

Strontium increasing calcium accessibility from calcium citrate

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ABSTRACT

Strontium chloride added to aqueous suspensions of metastable calcium citrate tetrahydrate increased calcium ion activity measured electrochemically without transition of metastable tetrahydrate to stable calcium citrate hexahydrate as shown by DSC. Calcium activity increase was explained by lower solubility of strontium citrate pentahydrate formed (8.9×10^{-4} M at 25 °C) increasing with temperature compared to calcium citrate tetrahydrate (1.6×10^{-3} M) decreasing with temperature. Strontium binding to citrate was found endothermic, $\Delta H^0 = 45 \text{ kJ}\cdot\text{mol}^{-1}$ at 25 °C, while calcium binding shows variation from $\Delta H^0 = 94 \text{ kJ}\cdot\text{mol}^{-1}$ at 10 °C becoming exothermic above physiological temperature with $\Delta H^0 = -9 \text{ kJ}\cdot\text{mol}^{-1}$ at 45 °C as determined from temperature and concentration variation in electric conductivity. These differences in solution thermodynamics and pH effect on complex formation between calcium and strontium citrate are discussed in relation to biomineralization.

1. Introduction

Strontium and calcium are alkaline earth metals with comparable chemical properties due to a very similar ionic radius of Sr^{2+} and Ca^{2+} (Hammer, Wessels, Ettliger, & Hoppe, 2020). Calcium is essential and plays a vital role in human health especially for bone and teeth, while strontium has no documented physiological functions (Flynn, 2003; Theobald, 2005). Strontium is, however, absorbed in the intestine by parallel paracellular and transcellular transport by mechanisms very similar to calcium absorption although only half as efficient as for calcium (Cabrera, Schrooten, De Broe, & D'Haese, 1999). The small difference in ionic radius seems also to affect the precipitation of bovine caseins by these alkaline earth metal ions (Lopez, Cuomo, Nostro, & Ceglie, 2013).

It has, however, lately been proposed that strontium as a trace element could have physiological functions related to bone health and teeth strength (Marx, Rahimnejad Yazdi, Papini, & Towler, 2020; Wang, Chang, Chiang, Lin, & Lin, 2019; Zhao et al., 2020). Strontium salts, including strontium chloride, strontium phosphate, strontium ranelate, and strontium citrate, have in recent years been studied for their effects on bone mineral density (Bruyere et al., 2007; Malaise, Bruyere, & Reginster, 2007; Querido, Rossi, & Farina, 2016; Siccardi et al., 2010), and further in relation to osteopenia and osteoporosis therapy (Landi et al., 2007; Moise, Adachi, Chettle, & Pejovic-Milic, 2012). Intake of

strontium ranelate has been found to increase bone mineral density and reduce bone fracture risk (Bruyere et al., 2007). Strontium ranelate is now used for curing osteoporosis on an experimental basis and this strontium salt became available in some countries as a commercial drug (Doublier et al., 2011; Querido et al., 2016). The intake of strontium citrate has also been found to increase bone mineral density (Siccardi et al., 2010), and in North America strontium citrate has now been approved as a food supplement and is commercially available in health food stores (Moise, Chettle, & Pejovic-Milic, 2014). A more recent study reports that strontium citrate also could prevent the loss of hardness of teeth enamel under acidic conditions due to lower solubility and dissolution of mixed calcium/strontium minerals (Wang et al., 2019).

The positive effects of strontium on bone and teeth structure have mainly been assigned to interaction among Sr^{2+} and Ca^{2+} with the surrounding anions in bone mineral structures. Strontium seems to replace calcium to form strontium salts locally in bone structures in effect increasing bone mineral density, or for teeth through formation of a new layer on teeth surface protecting the enamel against dissolution through contact with aggressive environments during intake of food and beverages (Querido et al., 2016; Wang et al., 2019).

Any explanation of positive effects of strontium on teeth strength and bone health would clearly benefit from a better understanding of interaction between calcium and strontium during mineralization reactions. Citrate has lately been identified as crucial for bone

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mineralization in humans, where a high level of circulating citrate correlates with high bone mass (Hartley et al., 2020). Calcium citrate has also been found to promote bone growth under experimental animal surgery despite the low solubility of calcium citrate (Wang et al., 2012). Citrate seems accordingly to have a crucial role for calcium mobility probably related to the facile conversion between various crystal forms of calcium citrate with comparable energy and a marked tendency of calcium citrate spontaneously to form strongly supersaturated solutions (Kaduk, 2018; Liu, Kirkensgaard, & Skibsted, 2021). The aqueous solution chemistry of combinations of calcium and strontium citrate seems accordingly of interest for a better understanding of the effect of strontium as a trace element in the human diet on bone health and was accordingly studied using electrochemical and calorimetric methods in order to characterize the effect of strontium on calcium mobility during mineralization, including effects of variation in temperature and pH as typical for physiological conditions.

2. Materials and methods

2.1. Chemicals

Strontium chloride hexahydrate ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$), trisodium citrate dihydrate ($\text{Na}_3\text{Citr} \cdot 2\text{H}_2\text{O}$), trisodium isocitrate ($\text{Na}_3\text{Isocitr}$), tricalcium citrate tetrahydrate ($\text{Ca}_3\text{Citr}_2 \cdot 4\text{H}_2\text{O}$, CCT), and Murexide were from Sigma Aldrich (Steinheim, Germany). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), sodium hydroxide (NaOH), and calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) were from Merck (Darmstadt, Germany). A Milli-Q Plus purification train (Millipore Corp. Bedford, MA, USA) was used to produce the ultrapure water used throughout.

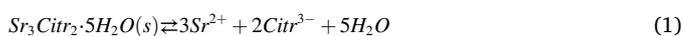
2.2. Synthesis

Strontium citrate pentahydrate ($\text{Sr}_3\text{Citr}_2 \cdot 5\text{H}_2\text{O}$, SCP) was made by mixing equal volumes of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ (0.030 M) and $\text{Na}_3\text{Citr} \cdot 2\text{H}_2\text{O}$ (0.045 M) solutions, and similarly, calcium isocitrate tetrahydrate ($\text{Ca}_3\text{Isocitr} \cdot 4\text{H}_2\text{O}$, CICT) was made from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.030 M) and $\text{Na}_3\text{Isocitr} \cdot 2\text{H}_2\text{O}$ (0.045 M) solutions, and calcium citrate hexahydrate ($\text{Ca}_3\text{Citr}_2 \cdot 6\text{H}_2\text{O}$, CCH) were made from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.030 M) and $\text{Na}_3\text{Citr} \cdot 2\text{H}_2\text{O}$ (0.045 M) solutions, the mixing was in each case followed by stirring for 24 h for reaction and precipitation at room temperature (Chattejee & Dhar, 1924; Cheng, Garcia, Tang, Danielsen, & Skibsted, 2018; Hammer et al., 2020). The precipitates formed were collected and washed with water followed by ethanol and dried at 25°C in a drying cabinet for one week.

2.3. Calculations of dissociation constants from electric conductivities

The electrical conductivity of 1.00×10^{-5} M, 2.00×10^{-5} M, 4.00×10^{-5} M, 8.00×10^{-5} M, 1.20×10^{-4} M, 1.6×10^{-4} M, 2.00×10^{-4} M of SCP, CCT, and of their 1/1 mixture (mixed citrate, M_3Citr_2 , MC) solutions at 10, 25, 35, 45 °C were measured in order to determine the dissociation constant (K_{diss}). The calculations for SCP, CCT, and MC were similar, and it is presented here only for SCP.

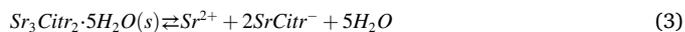
Concentration of dissolved SCP is set to b , and under the assumption of complete dissociation upon dissolution,



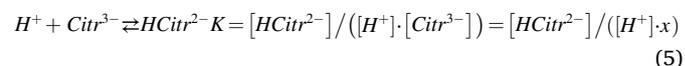
the ionic strength will be $I_{\text{ideal}} = 15b$ according to the ionic strength definition of Eq. 2, where $[X^{z\pm}]$ is the concentration of $X^{z\pm}$,

$$I = 0.5 \sum (z^2 \cdot [X^{z\pm}]) \quad (2)$$

Complex formation between strontium and citrate was taken into account in a two step dissolution process with the free citrate concentration $[\text{Citr}^{3-}]$ set as x ,



together with association between H^+ and Citr^{3-} (Eq. 5), since approximately 20% citrate will be protonated at pH around 7 (Apelblat & Barthel, 1991):



where K is the equilibrium constant similar to the reciprocal acid dissociation constant $1/K_{\text{a}3}$ valid for dilute aqueous solutions. The reciprocal acid dissociation constant depends on temperature as shown below in Eq. 23 (Apelblat & Barthel, 1991). $[\text{H}^+]$ was estimated from electrochemical pH measurement at each temperature (pH meter, HQ411d; Hach Company, CO. USA) using the international pH activity standards.

The equilibrium concentrations of HCitr^{2-} , Sr^{2+} and SrCitr^- were calculated from,

$$[\text{HCitr}^{2-}] = K[\text{H}^+] \cdot [\text{Citr}^{3-}] = K[\text{H}^+] \cdot x \quad (6)$$

$$[\text{Sr}^{2+}] = b + x + K[\text{H}^+] \cdot x \quad (7)$$

$$[\text{SrCitr}^-] = 2b - x - K[\text{H}^+] \cdot x \quad (8)$$

The dissociation constant for SrCitr^- based on concentration ($K_{\text{diss,c}}$) is defined as Eq. 9 corresponding to an ionic strength of $I_{\text{real}} = 3b + 6x + 3.5 \cdot K[\text{H}^+] \cdot x$:

$$K_{\text{diss,c}} = [\text{Sr}^{2+}] \cdot [\text{Citr}^{3-}] / [\text{SrCitr}^-] = (b + x + K[\text{H}^+] \cdot x) \cdot x / (2b - x - K[\text{H}^+] \cdot x) \quad (9)$$

The dissociation degree (α) of SrCitr^- defined as the ratio between the concentration of the dissociated complex and the initial complex concentration assuming no dissociation is,

$$\alpha = ([\text{Citr}^{3-}] + [\text{HCitr}^{2-}]) / ([\text{SrCitr}^-] + [\text{Citr}^{3-}] + [\text{HCitr}^{2-}]) = (x + K[\text{H}^+] \cdot x) / 2b \quad (10)$$

or

$$x = 2b\alpha / (1 + K[\text{H}^+]) \quad (11)$$

and the ratio between the ionic strength for complete dissociation and only partly dissociation is accordingly

$$I_{\text{ideal}} / I_{\text{real}} = 15b / (3b + 6x + 3.5 \cdot K[\text{H}^+] \cdot x) = 15b / (3b + (6 + 3.5 \cdot K[\text{H}^+]) \cdot 2b\alpha / (1 + K[\text{H}^+])) = 15 / (3 + (6 + 3.5 \cdot K[\text{H}^+]) \cdot 2\alpha / (1 + K[\text{H}^+])) \quad (12)$$

The conductivity for ideal conditions (λ_{ideal}) was calculated from the limiting molar conductivity (λ_{m}^0) of Sr^{2+} and Citr^{3-} (Apelblat & Barthel, 1991; Robinson & Stokes, 1959; Rodrigo, Ribeiro, Verissimo, Estes, & Leaist, 2019). The real conductivities of SCP solutions (λ_{real}) were measured by a 4-pole conductivity cell, model CDC866T (Radiometer, Copenhagen, Denmark), with a standard solution of potassium chloride in 0.0100 M ($1413 \mu\text{S} \cdot \text{cm}^{-1}$) at 25 °C. The curves of λ_{ideal} or λ_{real} as a function of concentration for SCP, CCT and the 1:1 mixture are shown in Fig. S1 for 15, 25, 35, and 45 °C.

Assuming $I_{\text{ideal}} / I_{\text{real}} = \lambda_{\text{ideal}} / \lambda_{\text{real}}$, Eq. 12 yields,

$$\lambda_{\text{ideal}} / \lambda_{\text{real}} = r = 15 / (3 + (6 + 3.5 \cdot K[\text{H}^+]) \cdot 2\alpha / (1 + K[\text{H}^+])) \quad (13)$$

or

$$\alpha = (15 - 3r) \cdot (1 + K[\text{H}^+]) / (6 + 3.5 \cdot K[\text{H}^+]) / r \quad (14)$$

The speciations, $K_{\text{diss,c}}$, and I_{real} , were estimated for the SCP solutions of varying concentrations by combining Eq. 9, 11, and 14 with λ_{ideal} calculated from λ_{m}^0 and the measured values for λ_{real} under the assumption that the effect of ionic strength on molar conductivity is the

same for ions with different charges. This assumption is considered to be valid at least for the very dilute solutions investigated (McCleskey, Nordstrom, & Ryan, 2012; Simón & García, 1999).

The dissociation constants based on activities ($K_{\text{diss},a}$) were adjusted from $K_{\text{diss},c}$ by the activity coefficients calculated from Eq. 15,

$$\log_{10} K_{\text{diss},a} = \log_{10} K_{\text{diss},c} + \log_{10} \gamma^{2+} + \log_{10} \gamma^{3-} - \log_{10} \gamma^{-} = \log_{10} K_{\text{diss},c} - A_{\text{DH}} (4 + 9 - 1) (\sqrt{I} / (1 + \sqrt{I}) - nI) \quad (15)$$

or

$$\log_{10} K_{\text{diss},c} - 12A_{\text{DH}} (\sqrt{I} / (1 + \sqrt{I})) = \log_{10} K_{\text{diss},a} + 12A_{\text{DH}} \cdot nI \quad (16)$$

Considering $\log_{10} K_{\text{diss},c} - 12A_{\text{DH}} (\sqrt{I} / (1 + \sqrt{I}))$ as a linear function of $12A_{\text{DH}} \cdot I$, allowed the determination of $\log_{10} K_{\text{diss},a}$ by linear regression, from which the value for the association constants based on activities was calculated,

$$K_{\text{ass},a} = 1/K_{\text{diss},a} \quad (17)$$

2.4. Calcium ion activity of dilute $\text{Ca}_3\text{Cit}_2 \cdot 4\text{H}_2\text{O}$ solutions

The calcium ion activity, $a(\text{Ca}^{2+})$, of CCT unsaturated solutions of 1.20×10^{-4} M, 1.6×10^{-4} M, 2.00×10^{-4} M were determined with a calcium ion-selective electrode ISE25Ca and a reference electrode REF251 (Radiometer, Copenhagen, Denmark). For investigation of the temperature effect on complex dissociation, each measurement was performed after stirring for equilibration for one hour at 10, 15, 20, or 25 °C. CaCl_2 solutions of 1.00×10^{-4} M, 1.00×10^{-3} M, and 1.00×10^{-2} M were used as standards with $a(\text{Ca}^{2+})$ calculated from Eq. 18,

$$a(\text{X}^{z\pm}) = \gamma^{z\pm} \cdot [\text{X}^{z\pm}] \quad (18)$$

where z is the charge of the ion $\text{X}^{z\pm}$, $a(\text{X}^{z\pm})$ is the activity of $\text{X}^{z\pm}$, and $[\text{X}^{z\pm}]$ is the concentration of $\text{X}^{z\pm}$. $\gamma^{z\pm}$ is the activity coefficient based on the extended Debye-Hückel equation (Eq. 15) with $n = 0.3$ (Davies, 1962).

2.5. Solubility of $\text{Sr}_3\text{Cit}_2 \cdot 5\text{H}_2\text{O}$

The aqueous solubilities of SCP at 10, 25, 35, 50, 70, 90 °C and at varying pH for 25 °C were determined by EDTA titration with 0.01000 M SrCl_2 aqueous solution as standard for calibrating the EDTA solution using 0.5 % Murexide as an indicator. SCP suspensions were prepared from 0.0010 mol SCP and 100 mL water under thermostated conditions and kept stirred for one hour for equilibration, and the pH of solutions for 10, 25, 35 °C were determined electrochemically (pH meter HQ411d; Hach Company, CO. USA). The equilibrated suspensions were filtered rapidly using syringe filters (0.45 μm , Frisenette ApS, Denmark) followed by titration with calibrated EDTA at room temperature.

2.6. Speciation and solubility products for $\text{Sr}_3\text{Cit}_2 \cdot 5\text{H}_2\text{O}$ and $\text{Ca}_3\text{Cit}_2 \cdot 4\text{H}_2\text{O}$

The speciations of SCP saturated solutions at 10, 25, 35 °C and at varying pH for 25 °C were calculated combining the pH and solubilities determined by EDTA titration, and the association constant based on activity and determined by conductivity for each temperature as described in section 2.3, including the principles of mass balance and electroneutrality:

$$[\text{Sr}]_{\text{total}} = [\text{Sr}^{2+}] + [\text{SrCit}^-] \quad (19)$$

$$2[\text{Sr}^{2+}] = 3[\text{Cit}^{3-}] + 2[\text{HCitr}^{2-}] + [\text{H}_2\text{Cit}^-] + [\text{SrCit}^-] \quad (20)$$

in combination with the equilibrium constants for dissociation of citric acid K_{a1} , K_{a2} , and K_{a3} (Apelblat & Barthel, 1991; Bates & Pinching, 1949):

$$\text{H}_3\text{Cit} \rightleftharpoons \text{H}^+ + \text{H}_2\text{Cit}^- \quad K_{a1} (10 \text{ }^\circ\text{C}) = a(\text{H}^+) \cdot a(\text{H}_2\text{Cit}^-) / a(\text{H}_3\text{Cit}) = 6.27 \times 10^{-4}$$

$$K_{a1} (25 \text{ }^\circ\text{C}) = 6.98 \times 10^{-4}$$

$$K_{a1} (35 \text{ }^\circ\text{C}) = 7.22 \times 10^{-4} \quad (21)$$

$$\text{H}_2\text{Cit}^- \rightleftharpoons \text{H}^+ + \text{HCitr}^{2-} \quad K_{a2} (10 \text{ }^\circ\text{C}) = a(\text{H}^+) \cdot a(\text{HCitr}^{2-}) / a(\text{H}_2\text{Cit}^-) = 1.33 \times 10^{-5}$$

$$K_{a2} (25 \text{ }^\circ\text{C}) = 1.40 \times 10^{-5}$$

$$K_{a2} (35 \text{ }^\circ\text{C}) = 1.43 \times 10^{-5} \quad (22)$$

$$\text{HCitr}^{2-} \rightleftharpoons \text{H}^+ + \text{Cit}^{3-} \quad K_{a3} (10 \text{ }^\circ\text{C}) = a(\text{H}^+) \cdot a(\text{Cit}^{3-}) / a(\text{HCitr}^{2-}) = 4.14 \times 10^{-7}$$

$$K_{a3} (25 \text{ }^\circ\text{C}) = 4.05 \times 10^{-7}$$

$$K_{a3} (35 \text{ }^\circ\text{C}) = 3.78 \times 10^{-7} \quad (23)$$

The calculations of speciation were performed by an iterative procedure, which was started assuming an initial ionic strength value of $I_0 = 0.001$ M. The concentrations of the relevant species were calculated by combining the determined solubility and pH of SCP saturated solutions, and solving the equations of Eq. 15, 17–23 simultaneously. Then a new value for ionic strength I_{new} was calculated by Eq. 2 according to the calculated speciation. A new calculation cycle was started by setting $I_0 = I_{\text{new}}$, and the iterative calculation ended when the ionic strength became constant. Afterward, the solubility product based on activities ($K_{\text{sp},a}$) from dissolution process of SCP shown in Eq. 1 was calculated by Eq. 24,

$$K_{\text{sp},a} = (a(\text{Sr}^{2+}))^3 \cdot (a(\text{Cit}^{3-}))^2 \quad (24)$$

A similar calculation for CCT was based on solubility data reported (Liu et al., 2021) and the conductivity of solutions of CCT at 10, 25, and 35 °C.

2.7. SrCl_2 or $\text{Na}_3\text{Isocitr}_2$ effects on calcium ion activity of $\text{Ca}_3\text{Cit}_2 \cdot 4\text{H}_2\text{O}$ suspensions

Four CCT suspensions were prepared, each from 0.0015 mol CCT and 100 mL water at 25 °C by stirring. After one hour of equilibration, 25, 50, or 100 μL of a 2.00 M SrCl_2 solution was added to three of the suspensions for investigating the effect of SrCl_2 on calcium activity. The total concentrations of SrCl_2 in the three suspensions were 0.00050, 0.00100, and 0.00200 M, respectively. In order to compare the effects of Sr^{2+} with another hydroxycarboxylate than citrate, a $\text{Na}_3\text{Isocitr}_2$ solution was added to an additional sample to obtain an isocitrate concentration of 0.00200 M. The calcium ion activity, $a(\text{Ca}^{2+})$, of the suspensions after SrCl_2 or $\text{Na}_3\text{Isocitr}_2$ addition were followed for up to 4 weeks at 25 °C.

2.8. Differential scanning calorimetry (DSC)

Samples of 10 mg were filled into 40 μL aluminum pans to obtain thermograms by a DSC STAR^e System from Mettler Toledo (Schwerzenbach, Switzerland) recorded with a scanning rate of $10 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$ in the range of 25–250 °C. The samples included CCT, CCH, CICT, SCP, and the precipitations from the CCT suspensions with 0.00200 M SrCl_2 or $\text{Na}_3\text{Isocitr}_2$ as described in section 2.7.

3. Results

Solubility of strontium citrate pentahydrate (SCP) in water at varying temperatures is shown in Fig. 1A together with the solubilities of calcium citrate tetrahydrate (CCT) and hexahydrate (CCH) determined in a previous study (Liu et al., 2021). SCP solubility increases with temperature, most significantly below 50 °C, as may be seen in Table S1. In

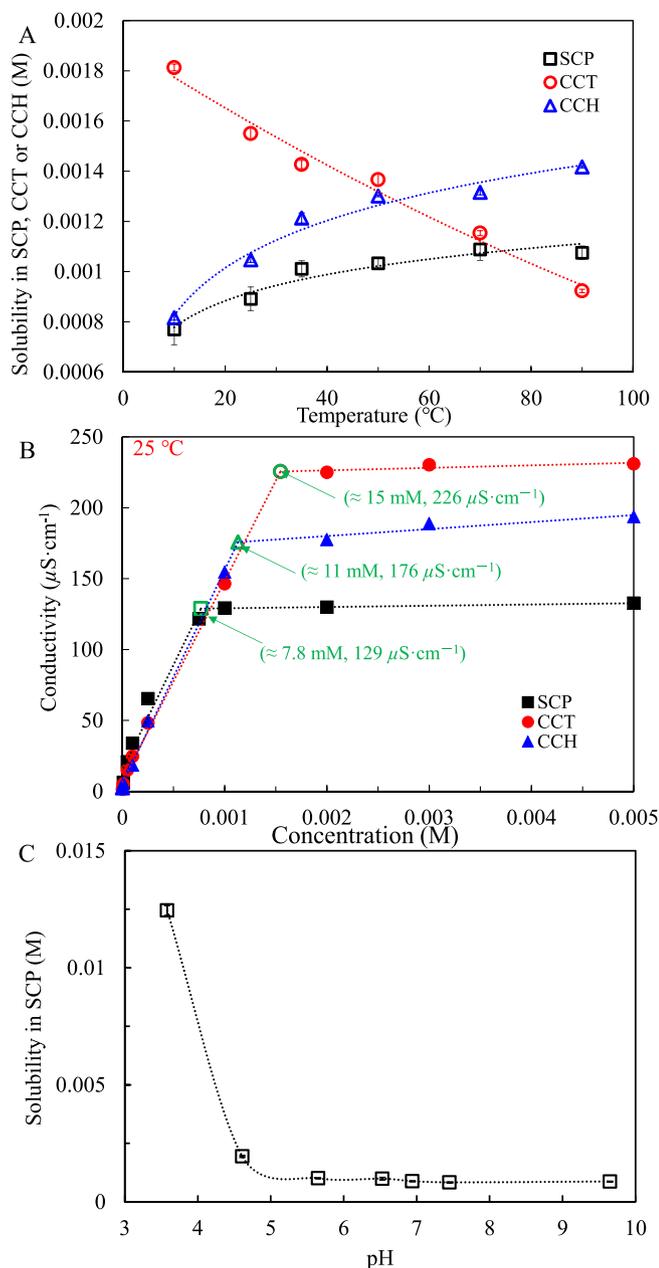


Fig. 1. (A) Solubilities of $\text{Sr}_3\text{Cit}_2\cdot 5\text{H}_2\text{O}$ (SCP), $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ (CCT) and $\text{Ca}_3\text{Cit}_2\cdot 6\text{H}_2\text{O}$ (CCH) based on SCP, CCT or CCH concentrations at 10, 25, 35, 50, 70, 90 °C from EDTA titration. The data for CCT and CCH are from (Liu et al., 2021). (B) Conductivities of SCP, CCT and CCH at indicated concentrations at 25 °C, the green unfilled marks are the conductivities for the saturated aqueous solutions of SCP, CCT and CCH. (C) Solubilities (in M) of SCP at the indicated pH determined by EDTA titration at 25 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contrast to calcium citrate only a single hydrate of strontium citrate is known to be in equilibrium with solutions of strontium citrate in this temperature range. The conductivities of SCP solutions and suspensions measured at the indicated concentrations at 25 °C are shown in Fig. 1B. The circle point in green was obtained as the crossing of the line obtained for concentrations up to saturation with the value for the suspensions only slightly increasing with temperature for higher concentrations. The value of 7.8×10^{-4} M for this crossing point is in agreement with the measured solubility of SCP (8.9 ± 0.5) $\times 10^{-4}$ M at 25 °C. The conductivities of CCT and CCH solutions or suspension show

a similar dependence on concentration, as also shown in Fig. 1B. For pH effect on the solubilities of SCP, new experimental data in Fig. 1C show that lower pH promotes the dissolution of SCP especially for pH < 5, as typical for gastric digestion.

In aqueous solutions, strontium citrate dissociates into strontium ions, citrate ions, and the strontium citrate complex (SrCit^-) with a comparable degree of formation for the complex as also found for the calcium citrate complex (CaCit^-), which accounted for more than half of total calcium species in saturated calcium citrate solutions (Liu et al., 2021). The association ($K_{\text{ass},a}$) constants for SrCit^- and CaCit^- were determined from comparing the ideal conductivities (λ_{ideal}) calculated from the limiting molar conductivity and the measured real conductivities (λ_{real}) for dilute SCP and CCT aqueous solutions at 10, 25, 35, 45 °C, and the curves for λ_{ideal} and λ_{real} for varying concentrations are shown in Fig. S1. For comparison between SCP, CCT, and their mixture of SCP/CCT = 1/1 (mixed citrate, MC), the association constants for complex formation in SCP, CCT, and MC solutions were compared as seen in the van't Hoff plot of Fig. 2A.

The $K_{\text{ass},a}$ of SCP, MC, and CCT all increase for increasing temperature, as seen in Fig. 2A with $K_{\text{ass},a}$ SCP < MC < CCT for each investigated temperature, however, with this difference decreasing for increasing temperature. Speciations for SCP and CCT solutions obtained from the

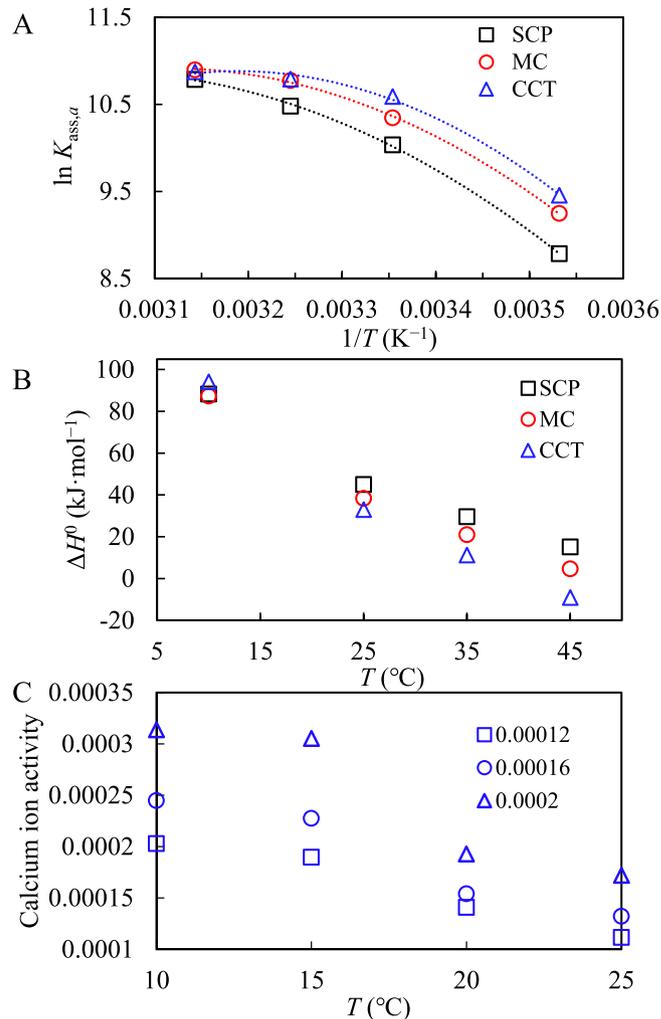


Fig. 2. (A) van't Hoff plots of activity-based association constants ($K_{\text{ass},a}$) for $\text{Sr}_3\text{Cit}_2\cdot 5\text{H}_2\text{O}$ (SCP), $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ (CCT), and the mixed citrate (MC) with a ratio of SCP/CCT = 1/1. (B) Enthalpy of complex formation in SCP, MC, and CCT solutions at varying temperatures. (C) The calcium ion activity of unsaturated $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ solutions in indicated concentrations measured at 10, 15, 20, 25 °C, respectively.

calculations of the $K_{\text{ass},a}$ are shown in Table 1, from which table it may be seen that the concentration of calcium ion and strontium ion are comparable with the concentration of the complex, especially for the higher concentration and the higher temperature. The limiting molar conductivities (Fig. S2) of complexes, SrCit^- and CaCit^- , were calculated by combining limiting molar conductivities of Sr^{2+} , Ca^{2+} , and Cit^{3-} and the speciation obtained for the solutions as shown in Table 1. The limiting molar conductivity (in $\text{S}\cdot\text{equiv}^{-1}$) of SrCit^- increases with temperature according to Eq. 25,

$$\lambda_m^0(\text{SrCit}^-) = 0.353 \times T(^{\circ}\text{C}) + 11.8 \quad (25)$$

very similar to CaCit^- ,

$$\lambda_m^0(\text{CaCit}^-) = 0.294 \times T(^{\circ}\text{C}) + 12.8 \quad (26)$$

showing that the two complex ions have very similar mobility in aqueous solution.

The Eq. 27–30 derived from Kirchoff law were used to calculate enthalpy change ($\Delta H_{\text{complex}}^0$), entropy change ($\Delta S_{\text{complex}}^0$), and heat capacity change ($\Delta C_{p\text{complex}}^0$) for complex formation in SCP, MC, and CCT solutions from the values of $K_{\text{ass},a}$ determined at different temperatures (Vailaya & Horváth, 1996):

$$\ln K_{\text{ass},a} = A + B/T + C/T^2 \quad (27)$$

where A , B , and C are parameters estimated from experimental data using Eq. 27 and non-linear regression. Calculating of the thermodynamic parameters for complex formation:

$$\Delta H_{\text{complex}}^0 = -R \cdot (B + 2C/T) \quad (28)$$

$$\Delta S_{\text{complex}}^0 = R \cdot (A - C/T^2) \quad (29)$$

$$\Delta C_{p\text{complex}}^0 = 2RC/T^2 \quad (30)$$

where R is the gas constant, and T is the absolute temperature in K. Values of $\Delta H_{\text{complex}}^0$, $\Delta S_{\text{complex}}^0$, and $\Delta C_{p\text{complex}}^0$ are shown in Table S2, and the parameters A , B , and C for SCP, MC, and CCT in Table S3 allowing calculations at other intermediate temperatures. $\ln K_{\text{ass},a}$ and $\Delta H_{\text{complex}}^0$ shown in Fig. 2A–B are seen to follow an interesting pattern, since $\ln K_{\text{ass},a}$ is rather similar at the highest temperature of 45 °C, while the calcium complex is more stable at lower temperatures compared to the strontium complex due to a less positive or even negative $\Delta H_{\text{complex}}^0$ for the calcium complex. The large variation in $\Delta H_{\text{complex}}^0$ as seen in Fig. 2B is rather unusual and caused by very large and negative $\Delta C_{p\text{complex}}^0$ indicating significant change in hydration of calcium upon binding of citrate.

Speciation of saturated solutions of SCP and CCT at 10, 25, and 35 °C were based on $K_{\text{ass},a}$ for complex formation as determined by conductivity measurement, the solubility of SCP and CCT, the measured pH, and calculated by the iterative procedure described in section 2.6. Distribution of species based on concentration and the solubility products for SCP and CCT based on activity ($K_{\text{sp},a}$) are shown in Table 2. From the temperature dependence of $K_{\text{sp},a}$, enthalpy ($\Delta H_{\text{dissol}}^0$) and entropy for dissolution ($\Delta S_{\text{dissol}}^0$) of SCP and CCT were calculated according to van't Hoff equation (Eq. 31) giving $-70 \text{ kJ}\cdot\text{mol}^{-1}$ for $\Delta H_{\text{dissol}}^0$ and a value of $-567 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for $\Delta S_{\text{dissol}}^0$ for SCP, and $\Delta H_{\text{dissol}}^0$ is $-94 \text{ kJ}\cdot\text{mol}^{-1}$ and $\Delta S_{\text{dissol}}^0$ is $-643 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ for CCT, as shown in Fig. S3.

$$\ln K_{\text{sp},a} = -\Delta H_{\text{dissol}}^0 / R \cdot 1/T + \Delta S_{\text{dissol}}^0 / R \quad (31)$$

The dissolution of SCP and CCT are both exothermic processes and $K_{\text{sp},a}$ decreases with increasing temperature. The total solubility of CCT also decreases with increasing temperature, as seen in Table 2 and Fig. 1. However, the total solubility of SCP increases with temperature in an apparent endothermic dissolution process. The difference between the temperature effect of the dissolution process on the molecular level and on what is observed on the macroscopic level is due to a contribution from the endothermic complex formation.

The speciations of saturated SCP aqueous solutions at varying pH at 25 °C were calculated by a similar procedure as described in section 2.6, and the speciation is shown in Table S4 and Fig. S4. The SrCit^- dominates at pH higher than 6, while CaCit^- dominates from pH = 5 due to the stronger binding of Ca^{2+} to citrate than of Sr^{2+} (Liu et al., 2021).

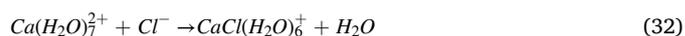
Calcium is more accessible from the CCT than from CCH due to the higher solubility of CCT when the temperature is below 50 °C, and below this temperature the transition of CCT → CCH should be spontaneous according to thermodynamics (Liu et al., 2021). The calcium ion activity of a saturated solution of CCT is higher than of a saturated solution of CCH by more than 10 %, and a transition from CCT to CCH is expected (Vavrusova & Skibsted, 2016). However, this transition is not seen, and strontium cation and isocitrate anion were found to have no effect on the CCT to CCH transition at 25 °C as was followed by $a(\text{Ca}^{2+})$ measurement for up to 4 weeks for CCT suspensions without or with Sr^{2+} or Isocitr^{3-} added, as may be seen in Fig. 3A and 3B. The effect of Sr^{2+} on the $a(\text{Ca}^{2+})$ of the saturated CCT solution was the opposite of what was expected for a CCT to CCH transition, as seen in Fig. 3A. The suspension of CCT without additions (blank) showed no change in $a(\text{Ca}^{2+})$, while strontium ions induced an increase in $a(\text{Ca}^{2+})$. Isocitrate had the opposite and decreased $a(\text{Ca}^{2+})$. The precipitates isolated after two weeks of equilibration were mainly CCT with a little SCP or calcium isocitrate, as seen from the DSC thermograms of Fig. 3C, indicating that CCT does not recrystallized to CCH under the present conditions, observations inviting further speculations.

4. Discussion

The association constants for SCP and CCT based on the activity are 2.3×10^4 and 4.0×10^4 at 25 °C, respectively, which are comparable to constants previously reported, $1.4\text{--}2.2 \times 10^4$ for SCP (Joseph, 1946; Schubert & Richter, 1948) and $3.6\text{--}7.2 \times 10^4$ for CCT at 25 °C (Davies & Hoyle, 1953; Singh, Yeboah, Pambid, & Debayle, 1991; Vavrusova & Skibsted, 2016). The activity-based association constants for the 1:1 mixture were found to have a value close to the average of the constant for binding of Ca^{2+} to Cit^{3-} and for binding of Sr^{2+} to Cit^{3-} and gave no indication of specific interaction between Sr^{2+} and Ca^{2+} in their citrate complexes.

The values of $\Delta H_{\text{complex}}^0$ for CCT obtained from the dilute CCT solutions with $11.2 \text{ kJ}\cdot\text{mol}^{-1}$ at 35 °C and $-9.1 \text{ kJ}\cdot\text{mol}^{-1}$ at 45 °C are comparable to the results from previous determinations with $<10 \text{ kJ}\cdot\text{mol}^{-1}$ (Hummel, Anderegg, Rao, Puigdomenech, & Tochiyama, 2005; Singh et al., 1991; Vavrusova & Skibsted, 2016). The binding of calcium to citrate is almost athermal but for lower temperature, $a(\text{Ca}^{2+})$ was found to decrease in calcium citrate solutions for increasing temperature at least up to 25 °C, see Fig. 2C. All the methods used for determination of $\Delta H_{\text{complex}}^0$ included contributions from the enthalpy of ion hydration or dehydration during ligand binding. $\Delta H_{\text{complex}}^0$ seems, however, to be affected by the presence of other ions like from NaCl or from other salts used for maintaining a constant ionic strength which may explain the differences in the values for $\Delta H_{\text{complex}}^0$ reported (Hummel et al., 2005; Singh et al., 1991; Vavrusova & Skibsted, 2016).

The presence of NaCl has been shown to affect the hydration process Ca^{2+} (Di Tommaso, Ruiz-Agudo, De Leeuw, Putnis, & Putnis, 2014). Generally, one Ca^{2+} is reported to bind to 5–7 water molecules in the first coordination sphere (Rodriguez-Cruz, Jockusch, & Williams, 1998, 1999), but the chloride ion seems to affect the process by binding to Ca^{2+} (Di Tommaso et al., 2014),



could also affect the binding between Ca^{2+} and Cit^{3-} . Using electrochemical measurements of CCT and SCP in water and extrapolating the obtained association constant to ionic strength zero without the presence of any background electrolyte like sodium chloride should provide association constants more reliable than the constants

Table 1
Main ion speciation (M) of dilute $\text{Sr}_3\text{Cit}_2 \cdot 5\text{H}_2\text{O}$ and $\text{Ca}_3\text{Cit}_2 \cdot 4\text{H}_2\text{O}$ solutions based on conductivity measurements and limiting molar conductivities.

SCP (M)	10 °C					25 °C					35 °C					45 °C				
	[Sr^{2+}]	[Cit^{3-}]	[SrCit^-]	[HCitr^{2-}]	[H^+]	[Sr^{2+}]	[Cit^{3-}]	[SrCit^-]	[HCitr^{2-}]	[H^+]	[Sr^{2+}]	[Cit^{3-}]	[SrCit^-]	[HCitr^{2-}]	[H^+]	[Sr^{2+}]	[Cit^{3-}]	[SrCit^-]	[HCitr^{2-}]	[H^+]
1.00E-05	3.43E-05	2.18E-05	-4.30E-06*	2.52E-06	4.86E-08	2.61E-05	1.47E-05	3.90E-06	1.41E-06	3.94E-08	2.30E-05	1.18E-05	7.04E-06	1.14E-06	3.67E-08	2.13E-05	1.03E-05	8.67E-06	1.05E-06	3.51E-08
2.00E-05	5.44E-05	3.23E-05	5.65E-06	2.05E-06	2.68E-08	4.21E-05	2.02E-05	1.79E-05	1.86E-06	3.77E-08	3.77E-05	1.62E-05	2.23E-05	1.45E-06	3.44E-08	3.50E-05	1.36E-05	2.50E-05	1.33E-06	3.36E-08
4.00E-05	9.33E-05	4.95E-05	2.67E-05	3.78E-06	3.24E-08	7.36E-05	3.04E-05	4.64E-05	3.13E-06	4.25E-08	6.53E-05	2.31E-05	5.47E-05	2.27E-06	3.79E-08	6.07E-05	1.90E-05	5.93E-05	1.69E-06	3.08E-08
8.00E-05	1.60E-04	7.33E-05	8.03E-05	6.42E-06	3.74E-08	1.25E-04	4.07E-05	1.15E-04	4.19E-06	4.28E-08	1.14E-04	3.09E-05	1.26E-04	2.83E-06	3.55E-08	1.06E-04	2.38E-05	1.34E-04	2.12E-06	3.09E-08
1.20E-04	2.21E-04	9.74E-05	1.39E-04	3.91E-06	1.72E-08	1.75E-04	5.31E-05	1.85E-04	2.28E-06	1.79E-08	1.58E-04	3.68E-05	2.02E-04	1.47E-06	1.56E-08	1.48E-04	2.70E-05	2.12E-04	9.56E-07	1.24E-08
1.60E-04	2.80E-04	1.08E-04	2.00E-04	1.25E-05	4.97E-08	2.26E-04	5.49E-05	2.54E-04	1.13E-05	8.60E-08	2.00E-04	3.71E-05	2.80E-04	2.83E-06	2.97E-08	1.89E-04	2.43E-05	2.91E-04	4.73E-06	6.81E-08
2.00E-04	3.29E-04	1.23E-04	2.71E-04	6.36E-06	2.23E-08	2.65E-04	6.10E-05	3.35E-04	3.79E-06	2.60E-08	2.38E-04	3.60E-05	3.62E-04	1.77E-06	1.92E-08	2.25E-04	2.35E-05	3.75E-04	1.45E-06	2.16E-08
CCT (M)	10 °C					25 °C					35 °C					45 °C				
	[Ca^{2+}]	[Cit^{3-}]	[CaCit^-]	[HCitr^{2-}]	[H^+]	[Ca^{2+}]	[Cit^{3-}]	[CaCit^-]	[HCitr^{2-}]	[H^+]	[Ca^{2+}]	[Cit^{3-}]	[CaCit^-]	[HCitr^{2-}]	[H^+]	[Ca^{2+}]	[Cit^{3-}]	[CaCit^-]	[HCitr^{2-}]	[H^+]
1.00E-05	2.98E-05	1.75E-05	1.88E-07	2.27E-06	5.44E-08	2.23E-05	1.08E-05	7.69E-06	1.50E-06	5.69E-08	1.99E-05	8.68E-06	1.01E-05	1.18E-06	5.19E-08	1.84E-05	7.39E-06	1.16E-05	1.02E-06	4.73E-08
2.00E-05	4.96E-05	2.56E-05	1.04E-05	3.99E-06	6.57E-08	3.68E-05	1.49E-05	2.32E-05	1.93E-06	5.32E-08	3.28E-05	1.13E-05	2.72E-05	1.46E-06	4.96E-08	3.10E-05	9.77E-06	2.90E-05	1.20E-06	4.22E-08
4.00E-05	8.21E-05	3.52E-05	3.79E-05	6.90E-06	8.30E-08	6.34E-05	1.96E-05	5.66E-05	3.76E-06	7.90E-08	5.63E-05	1.35E-05	6.37E-05	2.72E-06	7.72E-08	5.27E-05	1.05E-05	6.73E-05	2.24E-06	7.37E-08
8.00E-05	1.38E-04	5.13E-05	1.02E-04	6.50E-06	5.39E-08	1.08E-04	2.59E-05	1.32E-04	2.49E-06	3.98E-08	9.70E-05	1.55E-05	1.43E-04	1.42E-06	3.54E-08	9.10E-05	1.00E-05	1.49E-04	1.05E-06	3.62E-08
1.20E-04	1.88E-04	6.20E-05	1.72E-04	6.39E-06	4.40E-08	1.51E-04	2.91E-05	2.09E-04	2.33E-06	3.32E-08	1.35E-04	1.41E-05	2.25E-04	1.12E-06	3.10E-08	1.27E-04	6.64E-06	2.33E-04	6.64E-07	3.47E-08
1.60E-04	2.38E-04	7.53E-05	2.42E-04	3.02E-06	1.72E-08	1.93E-04	3.14E-05	2.87E-04	1.26E-06	1.67E-08	1.73E-04	1.21E-05	3.07E-04	4.43E-07	1.42E-08	1.64E-04	3.75E-06	3.16E-04	1.24E-07	1.15E-08
2.00E-04	2.84E-04	8.06E-05	3.16E-04	3.38E-06	1.80E-08	2.32E-04	2.99E-05	3.68E-04	1.98E-06	2.78E-08	2.08E-04	7.80E-06	3.92E-04	3.76E-07	1.87E-08	1.97E-04	-2.45E-06*	4.03E-04	-1.20E-07*	1.71E-08

* Lower than the limitation for speciation calculation.

Table 2

Speciations (in M) of saturated aqueous solutions at 10, 25, 35 °C, and ionic strengths (*I*, in M), and solubility products based on activity ($K_{sp,a}$) for $\text{Sr}_3\text{Cit}_2\cdot 5\text{H}_2\text{O}$ and $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$.

Temp. (°C)	[Sr] _{total} *	[Sr ²⁺]	[Cit ³⁻]	[SrCit ⁻]	[HCitr ²⁻]	[H ₂ Citr ⁻]	[H ₃ Citr]	[H ⁺]	<i>I</i>	$K_{sp,a}$
10	2.31E-03	1.18E-03	3.54E-04	1.13E-03	5.29E-05	2.85E-07	3.76E-11	8.28E-08	4.62E-03	2.28E-17
25	2.67E-03	1.06E-03	1.47E-04	1.62E-03	2.07E-05	9.67E-08	1.03E-11	7.44E-08	3.63E-03	3.59E-18
35	3.03E-03	1.13E-03	1.05E-04	1.90E-03	1.44E-05	5.98E-08	5.62E-12	6.78E-08	3.71E-03	2.17E-18
Temp. (°C)	[Ca] _{total} *	[Ca ²⁺]	[Cit ³⁻]	[CaCit ⁻]	[HCitr ²⁻]	[H ₂ Citr ⁻]	[H ₃ Citr]	[H ⁺]	<i>I</i>	$K_{sp,a}$
10	5.44E-03	2.65E-03	2.68E-04	2.79E-03	5.33E-04	4.02E-05	7.84E-08	1.23E-06	8.99E-03	6.97E-17
25	4.65E-03	1.83E-03	1.03E-04	2.82E-03	1.69E-04	9.46E-06	1.25E-08	9.24E-07	5.88E-03	5.65E-18
35	4.28E-03	1.72E-03	7.88E-05	2.56E-03	2.03E-04	1.62E-05	2.99E-08	1.34E-06	5.49E-03	2.97E-18

*[Sr]_{total} = [Sr²⁺] + [SrCit⁻], [Ca]_{total} = [Ca²⁺] + [CaCit⁻].

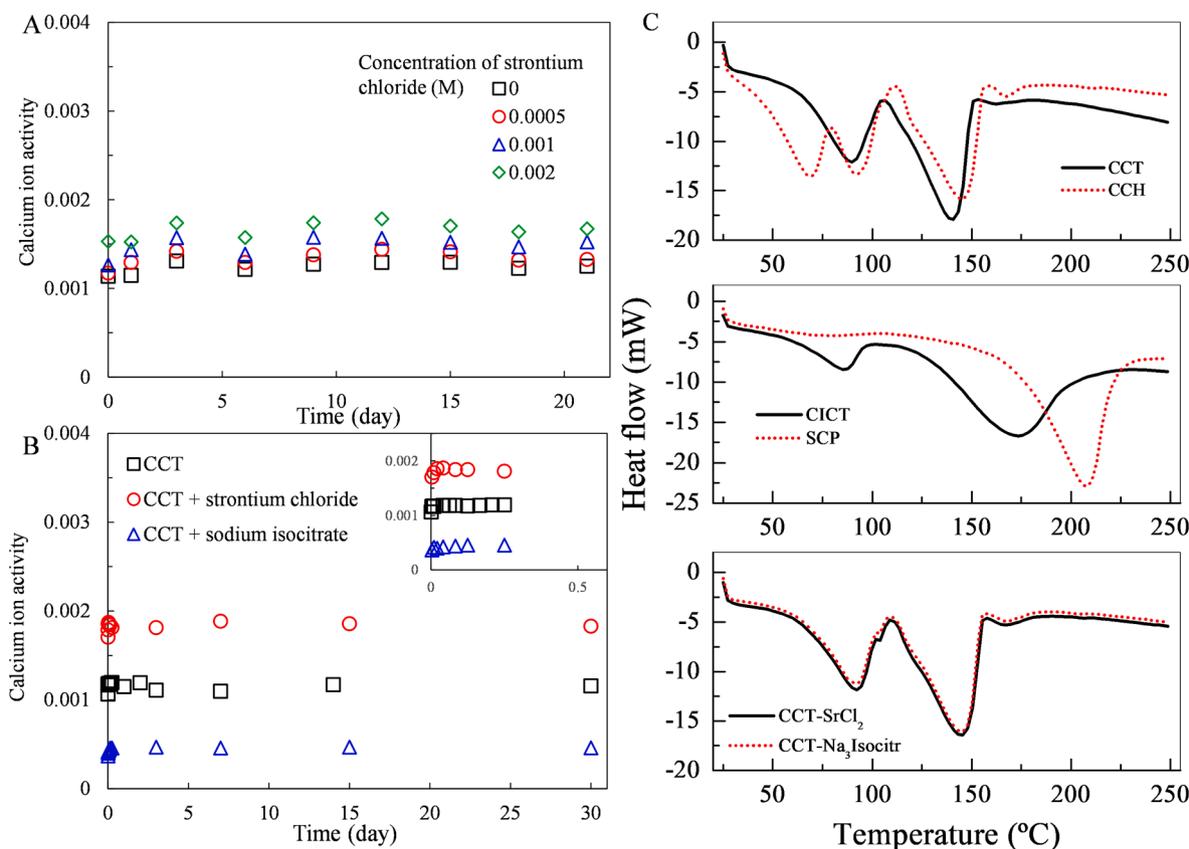


Fig. 3. (A) Calcium ion activity ($a_{\text{Ca}^{2+}}$) changes of saturated $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ (CCT) suspensions with 0, 0.0005, 0.001, and 0.002 M strontium chloride at 25 °C. (B) $a_{\text{Ca}^{2+}}$ changes of saturated CCT solutions with 0.002 M strontium chloride or sodium isocitrate at 25 °C, the insert is the magnification of the initial period. (C) DSC curves of standards of $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ (CCT), $\text{Ca}_3\text{Cit}_2\cdot 6\text{H}_2\text{O}$ (CCH), $\text{Ca}_3\text{Isocitr}_2\cdot 4\text{H}_2\text{O}$ (CICT), and $\text{Sr}_3\text{Cit}_2\cdot 5\text{H}_2\text{O}$ (SCP), and the precipitations from CCT suspensions with 0.002 M strontium chloride or sodium isocitrate after equilibrating for two weeks at 25 °C.

previously determined in the presence of other ions.

Increasing concentration of strontium chloride increases the $a_{\text{Ca}^{2+}}$ of suspensions of the metastable CCT. The speciation of the non-homogeneous solutions as calculated by the same iterative procedure as used for saturated CCT and saturated SCP solutions provides an

explanation for this effect, see Table 3. Citrate is partly precipitating strontium due to the lower solubility of SCP compare to CCT, see Table 2, and the heterogeneous equilibrium,

**Table 3**

Speciation (in M) and ionic strength (*I*, in M) for saturated suspensions of $\text{Ca}_3\text{Cit}_2\cdot 4\text{H}_2\text{O}$ with different concentrations of SrCl_2 added after equilibrating for 3 weeks at 25 °C.

[SrCl ₂] _{added}	[Sr ²⁺]	[SrCit ⁻]	[Sr] _{total} *	[Ca ²⁺]	[CaCit ⁻]	[Ca] _{total} *	[Cit ³⁻]	[HCitr ²⁻]	[H ₂ Citr ⁻]	[H ₃ Citr]	[H ⁺]	<i>I</i>
0	0	0	0	1.68E-03	1.91E-03	3.59E-03	6.93E-05	3.92E-05	7.43E-07	3.28E-10	3.56E-07	4.70E-03
0.0005	2.12E-04	1.71E-04	3.83E-04	1.98E-03	2.77E-03	4.74E-03	1.01E-04	6.71E-05	1.53E-06	8.40E-10	4.55E-07	6.93E-03
0.001	4.74E-04	3.25E-04	7.99E-04	2.23E-03	2.67E-03	4.90E-03	9.51E-05	6.65E-05	1.63E-06	9.68E-10	5.03E-07	8.47E-03
0.002	1.01E-03	5.38E-04	1.55E-03	2.71E-03	2.52E-03	5.23E-03	8.71E-05	6.38E-05	1.68E-06	1.11E-09	5.72E-07	1.15E-02

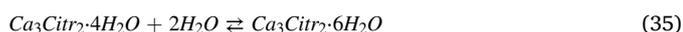
*[Sr]_{total} = [Sr²⁺] + [SrCit⁻], [Ca]_{total} = [Ca²⁺] + [CaCit⁻].

liberates calcium ions resulting in increasing $a(\text{Ca}^{2+})$ as confirmed experimentally, as seen in Fig. 3A, and by speciation calculation, as shown in Table 3. Strontium ions are accordingly concluded to increase the activity of calcium ions from calcium citrate without inducing transformation of CCT with higher accessibility of calcium to the more stable CCH with lower accessibility of calcium.

Isocitrate has the opposite effect as precipitation of calcium isocitrate will lower the $a(\text{Ca}^{2+})$,



The effect of strontium increasing the $a(\text{Ca}^{2+})$ of the suspensions of metastable CCT is of more significance for tissue mineralization than the opposite effect of isocitrate (Cheng et al., 2018). However, even more important is the observation that both cations like Sr^{2+} increasing the $a(\text{Ca}^{2+})$ of CCT suspension or anions like isocitrate with the opposite effect decreasing calcium ion activity result in no transformation of the metastable CCT to the stable CCH for these temperature conditions,



Isocitrate and other hydroxycarboxylates may precipitate calcium in effect lowering the calcium ion activity with effects on biomatrices which need further attention.

During the prolonged storage period, CCT will dissolve under the influence of precipitation of SCP or dissolved calcium will precipitate as isocitrate precipitation. The reprecipitation of calcium citrate will, however, under all conditions investigated result in reprecipitation of the metastable tetrahydrate, not of the stable hexahydrate as shown by DSC. Since the metastable calcium citrate tetrahydrate has a higher accessibility of calcium than the stable calcium citrate hexahydrate, the effect of increasing $a(\text{Ca}^{2+})$ by the presence of strontium is a clear indication of a positive effect of strontium on accessibility of calcium from calcium citrate tetrahydrate as an important vehicle for calcium in foods and as supplement (Liu et al., 2021).

5. Conclusion

The positive effect of strontium ion on accessibility of calcium from calcium citrate seems to depend on two factors: (i) strontium ions increase calcium citrate ion activity from solid calcium citrate tetrahydrate in saturated aqueous systems; and (ii) strontium ions do not induce transformation of the metastable calcium citrate tetrahydrate to the stable and less soluble calcium citrate hexahydrate with lower calcium ion accessibility.

CRediT authorship contribution statement

Xiao-Chen Liu: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing - original draft, Resources. **Leif H. Skibsted:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.130674>.

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