proton transfer from hydrogencitrate to hydrogenphosphate, was found to form homogenous solutions supersaturated by a factor up to 8 in calcium citrate tetrahydrate. A critical hydrogencitrate concentration for formation of homogeneous solutions was found to depend linearly on dissolved calcium hydrogenphosphate: $[\text{HCitr}^{2-}] = 14[\text{CaHPO}_4] - 0.05$ at 25 °C. The lag phase for precipitation of calcium citrate tetrahydrate, as identified from FT-IR spectra, from these spontaneously formed supersaturated solutions was several hours, and the time to reach solubility equilibrium was several days. Initial calcium ion activity was found to be almost independent of the degree of supersaturation as determined electrochemically. The supersaturated solutions had a pH around 4.7, and calcium binding to hydrogencitrate as the dominant citrate species during precipitation was found to be exothermic with a determined association constant of 357 L mol^{-1} at 25 °C for unit ionic strength, and $\Delta H^\circ = -22 \pm 2 \text{ kJ mol}^{-1}$, $\Delta S^\circ = -26 \pm 8 \text{ J K}^{-1} \text{ mol}^{-1}$. Calcium binding to hydrogencitrate and, more importantly, to citrate is suggested to decrease the rate of precipitation by lowering the driving force of precipitation, and becoming important for the robust spontaneous supersaturation with perspectives for design of functional foods with increased calcium bioavailability.

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1. Introduction

Osteoporosis, as caused by calcium malabsorption often also for individuals with a high dietary calcium intake, affects 75 million people worldwide and is specially a problem for the elderly [1]. Current theories do not offer explanations for the apparent paradox of low bioavailability of calcium even from foods for which the dietary calcium is known to dissolve in the gastric juice as calcium ions during digestion [2].

Calcium absorption mainly occurs in the intestines (i) through transcellular, saturable transport through cells, as regulated by vitamin D, and (ii) through paracellular, non-saturable transport between cells as regulated by diffusion [3]. Both of these absorption processes depend, however, on the concentration of free calcium, and calcium absorption is hampered by precipitation by phosphates, oxalate, phytates and carbonate for the conditions of increasing pH in the intestines. The paracellular path seems quantitatively the most important although the two absorption paths seem to interact depending on individual physiological conditions [4].

Complex binding of calcium by peptides, amino acids and hydroxycarboxylates may prevent precipitation, but will also lower the free calcium concentration below the critical value for spontaneous diffusion [2]. Supersaturation of calcium salts in the intestine may, accordingly, be important for the calcium gradient from the chyme in the intestines to the free calcium level around 10^{-3} mol L⁻¹ in the extracellular fluid behind the epithelium.

Hydroxycarboxylates like gluconate and citrate are known to form supersaturated calcium salt solutions [5,6]. Isothermal dissolution of combinations of sparingly soluble calcium salts and sodium salts of potential ligands for calcium have been shown spontaneously to form highly supersaturated solutions of remarkable robustness. Such solubility overshooting could explain the positive effect of citrate on calcium absorption, bone formation and fracture healing in bones through increased calcium mobility despite the low solubility of calcium citrate [7–10].

Injection fluids for veterinary calcium therapy have been formulated as supersaturated aqueous calcium gluconate solutions made by heating and stabilized through addition of other hydroxycarboxylates for long term storage apparently without a detailed understanding of the mechanism behind the surprising robustness of supersaturation at ambient temperatures [11]. A breakthrough in such understanding seems, however, possible expanding the kinetic models recently published for spontaneous supersaturation of calcium hydroxycarboxylates in the presence of citrate [6]. A further step forwards in the development of novel functional foods with high mineral bioavailability and of food supplements for treatment of calcium deficiency especially for the elderly seems to depend on combining calcium phosphates and citrates [12]. Results of studies of such combinations are now reported, which will hopefully lead to development of new functional foods and novel drug products.

2. Methods and materials

2.1. Materials

Calcium hydrogenphosphate dihydrate, sodium hydrogencitrate sesquihydrate and nitric acid were from Sigma Aldrich (Steinheim, Germany). Calcium chloride dihydrate was from Merck (Darmstadt, Germany). All aqueous solutions were made from purified water from Milli-Q Plus (Millipore Corporation, Bedford, MA).

2.2. Electrochemical measurement of calcium ion activity

Calcium ion activity, $a_{\text{Ca}^{2+}}$, was measured using a calcium ion selective electrode ISE25Ca with a reference REF251 electrode from Radiometer (Copenhagen, Denmark). The calibration solutions used for calibration of electrode were prepared as aqueous CaCl₂ solutions with concentration of 1.00×10^{-4} , 1.00×10^{-3} , 1.00×10^{-2} mol L⁻¹ prepared from a 1.000 mol L⁻¹ CaCl₂ stock solution at 10, 20, 25 °C, and 30 °C. Calcium ion activity, $a_{\text{Ca}^{2+}}$, in the standard solutions was calculated based on the relationship between activity and concentration according to

$$a_{\text{Ca}^{2+}} = c_{\text{Ca}^{2+}} \gamma^{2+} \quad (1)$$

where γ^{2+} is the activity coefficient calculated from the Davies' equation as described previously [13]

$$\log \gamma^{2+} = -A_{\text{DH}} z^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.30I \right) \quad (2)$$

where A_{DH} is the Debye-Hückel constant with the numerical value of $A_{\text{DH}} = 0.498, 0.506, 0.510,$ and $0.515,$ at 10 °C, 20 °C, 25 °C, and 30 °C, respectively, and $z = 2$ for calcium ions [14]. The calcium ion activity in the test solutions was calculated as described previously [13].

2.3. ICP-OES determination of total calcium and total phosphate

The samples were filtered (589/3, Whatman, Dassel, Germany) and 10 µL were added to 9.99 mL of HNO₃ 5%. The samples were analysed by inductively coupled plasma-optical emission spectroscopy using an Agilent 5100 ICP-OES (Santa Clara, CA, USA) and the wavelengths of 396.847 nm and 177.434 nm were monitored to quantify total calcium and total phosphorus, respectively.

2.4. FTIR of precipitates

The precipitates collected from the experiments after equilibrium was reached were characterized by infrared spectroscopy using a FT-IR spectrometer (Bomen MB100, ABB, Quebec, Canada) equipped with ATR attachment. All the spectra were obtained by accumulation of 64 scans, with resolution of 4 cm⁻¹, at 550–4000 cm⁻¹.

2.5. Dissolution of solid calcium hydrogenphosphate dihydrate and sodium hydrogencitrate sesquihydrate and determination of the critical ratio

Several combinations of calcium hydrogenphosphate dihydrate and sodium hydrogencitrate sesquihydrate were investigated in order to determine the critical amount of sodium hydrogencitrate sesquihydrate required to dissolve a specified amount of calcium hydrogenphosphate dihydrate resulting in supersaturated solutions of calcium citrate tetrahydrate. The following combinations of sodium hydrogencitrate sesquihydrate and calcium hydrogenphosphate dihydrate resulted in supersaturated (homogeneous) solutions: 1.00, 2.00, 2.50, and 3.00 g (5.81, 11.6, 14.5, and 17.4 mmol) of solid calcium hydrogenphosphate dihydrate combined with 10.00, 30.00, 40.00, and 55.00 g ($3.80 \cdot 10^{-2}$, 0.114, 0.152, and 0.209 mol) of solid sodium hydrogencitrate sesquihydrate, respectively. To each of these combinations of solids, 100 mL of water was added. The samples (A, B, C, and D) were stored at 25 °C under constant stirring, pH was measured using a 713 pH Meter (Metrohm, Herisau, Switzerland), and the samples were analysed for calcium ion activity using a calcium ion selective electrode up to 144 h while precipitation occurred. The sample made from 11.6 mmol of calcium hydrogenphosphate and 0.114 mol of sodium hydrogencitrate in 100 mL of water was further analysed for total calcium and total phosphorus, both quantified by ICP during precipitation. All these analyses were made periodically starting when the solutions became supersaturated and continued until equilibrium was reached in the samples. All the samples and analyses were made in duplicates.

2.6. Potentiometric determination of association constant

Aqueous solutions of hydrogencitrate were prepared from sodium hydrogencitrate sesquihydrate in concentrations of $0.00100 \text{ mol L}^{-1}$ and $0.0100 \text{ mol L}^{-1}$. Solution of CaCl_2 was added to each sample with the final concentration of $0.000500 \text{ mol L}^{-1}$ of calcium in the samples. All samples remained homogenous during equilibration for 1 h at 10.0 °C, 20.0 °C, 25.0 °C, and 30.0 °C. The calcium ion activity was determined by the calcium ion selective electrode at each of the four investigated temperatures. The calcium ion activity was used for calculation of an association constant. All samples were prepared in duplicates.

3. Results and discussion

Supersaturated homogeneous solutions appeared after 30–60 min after the addition of water to mixtures of solid calcium hydrogenphosphate dihydrate and sodium hydrogencitrate sesquihydrate under constant stirring at 25 °C for certain combinations of the two salts. The higher the amount of the salts, the longer period of time was needed for complete dissolution of the solids. Among the different combinations of these two salts, the critical mass of Na_2HCitr required to completely dissolve a certain mass of CaHPO_4 , as determined by visual inspection, was found to depend linearly on the

mass of CaHPO_4 dissolved. By linear regression, the critical hydrogencitrate concentration for formation of homogenous solutions was found to depend on dissolved calcium hydrogencitrate according to $[\text{HCitr}^{2-}] = 14[\text{CaHPO}_4] - 0.05$, as may be seen from Fig. 1.

Extrapolation of the linear curve to zero in relation to the amount of Na_2HCitr leads to $3.51 \pm 1.61 \text{ mmol}$ (35.1 mmol L^{-1}), indicating supersaturation, since the solubility of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, has been reported to be approximately 1.5 mmol L^{-1} [15].

Different from our previous results involving spontaneous supersaturated solutions [6], the robustness of the supersaturation of the calcium hydrogencitrate/hydrogenphosphate system seems to be independent of the degree of supersaturation in the concentration range studied. Fig. 2 shows total calcium, total phosphate, and calcium ion activity for one of the studied samples (B).

The rate of precipitation of calcium citrate could be describe by a first order reaction as determined for experiment B by the decreasing calcium ion concentration (Fig. 2 A): $[\text{Ca}^{2+}] = 0.137 \cdot e^{-(0.12t)} + 0.0236$ and for the decreasing calcium ion activity (Fig. 2 B): $a_{\text{Ca}^{2+}} = 1.79 \cdot 10^{-4} \cdot e^{-(0.11t)} + 1.78 \cdot 10^{-5}$. Practically identical values for the pseudo first order rate constant based on concentration of calcium and calcium ion activity were obtained and the electrochemical method based on calcium ion activity is, accordingly, to be recommended for characterization of supersaturation as this method does not required individual sampling for each analysis. Rate constants based on electrochemical registrations of calcium ion activity during precipitation were determined for each experiment by exponential fitting as seen in Fig. 3.

The first order rate constants for precipitation of calcium citrate was found to increase linearly for increasing amount of CaHPO_4 dissolved, see insert in Fig. 3. In order to identify the factors controlling the precipitation and precipitation rate,

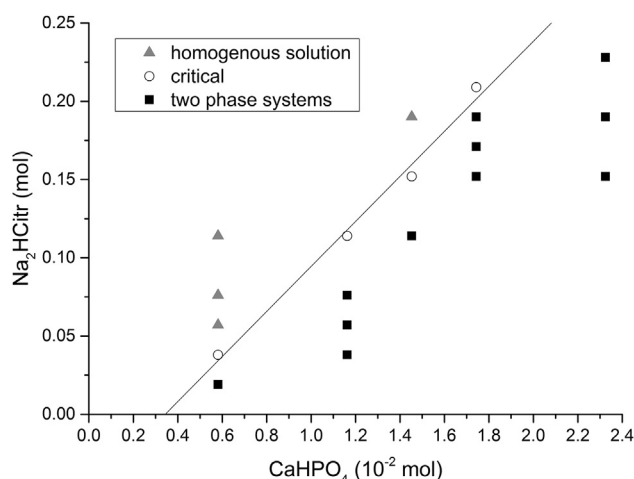


Fig. 1 – Different combinations of sodium hydrogencitrate sesquihydrate and calcium hydrogenphosphate dihydrate in 100 mL of water. The black squares represent the samples in which the dissolution was not complete resulting in two phase systems, the grey triangles represent the samples that formed homogenous supersaturated solutions, and the white circles represent the samples for which complete dissolution just occurred.

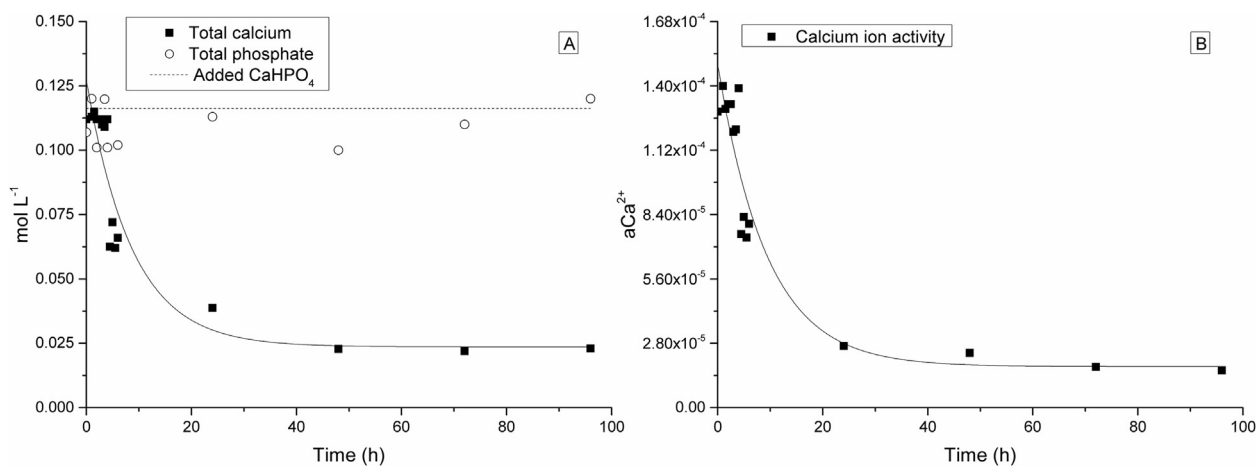


Fig. 2 – Time evolution during precipitation of calcium citrate tetrahydrate for total calcium, total phosphate and calcium ion activity for sample B, consisting of 2.00 g of CaHPO_4 (11.6 mmol) and 30.00 g of Na_2HCitr (0.114 mol) in 100 mL of water.

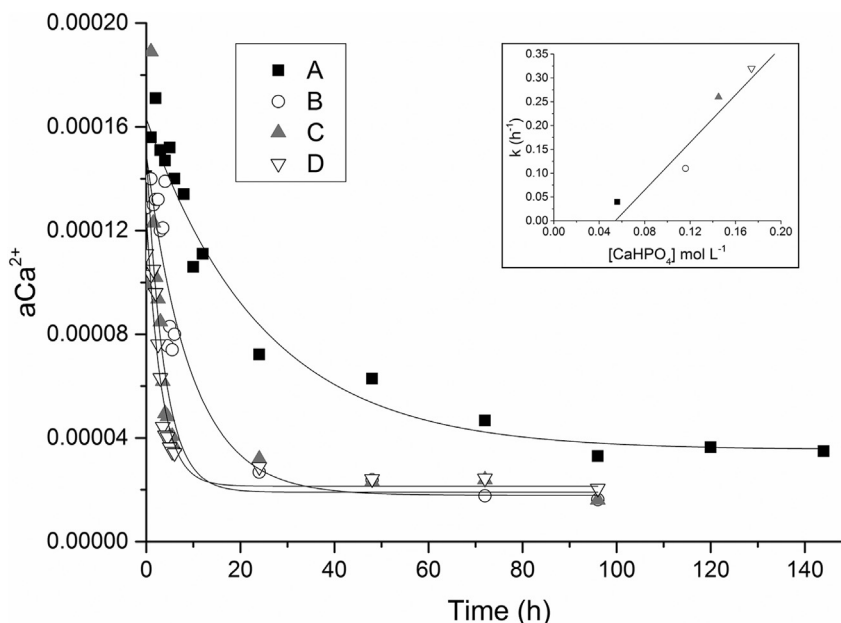


Fig. 3 – Calcium ion activity during precipitation for samples A, B, C and D, consisting of 1.00, 2.00, 2.50, and 3.00 g (5.81, 11.6, 14.5, and 17.4 mmol) of CaHPO_4 combined with 10.00, 30.00, 40.00, and 55.00 g ($3.80 \cdot 10^{-2}$, 0.114, 0.152, and 0.209 mol) of Na_2HCitr in 100 mL of water, respectively. First order rate constants for precipitation determined by exponential fitting for increasing concentration of CaHPO_4 , are seen in insert.

speciation of calcium in the supersaturated solutions were established.

The thermodynamic equilibrium constant for binding of calcium to citrate has previously been determined [16] to have the value of $3.6 \cdot 10^4$ at 25 °C. In the current work we have corrected this activity based equilibrium constant to a concentration based constant valid for an ionic strength of 1.0 M using Davies' equation (2) leading to a value of $2.72 \cdot 10^3 \text{ L mol}^{-1}$. The association constant for binding calcium to hydrogencitrate was studied electrochemically at four temperatures and the activity base constant has also been corrected to concentration under the same conditions. Fig. 4 shows the

van't Hoff plot for the concentration based association constant for calcium hydrogencitrate. From the plot, the association constant between calcium and hydrogencitrate at 25 °C is found to have a value of 357 L mol^{-1} , which is reasonable when compared to the value reported by Davies [17] of $1.2 \cdot 10^3$ for the thermodynamic association constant at low ionic strength.

The results from determination of total phosphorus in solution during precipitation show that phosphate remains in solution, see Fig. 2. In agreement with this, the precipitate formed was identified by FT-IR to be calcium citrate tetrahydrate, see Fig. 5.

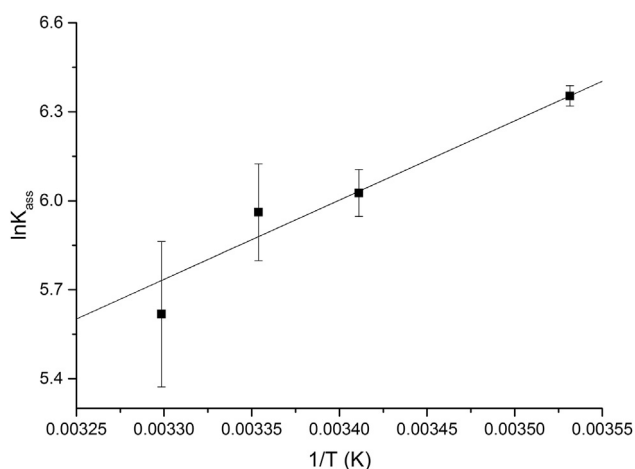


Fig. 4 – van't Hoff plot for calcium hydrogencitrate association constant in water based on concentration at 1.0 M of ionic strength. Enthalpy and entropy have the values $\Delta H^\circ = -22 \pm 2 \text{ kJ mol}^{-1}$ and $\Delta S^\circ = -26 \pm 8 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively.

Calcium ion activity was similar in all supersaturated systems studied; this is strong evidence that only a small fraction of calcium is found as free calcium ions in the supersaturated solutions, but is rather associated to the ligands present. To calculate the calcium speciation in the supersaturated solutions several equations were taken into account:



corresponding to the second dissociation of phosphoric acid, equal to $2.51 \cdot 10^{-7} \text{ mol L}^{-1}$ for ionic strength equals to 1.0 M [18], defined as

$$K_{a_2}^{\text{H}_3\text{PO}_4} = \frac{[\text{H}_3\text{O}^+][\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \quad (4)$$



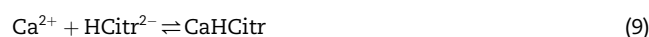
corresponding to the third dissociation of citric acid, equal to $6.34 \cdot 10^{-6} \text{ mol L}^{-1}$ for ionic strength equals to 3.2 M [19], defined as

$$K_{a_3}^{\text{H}_3\text{Citr}} = \frac{[\text{H}_3\text{O}^+][\text{Citr}^{3-}]}{[\text{HCitr}^{2-}]} \quad (6)$$



corresponding to an equilibrium constant, equal to 76 L mol^{-1} corrected to a concentration constant adjusted to ionic strength equal to 1.0 M from the thermodynamic constant of 500 reported by Davies [17], defined as

$$K_1 = \frac{[\text{CaHPO}_4]}{[\text{Ca}^{2+}][\text{HPO}_4^{2-}]} \quad (8)$$



corresponding to the equilibrium constant, equal to 357 L mol^{-1} , defined as

$$K_2 = \frac{[\text{CaHCitr}]}{[\text{Ca}^{2+}][\text{HCitr}^{2-}]} \quad (10)$$



corresponding to the equilibrium constant, equal to $2.72 \cdot 10^3 \text{ L mol}^{-1}$, defined as

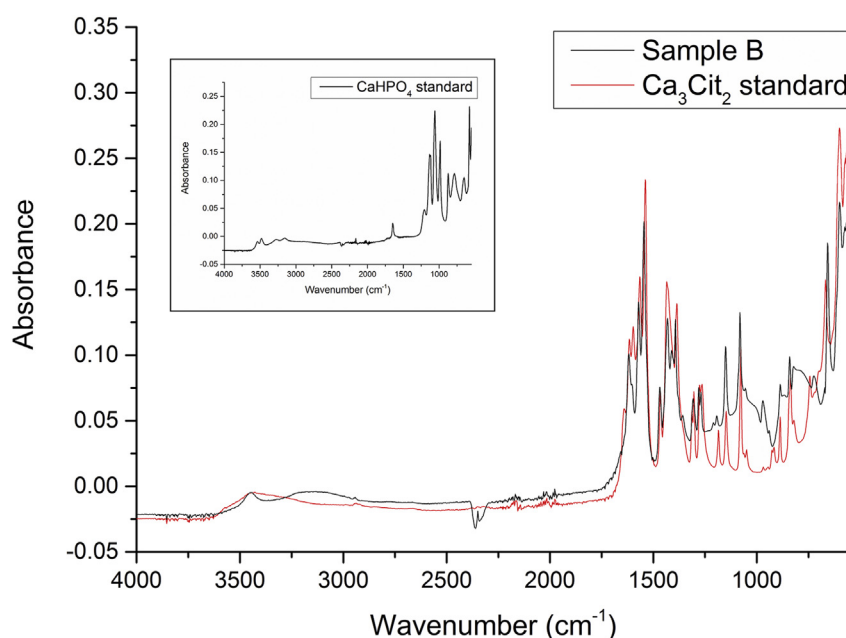


Fig. 5 – FT-IR spectra of the solid collect from experiment B, calcium citrate tetrahydrate standard and, in the insert, the spectrum of calcium hydrogenphosphate dihydrate standard.

$$K_3 = \frac{[\text{CaCitr}^-]}{[\text{Ca}^{2+}][\text{Citr}^{3-}]} \quad (12)$$

and the three mass balance equations

$$t_{\text{Ca}} = [\text{CaHPO}_4] + [\text{CaHCitr}] + [\text{CaCitr}^-] + [\text{Ca}^{2+}] \quad (13)$$

$$t_{\text{P}} = [\text{CaHPO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] \quad (14)$$

$$t_{\text{Citr}} = [\text{CaHCitr}] + [\text{CaCitr}^+] + [\text{HCitr}^{2-}] + [\text{Citr}^{3-}] \quad (15)$$

from which t_{Ca} correspond to total calcium concentration, t_{P} corresponds to total phosphorus, both equal to the concentration of added CaHPO_4 , and t_{Citr} correspond to total citrate, which is equal to the concentration of added Na_2HCitr . All the equilibria and mass balance equations contain eight unknowns, $[\text{H}_2\text{PO}_4^-]$, $[\text{HPO}_4^{2-}]$, $[\text{HCitr}^{2-}]$, $[\text{Citr}^{3-}]$, $[\text{CaHPO}_4]$, $[\text{CaHCitr}]$, $[\text{CaCitr}^-]$, and $[\text{Ca}^{2+}]$, which were calculated neglecting the variation in the volume of the samples due to the added salts. All the equilibrium constants used for these calculations were valid at an ionic strength of 1.0 M. For many electrolytes, the activity coefficients are constant in an ionic strength interval around unity. The speciation is, accordingly, not sensitive to the somewhat higher ionic strength of the most concentrated supersaturated solutions. Table 1 shows the ion speciation in experiments A, B, C, and D prior to precipitation. The supersaturation factor is the ratio between calcium ion activity in supersaturated solutions and in the equilibrium solutions, I is the ionic strength of the samples, γ^{2+} is the coefficient of activity of calcium ions, calculated as the ratio between measured calcium ion activity and the calculated ion concentration, equation (1), and Q is the ionic product of Ca_3Citr_2 , which can be compared to the solubility product, K_{sp} , reported to be $(7 \pm 2) \cdot 10^{-14} \text{ mol}^5 \text{ L}^{-5}$ at ionic strength of 1.0 M [6]. The calculated coefficient of activity is close to unit in the supersaturated solutions, which is in agreement with the mean of the coefficients reported for calcium chloride solutes of high ionic strength, conforming the validity of the calculation method [20].

From the results reported in Table 1, it is seen that the calculated ion product is larger than or close to the solubility product. However, the ratio between $Q_{\text{Ca}_3\text{Citr}_2}/K_{\text{sp}}(\text{Ca}_3\text{Citr}_2)$ is smaller than the apparent supersaturation as calculated from the activity of calcium ions in the supersaturated solutions and the equilibrium calcium ion activity, see Fig. 3. This provides an explanation for the robustness of the supersaturation. The driving force for precipitation is becoming smaller due to the complexation of calcium by hydrogencitrate and especially citrate. The supersaturated homogenous solutions resulting from dissolution of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Na}_2\text{HCitr} \cdot 1\frac{1}{2}\text{H}_2\text{O}$ may contain up to approximately 10 times as much calcium as compared to the equilibrated solutions, providing a unique example of solubilization of an inorganic nutrient by complexation, which could form the basis for development of novel foods with high calcium bioavailability.

Bioavailability of mineral nutrients always needs to be confirmed in human intervention studies. Citrate has already been found to increase calcium absorption in several studies [7,8]. The method developed in the present study may thus serve as a convenient preclinical test of new mineral drug formulations or of novel mineral supplements, but will need

Table 1 – Ion speciation in supersaturated systems A, B, C and D. All concentrations are expressed as mol L⁻¹. Calcium activity, $a_{\text{Ca}^{2+}}$, corresponds to the average of the first four measurements.

System	Added CaHPO ₄	Added Na ₂ HCitr	Supersaturation factor	pH	[H ₂ PO ₄ ⁻]	[HPO ₄ ²⁻]	[HCitr ²⁻]	[Citr ³⁻]	[CaHPO ₄]	[CaHCitr]	[CaCitr ⁻]	[Ca ²⁺]	I	γ^{2+} ^a	$Q_{\text{Ca}_3\text{Citr}_2}$ ^b
A	$5.81 \cdot 10^{-2}$	$3.80 \cdot 10^{-1}$	4.4	4.80 ± 0.01	$5.72 \cdot 10^{-2}$	$9.06 \cdot 10^{-4}$	$2.41 \cdot 10^{-1}$	$8.14 \cdot 10^{-1}$	$1.30 \cdot 10^{-5}$	$1.62 \cdot 10^{-2}$	$4.17 \cdot 10^{-2}$	$1.88 \cdot 10^{-4}$	1.24	0.82	$4.4 \cdot 10^{-14}$
B	$1.16 \cdot 10^{-1}$	1.14	8.1	4.62 ± 0.01	$1.15 \cdot 10^{-1}$	$1.21 \cdot 10^{-3}$	$8.36 \cdot 10^{-1}$	$1.88 \cdot 10^{-1}$	$1.32 \cdot 10^{-5}$	$4.28 \cdot 10^{-2}$	$7.33 \cdot 10^{-2}$	$1.43 \cdot 10^{-4}$	3.66	0.92	$1.0 \cdot 10^{-13}$
C	$1.45 \cdot 10^{-1}$	1.52	8.0	4.61 ± 0.01	$1.44 \cdot 10^{-1}$	$1.45 \cdot 10^{-3}$	1.13	$2.44 \cdot 10^{-1}$	$1.50 \cdot 10^{-5}$	$5.49 \cdot 10^{-2}$	$9.03 \cdot 10^{-2}$	$1.36 \cdot 10^{-4}$	4.88	0.94	$1.5 \cdot 10^{-13}$
D	$1.74 \cdot 10^{-1}$	2.09	5.1	4.58 ± 0.01	$1.73 \cdot 10^{-1}$	$1.66 \cdot 10^{-3}$	1.59	$3.25 \cdot 10^{-1}$	$1.51 \cdot 10^{-5}$	$6.81 \cdot 10^{-2}$	$1.06 \cdot 10^{-1}$	$1.20 \cdot 10^{-4}$	6.72	0.87	$1.8 \cdot 10^{-13}$

^a $\gamma^{2+} = a_{\text{Ca}^{2+}}/[\text{Ca}^{2+}]$.

^b $Q_{\text{Ca}_3\text{Citr}_2} = [\text{Ca}^{2+}]^3[\text{Citr}^{3-}]^2$.

to be followed up by intervention studies [21]. Such clinical studies should also include effects of other food or beverage components, since calcium is known to interact with browning products formed during heating of foods like shrimp or with antibacterial drugs [21,22]. Oligosaccharides are also known to interfere with calcium absorption [23,24].

Conflicts of interest

All authors declare no conflicts of interest.

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