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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: $[HCitr^{2-}] = 14[CaHPO_4] - 0.05$ 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 $\Delta H^\circ = -22 \pm 2$ kJ $mol^{-1}$, $\Delta S^\circ = -26 \pm 8$ J $K^{-1}$ $mol^{-1}$. 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, $a_{Ca}^{2+}$, 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 $CaCl_2$ solutions with concentration of $1.00 \times 10^{-4}$, $1.00 \times 10^{-3}$, $1.00 \times 10^{-2}$ mol $L^{-1}$ prepared from a 1.000 mol $L^{-1}$ $CaCl_2$ stock solution at 10, 20, 25 °C, and 30 °C. Calcium ion activity, $a_{Ca}^{2+}$, in the standard solutions was calculated based on the relationship between activity and concentration according to

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

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

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

where $A_{DH}$ is the Debye-Hückel constant with the numerical value of $A_{DH} = 0.498$, 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 $HNO_3$ 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 $cm^{-1}$.

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