The solubility of calcium phosphate in concentrated dairy effluent brines

*K. Kezia[1], J. Lee[2], B. Zisu[3], G. Q. Chen[1], S. L. Gras[1,4], S. E. Kentish[1]**

1. The ARC Dairy Innovation Hub, Department of Chemical & Biomolecular Engineering, University of Melbourne, Vic 3010 Australia

2. Department of Chemical and Process Engineering, University of Surrey Guildford, Surrey GU27XH United Kingdom

3. School of Applied Science, College of Science, Engineering and Health, RMIT University Melbourne, Vic 3000 Australia

4. The Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Vic 3010 Australia

Keywords: calcium phosphate; dairy; brine; solid phase equilibria; lactate; citrate.

Corresponding Author

*Email: sandraek@unimelb.edu.au Phone: +61 3 8344 6682

ABSTRACT

The solubility of calcium phosphate in concentrated dairy brine streams is important in understanding mineral scaling on equipment such as membrane modules, evaporators and heat exchangers, and in brine pond operation. In this study, the solubility of calcium phosphate has been assessed in the presence of up to 300 g/L sodium chloride as well as lactose, organic acids and anions at 10ºC, 30ºC and 50ºC. As a neutral molecule, lactose has a marginal, but still detectable effect upon calcium solubility. However, additions of sodium chloride up to 100 g/L result in a much greater increase in calcium solubility. Beyond this point, the concentrations of ions in the solution decreases significantly. These changes in calcium solubility can readily be explained through changes in the activity coefficients. There is little difference in calcium phosphate speciation between 10 and 30ºC. However, at 50ºC, the ratio of calcium to phosphate in the solution is lower than at the other temperatures and varies less with ionic strength. While the addition of sodium lactate has less effect upon calcium solubility than sodium citrate, it still has a greater effect than sodium chloride at an equivalent ionic strength. Conversely, when these organic anions are present in the solution in the acid form, the effect of pH dominates and results in much higher solubility and a calcium/phosphate ratio close to one, indicative of dicalcium phosphate dihydrate as the dominant solid phase.

1. INTRODUCTION

With increasingly stringent environmental regulation, dairy industries in Australia are under pressure to pretreat saline effluent so as to improve the quality and reduce the volume of discharged effluent. This effluent stream comprises small amounts of milk sugar, large concentrations of sodium chloride (NaCl) from cheese manufacturing plants, as well as smaller amounts of divalent salts such as calcium phosphates. Due to the low economic value of the discharged effluent, any concentration process should be performed at minimum cost. Such processes are commonly conducted by membrane filtration or thermal evaporation. During the concentration process, heterogenous precipitation of minerals on the surface of evaporators or membrane modules often occurs, creating a major fouling issue and reducing the lifetime of the equipment. If the salt concentration exceeds the limits set by local water authorities, it may then be necessary to divert the saline effluent to a brine pond, where further precipitation occurs.

The primary precipitating agent is calcium phosphate, which possesses low solubility in comparison to sodium chloride or lactose. Studies of the solubility of calcium phosphate are complex and ongoing, due to incongruent dissolution phenomenon and dynamic intermediate equilibria [1-4]. Calcium phosphate is a biomineral, which can exhibit various forms with different ratios of calcium to phosphate. The solubility of calcium phosphate is also affected by pH, temperature, the presence of ionic impurities and their concentration, the total ionic strength and the precipitation rate [5, 6]. Compared to the other parameters, pH has the most profound effect and has been extensively studied [7-9].

The solubility of different calcium phosphate compounds across variable pH ranges are listed in Table 1. In cheese whey where the pH is typically 4 – 5, dicalcium phosphate dihydrate (DCPD) is the most common precipitant. At higher pH, salts such as octacalcium phosphate

(OCP) or hydroxyapatite (HAP) are more thermodynamically stable (Table 1). However in dairy processing, DCPD often remains dominant. This is usually attributed to faster crystallization kinetics for DCPD[10 11, 12]. Inhibition of HAP and subsequent formation of DCPD has also been attributed to the presence of citrates in these dairy systems [13, 14].

At room temperature, phosphoric acid dissociates based on the pH of the environment, as shown below [15]. This ionization governs the cation pairing mechanism to maintain electro-neutrality within the solution.

$$H_3PO_4 \leftrightarrow H^+ + H_2PO_4^- \qquad pK_1 = 2.1 \qquad (1)$$

$$H_2PO_4^- \leftrightarrow H^+ + HPO_4{}^{2-} \qquad pK_2 = 7.2 \qquad (2)$$

$$HPO_4{}^{2-} \leftrightarrow H^+ + PO_4{}^{3-} \qquad pK_3 = 12.3 \qquad (3)$$

In dairy systems such as saline effluent and salty whey permeate, the solubility is also greatly affected by the presence of background electrolytes. In particular, high concentrations of NaCl and organic anions such as lactate and citrate are often present. For example, in a saline effluent such as salty whey permeate, the concentration of citrate ranges from 0.8 mM to 3.6 mM. Citrate is known to sequester calcium to form the soluble calcium citrate anion ($CaCit^-$), which increases calcium solubility [16, 17]. In fresh salty whey permeate, lactate concentration can vary from 1.6 mM to 37 mM. This lactate concentration can further increase with time due to the action of residual lactic acid bacteria which convert lactose into this acid. Indeed, we have observed lactate concentrations of over 100 mM for samples stored in our laboratory for six months.

The initial amount of NaCl can range between 30 g/L to 100 g/L (0.5 – 1.7M) and this could be concentrated up to an equivalent of 250-300 g/L (> 4 - 5.1M) during effluent treatment. Similarly, the calcium concentration might start at 40mM (7 g/L DCPD) but increase to 120 mM (20 g/L DCPD) during concentration[18]. Further, process temperatures can swing between production and effluent treatment steps, ranging from 4ºC to greater than 50ºC. There is currently little information regarding the solubility and precipitation behavior of calcium phosphate under such operating conditions.

The current study aims to deepen our understanding of these processes and to assess the possibility for selective precipitation of calcium phosphate salt. To achieve this objective, we mimic the impurities and ionic strength of saline effluent in a full-scale system. Understanding the precipitation of calcium phosphate in the presence of extreme concentrations of NaCl and organic anions and acids will aid in mitigating mineral scaling and thus prolong the lifetime of equipment through the adjustment of operating conditions. These findings have applications beyond the treatment of dairy saline effluent, encompassing water treatment processes within other chemical industries.

2. MATERIAL AND METHODS

Materials

All chemicals used were analytical grade and were used as received without further purification. For experiments and cleaning procedures, purified water (Elix Millipore, resistivity > 15 MΩ cm-1) was used. For analytical procedures and preparation of standard solutions, water of higher purity (Milli-Q Millipore, resistivity > 18 MΩ cm-1) was used.

Dicalcium phosphate dihydrate, DCPD ($CaHPO_4.2H_2O$, >98%) was obtained from Astral Scientific. Calcium chloride dihydrate ($CaCl_2.2H_2O$, >99%), di-Sodium hydrogen phosphate (Na_2HPO_4, >99%) and lactose monohydrate ($C_{12}H_{22}O_{11}.H_2O$) were purchased from Chem Supply. To adjust the pH and organic anion concentration, tri-sodium citrate (Na_3Cit, >99%), sodium lactate ($NaC_3H_5O_3$, >70%), citric acid ($C_6H_8O_7$, >99%) and lactic acid ($C_3H_6O_3$, 85%) were purchased from Chem Supply and nitric acid (HNO_3, 69.5%) was purchased from Scharlau.

Methods

The experiments were performed using an end-point equilibrium technique. This technique operates by incubating excess amounts of solid in a pre-conditioned background solution at constant temperature. It has been widely utilized for solubility measurement [19]. In the present case, a consistent excess amount (2 g unless otherwise stated) of solid DCPD (Ca/P =1) was added to 200 ml of a background solution in a glass container, as shown in Figure 1. The composition of the background solution was adjusted to be between 0 – 300 g/L (5.1 M) NaCl and 0 to 100 g/L (0.3M) of lactose. The role of organic acid and anions was determined using a background solution of 1 to 100 mM of either lactic or citric acid, sodium lactate or sodium citrate. The vessel was double-sealed using Parafilm M,R Laboratory film (SPI supplies) and capped to avoid evaporation. It was then incubated in a circulating water bath (Julabo) controlled at 10ºC, 30ºC or 50ºC for 168 hours (one week). A constant stirring rate was set at approximately 250 rpm. At the end of the incubation period, the supernatant was filtered using a 0.2 µm (polyethersulfone, PES) syringe filter (Millipore) and the composition of the supernatant liquor was analysed. The pH was measured at the beginning and at the end of the experiment, using a WP81 double junction glass pH electrode and temperature probe, connected to a digital benchtop meter. Each experiment was carried out at least in duplicate.

At the conclusion of experiments, the concentration of Ca, Na and phosphorus (P) was measured using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) (Varian 720-OES). The concentrations of the orthophosphate anions ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) and chloride (Cl^-) were determined using Ion Chromatography (Dionex 1000CS) with an AS 14 anion exchange column. The concentration of lactate and citrate was quantified using Reverse Phase High Performance Liquid Chromatography (RP-HPLC) (Shimadzu LC-20AT) employing an ion-exchange column (HPX-87H). Triplicate measurements were performed for each independent sample and the error margins are based on a single standard deviation of these results (n=6). Data was analysed using one way analysis of variance (ANOVA), with a significance level of $p = 0.01$.

The behavior of calcium and phosphate ions in the solution was also simulated through predictions of the activity coefficient of these ions at ionic strengths of 0 to 5M NaCl. ASPEN Plus V8.6 (Aspen One) was employed using the Pitzer thermodynamic package.

3. RESULTS AND DISCUSSION

Effect of neutral solute (lactose)

It was expected that lactose would not alter the solubility of calcium phosphate, as a neutral solute should not dictate ion speciation phenomena. However, there was a significant increase ($p < 0.01$) in the concentration of soluble calcium from 0.68 ± 0.06 mM to 1.33 ± 0.10 mM, as the lactose concentration increased from 0 to 100 g/L (Figure 2). It can also be seen from Figure 2b that the calcium to phosphate ratio (Ca/P) in the supernatant in the absence of lactose or NaCl is significantly less than unity ($p<0.01$), indicating that the solid phase has a Ca/P ratio greater than unity, indicative of significant quantities of HAP or OCP (Table 1). As the amount of the remaining excess solid in the solution is significantly greater than the amount dissolved, an accurate mass balance of the solid could not be conducted to confirm the exact solid phase composition.

Increasing the lactose concentration significantly increases ($p < 0.01$) the Ca/P ratio, from 0.54 ± 0.01 to 0.70 ± 0.01 mM. The effect of sugars on different calcium salts has also been observed by Doherty et al. [20] in a calcium oxalate-sucrose system. Besic [21] claims that sucrose in food products affects the solubility of calcium and phosphate in teeth. Other workers have shown that calcium salts can significantly influence the solubility and growth rate of lactose crystals due to the formation of a lactose complex [22, 23]. The changing solubility may also reflect a decrease in the water activity as the lactose concentration increased, consistent with the observation of a very small conductivity increase. However, while lactose clearly impacts calcium solubility, the effect is very small, when compared with NaCl, as also seen in Figure 2(a). The remainder of this work therefore focuses on the impact of such ionic species.

Effect of ionic strength

It can be seen from Figure 3 that the concentration of the calcium ion in the supernatant increases significantly for all temperatures as the NaCl concentration increases to 100g/L. Beyond this point, a significant ($p < 0.01$) decrease in both the soluble calcium and phosphate concentration is observed. This decreased solubility of calcium in solution at the highest NaCl concentrations is of significance in understanding fouling in equipment such as brine evaporators, which may operate under such conditions. The decreased solubility could cause rapid calcium scaling to occur.

These trends in solubility can be understood by reference to the activity coefficient of the ions in solution. With increasing ionic strength, the activity coefficient of both calcium and phosphate ions decrease (Figure 4) resulting in increased solubility. This loss in activity results from ion-pairing (association), which has been previously investigated [24-26], up to the ionic strength of seawater (approximately 35 g/L or 0.7M) at room temperature. Millero et al[26] uses the Pitzer equation to predict that the activity coefficient of Ca^{2+} should decrease from 0.851 at an ionic strength of 0.002 M down to 0.225 at an ionic strength of 0.7 M at 25 ^{o}C. Their study also predicts that the activity coefficients for $H_2PO_4^-$ decrease from 0.947 to 0.363 and for HPO_4^{2-} from 0.758 to 0.044. Comparable values were generated in the present case, as shown in Figure 4. However, beyond a salt concentration of 100 g/L the activity coefficients for calcium increase again.

Prediction of the supernatant concentration using these activity coefficients is a complex problem given the solid phase equilibria between OCP, HAP and DCPD, in addition to the phosphate speciation given by Equations 1 to 3. Figure 5 shows a simulation for the simplest case where DPCD is assumed to be the only solid phase, using the solubility constant provided in Table 1 at 25^{o}C. It is clear that even this simple simulation provides a reasonable fit to the supernatant calcium concentration. The phosphate concentrations are higher, again suggesting that the solid phase contains OCP or HAP in addition to DCPD.

With increasing ionic strength a small decrease in pH was observed. The pH fell from 7.67 ± 0.25 in the absence of NaCl to 7.00 ± 0.22 at 300 g/L NaCl. At comparable pH values and at zero NaCl concentration, the Ca/P ratio observed (Table 2) is similar to the value reported by Sutter et al. from their leaching experiments [27]. However, with increasing ionic strength, the Ca/P ratio increases significantly at both 10 ºC and 30 ºC, with values in the range of 0.72-0.77 at 10 to 300 g/L NaCl. This suggests a shift back from OCP or HAP towards DCPD, consistent with the common observations in dairy processing. Conversely, there is no significant shift in the Ca/P ratio at 50ºC, implying that at this temperature, the crystal morphology is unaffected by ionic strength. Figure 4(b) also shows that the ionic strength affects the activity of $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} to differing extents, which alters the equilibria of these species. These changes may also explain the observed pH variation.

Some workers have argued that the amount of excess solid existing in the system alters the solubility of calcium phosphate, with the lowest solubility occurring for the smallest amount of solid [1, 28, 29]. This is consistent with the present results shown in Figure 6. In the absence of NaCl, no significant difference could be observed ($p < 0.01$), as the amount of $[Ca^{2+}]$ dissolved in the solution is very low. However, at higher ionic strength, it can be seen that the solubility is significantly affected ($p<0.01$) by the amount of remaining excess solid in the solution. These differences have been reported to relate to the different calcium phosphate polymorphs that can form and the resulting equilibria between multiple solid phases, in addition to the liquid-solid phase equilibria. These authors argue that this is the reason that the solubility data reported by researchers using different techniques often shows wide variability.

Effect of Temperature

The solubility of both calcium and phosphate is identical within experimental error at 10 ºC to 30 ºC for NaCl concentrations of up to 50 g/L (Figure 3). However, beyond this concentration, the solubility at 30ºC is significantly lower than that at 10ºC ($p<0.01$). Changes in solubility with temperature are more apparent at 50ºC, with the solubility increasing significantly ($p<0.01$) at all NaCl concentrations. Sutter et al. [27] observed a slight decrease in the concentration of soluble calcium in the solution from temperatures of 5 ºC to 37 ºC using a leaching technique (at comparable pH ~ 6.5-6.8) and from 25ºC to 37ºC in batch experiments at near neutral and acidic conditions. On the other hand, Green and Perry [30] report an increasing solubility of monobasic calcium phosphate with respect to temperature. These literature results are all broadly consistent with the work presented here. However, the temperature effects in this case should not be oversimplified as the Ca/P ratio in the solution is also significantly altered, especially at 50 ºC where this ratio decreases to 0.45 ± 0.05 (Table 2).

The results appear initially inconsistent with many previous studies, which claim that calcium phosphate solubility declines with temperature [31-33]. Indeed, in our own work, we have also previously observed that increasing the temperature could shorten the time it takes for the first appearance of crystals in saline dairy effluent[34]. However, these references generally describe kinetic effects, where increasing temperature causes calcium salts to precipitate in a shorter time. The present work and that of the literature cited above considers the final equilibrium after at least 168 hours, where such kinetic effects become irrelevant.

Effect of Organic anions

Figure 6 shows the concentration of calcium in the solution in the presence of either the organic lactic and citric acids, or the strong acid HNO_3. The increasing solubility of calcium phosphate with respect to pH is well known ($pH < 6$), although this effect varies depending upon the pKa of the specific acid. The role of pH in governing the solubility of calcium phosphate salts by addition of orthophosporic acid has been reported [35, 36]. However, orthophosphoric acid exhibits both weak acid and polybasic properties, and will alter the amount of available phosphate in the solution through the common ion effect. In this study, HNO_3 is used to isolate the role of pH, as the formation of $Ca(NO_3)_2$ is unlikely to occur.

In 1 mM HNO_3, lactic acid or citric acid, the initial pH of the solution (with 20 g/L DCPD) is 4.61 ± 0.14, 5.67 ± 0.38 and 5.00 ± 0.33, respectively. Within this pH range the concentration of calcium recorded in solution is comparable to the value reported by Sutter et al. [27] obtained using a leaching method where the pH was adjusted using H_3PO_4. The values recorded at 1 mM are also comparable with that obtained by other workers using OCP as the starting solid [37]. The calcium solubility is lowest for the lactic acid ($p<0.01$), reflecting its weak acidity and hence the relatively high pH of the solution. The solubility of the nitric and citric acids are comparable within experimental error, even though the pH of the citric solution is higher than that of the nitric. This reflects the calcium sequestering capacity of the citrate anion.

Discrepancies with the literature are observed at higher acidity (10 mM, see Figure 7). The solubility of calcium in the solution is two to four times lower compared to the concentration reported by Sutter et al. [27]. At the end of experiments, these workers obtain a value of 47mM at similar pH (pH 2.90 and 3.53 for HNO_3 and lactic acid respectively in this work, versus

3.38 in the Sutter et al. study). This might be due to the utilisation of different acids here instead of orthophosphoric acid.

Figure 8a shows that the solubility of calcium ions increases significantly ($p<0.01$) as the sodium lactate concentration increases from 1 to 100 mM at 10 and 30 ºC. The use of sodium lactate in these experiments ensures that the pH of the solution changes little, within the range of pH 7.0 to 8.7. The increased solubility is partly associated with the increase in the background ionic strength. Nevertheless, the effect of sodium lactate on the solubility of calcium and phosphate is more pronounced than NaCl at an equivalent concentration. Specifically, the effect of 100 mM sodium lactate on calcium solubility is equivalent ($p>0.05$) to 170 mM (10g/L) of NaCl at 10 ºC and 30 ºC (see Table 3).

At 50ºC, the pH falls significantly upon addition of sodium lactate, suggesting that different calcium phosphate speciation is occurring. The calcium solubility increases further, but it is difficult to confirm whether this is due to the lactate or the shift in pH.

In dairy effluent, the amount of citrate is relatively low compared to lactate. However, much greater increases in the concentration of calcium in the solution are observed when the citrate anion is added to solution (Figure 8a). The contribution of pH is again minimized here through utilization of tri-sodium citrate (pH 7.3-8.2). It is clear that citrate shows a strong calcium sequestering capacity, as has been widely reported elsewhere [4, 35, 38-43]. The effect of temperature on the saturation concentration of calcium in the presence of the citrate anion is less clear than with lactate or chloride, due to the dominant effect of citrate sequestration (Figure 8a).

A steady increase in the calcium concentration in the solution is observed for both organic acids as shown in Figure 8b. The sequestering capacity of citrate dominates over the contribution of pH under all conditions, with the total calcium in the citric acid solution three to four times higher than in lactic acid. Further, analysis indicated that after 168 hours equilibration, from an initial concentration of 10 mM of organic acid, 5.4 ± 0.8 mM of citrate was consumed whereas only 1.5 ± 0.3 mM lactate was consumed for the same amount of DCPD. At 100mM (Figure 6), the concentration of calcium in the solution is largely the same for lactic and nitric acid, which confirms that the increase in solubility for lactic acid is solely due to phosphate dissociation at these extreme pH levels (DCPD in 100mM HNO_3 pH 1.75 and in 100 mM lactic acid pH 2.68).

The addition of sodium lactate has minimal impact on the Ca/P ratio at 30°C (see Figure 9). Conversely, the addition of trisodium citrate increases the amount of the available calcium in the solution due either to formation of $CaCit^-$ or to the preferential formation of DCPD [13, 14]. The addition of lactic acid causes the Ca/P ratio to increase to unity. This is consistent with the literature; DCPD is the most stable salt at pH less than 5.5 (Table 1). A similar increase was expected for citric acid, however this was not clearly observed, with the Ca/P in this case showing significant variability (data not shown).

As with the systems containing NaCl (Table 2) in systems at neutral pH, high temperature (50 °C) results in a lower Ca/P ratio in solution ($p<0.01$) implying preferential formation of HAP or OCP (Table 4). In the presence of lactic acid, the Ca/P ratio remains close to unity regardless of the temperature. This shows the dominating effect of pH in comparison to other parameters. Conversely, with citric acid, this ratio falls with temperature ($p < 0.01$).

Effect of high ionic strength in the presence of lactic acid

In real saline waste effluent, the background electrolyte comprises a mixture of ions. Figure 10 shows the saturation concentration of calcium in a mixture of NaCl with 10 mM of lactic acid at 30 ºC. The pH range throughout the whole experiment was 3.7 ± 0.6. It can be seen that the trends in solubility observed with NaCl alone (Figure 3) are no longer observed. Instead, the concentration of calcium in the solution remains relatively constant at the same value as for 10mM lactic acid alone (7 mM). This again reflects the stronger influence of pH, relative to those of ionic strength alone.

Despite the dominance of pH in determining the calcium solubility, the presence of NaCl still significantly affects the Ca/P ratio. In the absence of NaCl, the Ca/P ratio observed is 1.01 ± 0.03 which is consistent with the Ca/P ratio across a broad range of acidic conditions. However, in the presence of NaCl the Ca/P ratio falls significantly ($p<0.01$) to 0.8 ± 0.03.

4. DISCUSSION

In this work, the solubility of calcium phosphate in the presence of high concentrations of lactose, sodium chloride, lactate and citrate and under conditions of variable acidity and temperature has been investigated. Lactose has a marginal but significant effect upon the saturation concentration of calcium in the solution, possibly due to the formation of a complex of lactose with calcium salts. Increasing ionic strength through the addition of NaCl up to 100 g/L within the temperature range of 10ºC to 50ºC, reduces the activity of Ca^{2+} resulting in an increase of calcium in the solution. Above this NaCl concentration, the calcium solubility declines, particularly at 50ºC. This latter finding is of importance in understanding calcium scaling in high salt solutions. The addition of salts such as sodium chloride or trisodium lactate has differing effects on the solubility of $H_2PO_4^-$ and HPO_4^{2-} at different temperatures, so that at 10 and 30ºC, the Ca/P ratio increases from around 0.5 to 0.7-0.8, while at 50ºC this ratio remains low.

The lactate anion shows only a very small calcium sequestering capacity compared to the citrate anion, but is still a more effective sequestering agent than chloride. Specifically, at

10ºC and 30ºC, the same calcium solubility is achieved by 100 mM sodium lactate as 170 mM sodium chloride. However, when lactate is added as an organic acid, the role of pH dominates, providing large increases in the calcium concentration and resulting in the Ca/P ratio reaching unity.

In a real effluent solution comprising an abundant amount of both NaCl and lactic acid, the effect of pH dominates the saturation concentration of calcium, but crystal morphology is still affected by the NaCl concentration, as indicated by changes in the Ca/P ratio.

Lastly, experiments showed some evidence in support of previous work by other authors that have suggested that calcium solubility can be affected by the quantity of excess solid in the solution.

5. ACKNOWLEDGMENTS

This research was supported under Australian Research Council's Industrial Transformation Research Program (ITRP) funding scheme (Project number IH120100005). The ARC Dairy Innovation Hub is a collaboration between The University of Melbourne, The University of Queensland and Dairy Innovation Australia Ltd. Judy Lee acknowledges the support from an Australian Research Council Discovery Early Career Researcher Award (DE120101567).

REFERENCES

1. Pan, H. B.; Darvell, B. W., Calcium Phosphate Solubility: The Need for Re-Evaluation. *Cryst. Growth Des.* **2009**, *9*, 639-645.
2. Miyazaki, T.; Sivaprakasam, K.; Tantry, J.; Suryanarayanan, R., Physical characterization of dibasic calcium phosphate dihydrate and anhydrate. *J. Pharm. Sci.* **2009**, *98*, 905-916.
3. Pan, H. B.; Darvell, B. W., Solubility of Dicalcium Phosphate Dihydrate by Solid Titration. *Caries Res.* **2009**, *43*, 254-260.

4. Spanos, N.; Patis, A.; Kanellopoulou, D.; Andritsos, N.; Koutsoukos, P., Precipitation of calcium phosphate from simulated milk ultrafiltrate solutions. *Cryst. Growth Des.* **2007**, *7*, 25-29.
5. Dorozhkin, S., Calcium Orthophosphates: Occurrence, Properties and Major Applications. *Bioceram. Dev. Appl.* **2014**, *4*, 2.
6. Wang, L.; Nancollas, G. H., Calcium orthophosphates: crystallization and dissolution. *Chem. Rev.* **2008**, *108*, 4628-4669.
7. Johnsson, M. S.-A.; Nancollas, G. H., The Role of Brushite and Octacalcium Phosphate in Apatite Formation. *Crit. Rev. Oral Biol. & Med.* **1992**, *3*, 61-82.
8. Lundager Madsen, H. E.; Thorvardarson, G., Precipitation of calcium phosphate from moderately acid solution. *J. Cryst. Growth* **1984**, *66*, 369-376.
9. Madsen, H. E. L.; Christensson, F., Precipitation of calcium phosphate at 40 C from neutral solution. *J. Cryst. Growth* **1991**, *114*, 613-618.
10. Ferreira, A.; Oliveira, C.; Rocha, F., The different phases in the precipitation of dicalcium phosphate dihydrate. *J. Cryst. Growth* **2003**, *252*, 599-611.
11. House, W. A., The physico-chemical conditions for the precipitation of phosphate with calcium. *Environ. Technol.* **1999**, *20*, 727-733.
12. Van Kemenade, M.; De Bruyn, P., A kinetic study of precipitation from supersaturated calcium phosphate solutions. *J. Colloid Interface Sci.* **1987**, *118*, 564-585.
13. Rosmaninho, R.; Melo, L. F., The effect of citrate on calcium phosphate deposition from simulated milk ultrafiltrate (SMUF) solution. *J. Food Eng.* **2006**, *73*, 379-387.
14. Van Der Houwen, J. A. M.; Valsami-Jones, E., The Application of Calcium Phosphate Precipitation Chemistry to Phosphorus Recovery: The Influence of Organic Ligands. *Environ. Technol.* **2001**, *22*, 1325-1335.
15. *CRC Handbook of Chemistry and Physics*. 58th ed.; CRC Press: Cleveland Ohio, 1978.
16. Mekmene, O.; Gaucheron, F., Determination of calcium-binding constants of caseins, phosphoserine, citrate and pyrophosphate: A modelling approach using free calcium measurement. *Food Chem.* **2011**, *127*, 676-682.
17. Sharma, V. K.; Johnsson, M.; Sallis, J. D.; Nancollas, G. H., Influence of citrate and phosphocitrate on the crystallization of octacalcium phosphate. *Langmuir* **1992**, *8*, 676-679.
18. Kezia, K.; Lee, J.; Weeks, M.; Kentish, S., Direct contact membrane distillation for the concentration of saline dairy effluent. *Water Res.* **2015**.
19. Goss, S. L.; Lemons, K. A.; Kerstetter, J. E.; Bogner, R. H., Determination of calcium salt solubility with changes in pH and PCO2, simulating varying gastrointestinal environments. *J. Pharm. Pharmacol.* **2007**, *59*, 1485-1492.
20. Doherty, W.; Wright, P. A solubility model for calcium oxalate formation in a sugar mill, in Proceedings-Australian Society Of Sugar Cane Technologists, **2004**; PK Editorial Services; 1999: 2004; pp 50.
21. Besic, F. C., Food materials for prevention of carious degradation of teeth.United States Patent No. 4515770 A **1985**.
22. Jelen, P.; Coulter, S. T., Effects Of Certain Salts And Other Whey Substances On The Growth Of Lactose Crystals. *J. Food Sci.* **1973**, *38*, 1186-1189.
23. Jensen, O.; Hanford, Z.; Supplee, G., Equilibrium solutions of certain lactose-salt mixtures. *J. Dairy Sci.* **1940**, *23*, 745-753.
24. Atlas, E.; Culberson, C.; Pytkowicz, R., Phosphate association with Na^+, Ca^{2+} and Mg^{2+} in seawater. *Marine Chem.* **1976**, *4*, 243-254.
25. Kester, D. R.; Pytkowicz, R. M., Theoretical model for the formation of ion-pairs in seawater. *Marine Chem.* **1975**, *3*, 365-374.

26. Millero, F. J.; Schreiber, D., Use of the ion pairing model to estimate activity coefficients of the ionic components of natural waters. *Am. J. Sci.* **1982**, *282*, 1508-1540.
27. Sutter, J.; McDowell, H.; Brown, W. E., Solubility study of calcium hydrogen phosphate. Ion-pair formation. *Inorg. Chem.* **1971**, *10*, 1638-1643.
28. Greenwald, I., The solubility of calcium phosphate I. The effect of pH and of amount of solid phase. *J. Biol. Chem.* **1942**, *143*, 703-710.
29. Pan, H. B.; Darvell, B. W., Solubility of hydroxyapatite by solid titration at pH 3–4. *Arch. Oral Biol.* **2007**, *52*, 618-624.
30. Green, D.; Perry, R., *Perry's Chemical Engineers' Handbook,* Eighth Edition. McGraw-Hill Education: 2007.
31. Koutsoukos, P. G. *Current knowledge of calcium phosphate chemistry and in particular solid surface–water interface interactions*, In Proceedings of the Second International Conference on Phosphorus Recovery for Recycling from Sewage and Animal Wastes, Institute of Chemical Engineering and High Temperature Chemical Processes, Univ. of Patras, 2000; pp 12-14.
32. Rice, G.; Barber, A.; O'Connor, A.; Stevens, G. W.; Kentish, S. E., Fouling of NF membrane by dairy ultrafiltration permeate. *J. Membr. Sci.* **2009**, *330*, 117-126.
33. Schäfer, A.; Fane, A. G.; Waite, T., Nanofiltration of natural organic matter: removal, fouling and the influence of multivalent ions. *Desalin.* **1998**, *118*, 109-122.
34. Kezia, K.; Lee, J.; Zisu, B.; Weeks, M.; Chen, G.; Gras, S.; Kentish, S., Crystallisation of minerals from concentrated saline dairy effluent. *Water Res.* **2016**, *101*, 300-308.
35. Amjad, Z. (Ed.), *Calcium Phosphates in Biological and Industrial Systems*. Springer US: 2013.
36. Chow, L. C.; Eanes, E. D., *Octacalcium Phosphate*. Karger: 2001; Vol. 18.
37. Tung, M.; Eidelman, N.; Sieck, B.; Brown, W., Octacalcium phosphate solubility product from 4 to 37 C. *J. Res. Nat. Bur. Stand.* **1988**, *93*, 613-624.
38. Mekmene, O.; Mekmene, F.; Gaucheron, Determination of calcium-binding constants of caseins, phosphoserine, citrate and pyrophosphate: A modelling approach using free calcium measurement. *Food Chem.* **2011**, *127*, 676-682.
39. Mekmene, O.; Mekmene, T.; Rouillon, S.; Quillard, P.; Pilet, J.-M.; Bouler, S.; Pezennec, F.; Gaucheron, Effects of citrate and NaCl on size, morphology, crystallinity and microstructure of calcium phosphates obtained from aqueous solutions at acidic or near-neutral pH. *J. Dairy Res.* **2012**, *79*, 238-248.
40. Sharma, V.; Johnsson, M.; Sallis, J.; Nancollas, G., Influence of citrate and phosphocitrate on the crystallization of octacalcium phosphate. *Langmuir* **1992**, *8*, 676-679.
41. Duputel, M.; Devau, N.; Brossard, M.; Jaillard, B.; Jones, D. L.; Hinsinger, P.; Gérard, F., Citrate adsorption can decrease soluble phosphate concentration in soils: Results of theoretical modeling. *Appl. Geochem.* **2013**, *35*, 120-131.
42. Holt, C., Calcium phosphate nanoclusters and their applications. United States Patent No. US 7517850 B2, 2009.
43. Holt, C.; Jenness, R., Interrelationships of constituents and partition of salts in milk samples from eight species. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **1984**, *77*, 275-282.

TABLE CAPTIONS

Table 1. The various forms of calcium phosphate that can form in aqueous solution and their solubility at 25°C and neutral pH[5].

Table 2. The Ca/P ratio in the supernatant after 168 hours equilibration of 10 g/L DCPD, with a background concentration of NaCl. Superscripts indicate samples that are not significantly different ($p > 0.01$).

Table 3. The Calcium and phosphorus concentrations in the supernatant after 168 hours equilibration of 10 g/L DCPD, with a background concentration of NaCl or sodium lactate. Superscripts indicate samples that are not significantly different ($p > 0.01$).

Table 4. The Ca/P ratio in the supernatant after 168 hours equilibration of 10g/L DCPD, with a background concentration of organic anions. Superscripts indicate samples that are not significantly different ($p > 0.01$).

FIGURE CAPTIONS

Figure 1. Schematic diagram of the end-point equilibrium technique.

Figure 2. (a) The concentration of calcium and (b) the calcium to phosphate ratio in the supernatant after 168 hours of equilibration at 30ºC of 10 g/L DCPD, with a background concentration of NaCl or lactose.

Figure 3. The concentration of calcium in the supernatant after 168 hours of equilibration of 10 g/L DCPD, with a background concentration of NaCl.

Figure 4. (a) Activity coefficient of Ca^{2+} (b) Activity coefficient of orthophosphates simulated using the Pitzer model within ASPEN Plus V8.6 at 25ºC. Concentrations of calcium, phosphate and hydronium simulated were 10mM.

Figure 5. Comparison of the experimental data for calcium and phosphorus concentrations in the supernatant at 30ºC with a simulation that assumes that DCPD is the only salt (Ca/P = 1) and uses the activity coefficients determined from the Pitzer model and the solubility constant from Table 1 at 25ºC.

Figure 6. Concentration of Ca^{2+} in the supernatant after 168 hours equilibration of 10g/L DCPD and 1.25g/L DCPD at 30ºC.

Figure 7. Concentration of calcium in the supernatant after 168 hours of equilibration of 20g/L DCPD at 30ºC, with a background acid concentration (a) absolute concentrations (b) concentrations relative to the nitric acid case.

Figure 8. (a) The concentration of Ca^{2+} in the supernatant with a background concentration of an organic anion i.e. sodium lactate and trisodium citrate (pH 7.0-8.7) (b) The concentration of Ca^{2+} in the supernatant with a background concentration of organic acid i.e.

lactic acid and citric acid (pH 2.68 - 6.00) after 168 hours of equilibration of 10g/L DCPD. The NaCl concentration is zero. Note the different x-axis scales on the two graphs.

Figure 9. The calcium to phosphate ratio in the supernatant after 168 hours of equilibration at 30ºC of 10 g/L DCPD, with a background concentration of lactic acid, trisodium citrate or sodium lactate.

Figure 10. Concentration of calcium and phosphorous in the supernatant after 168 hours of equilibration at 30ºC of 10g/L in 10 mM lactic acid, with a background concentration of NaCl.

Table 1

Ca/P mol ratio	Compound	Formula	Solubility 25ºC -log (Ksp)	pH* 25ºC
0.5	Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2.H_2O$	1.14	0.0-2.0
1.0	Dicalcium phosphate dihydrate (DCPD, brushite)	$CaHPO_4.2H_2O$	6.59	2.0-6.0
1.3	Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$	96.6	5.5-7.0
1.2-2.2	Amorphous calcium phosphates (ACP)	$Ca_xH_y(PO_4)_z.nH_2O$ n=3-4.5	***	5.0-12.0
1.5-1.7	Calcium deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0<x<1)$	≈ 85	6.5-9.5
1.7	Hydroxyapatite (HAP)	$Ca_{10}(PO_4)_6(OH)_2$	116.8	9.5-12.0

*pH where the solid can exist in aqueous solution at room temperature.

***cannot be precisely measured, in acidic buffer ACP<CDHA<HAP.

Table 2

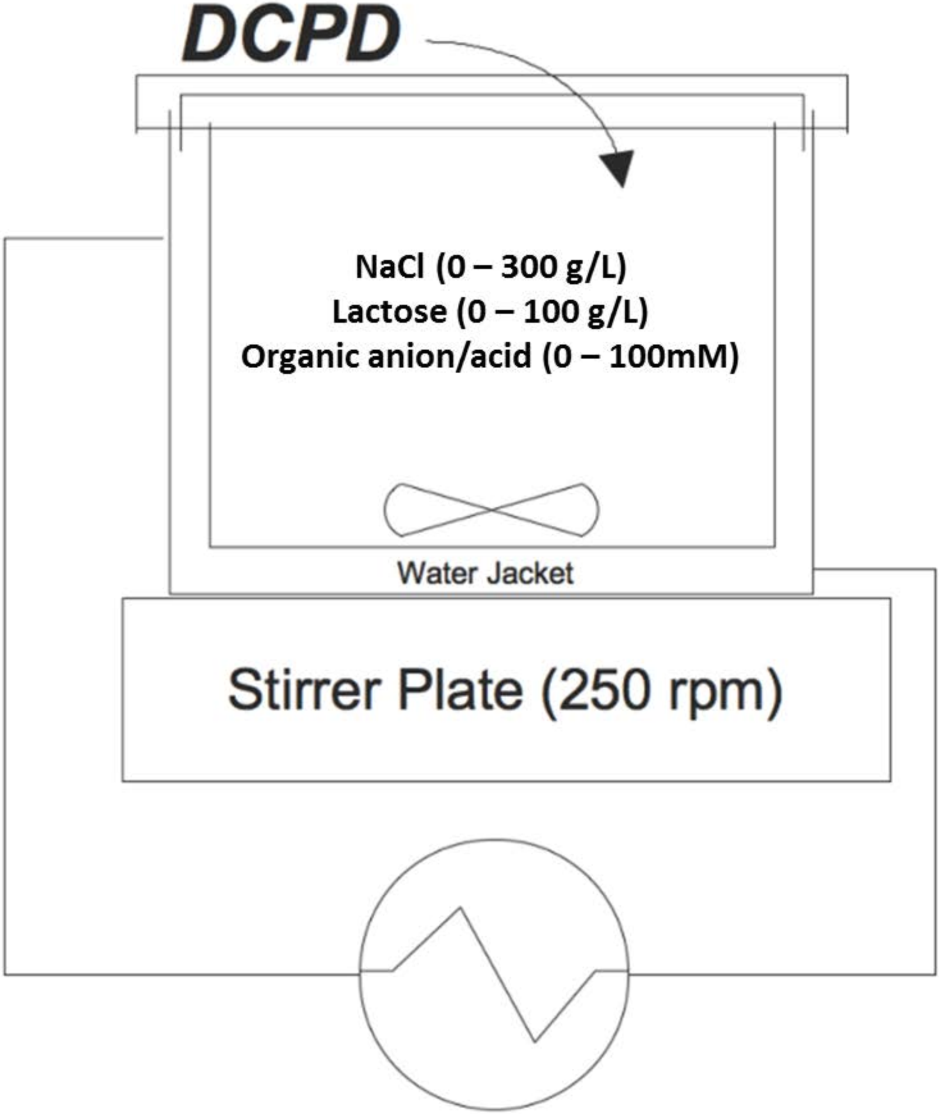
	0 g/L NaCl	10 g/L NaCl	100 g/L NaCl	300 g/L NaCl
10ºC	0.52 ± 0.06^{a}	0.74 ± 0.04^{b}	0.74 ± 0.03^{b}	0.77 ± 0.01^{b}
30ºC	0.54 ± 0.03^{a}	0.72 ± 0.01^{b}	0.77 ± 0.02^{b}	0.72 ± 0.04^{b}
50ºC	0.45 ± 0.03^{c}	0.46 ± 0.01^{c}	0.44 ± 0.02^{c}	0.46 ± 0.04^{c}

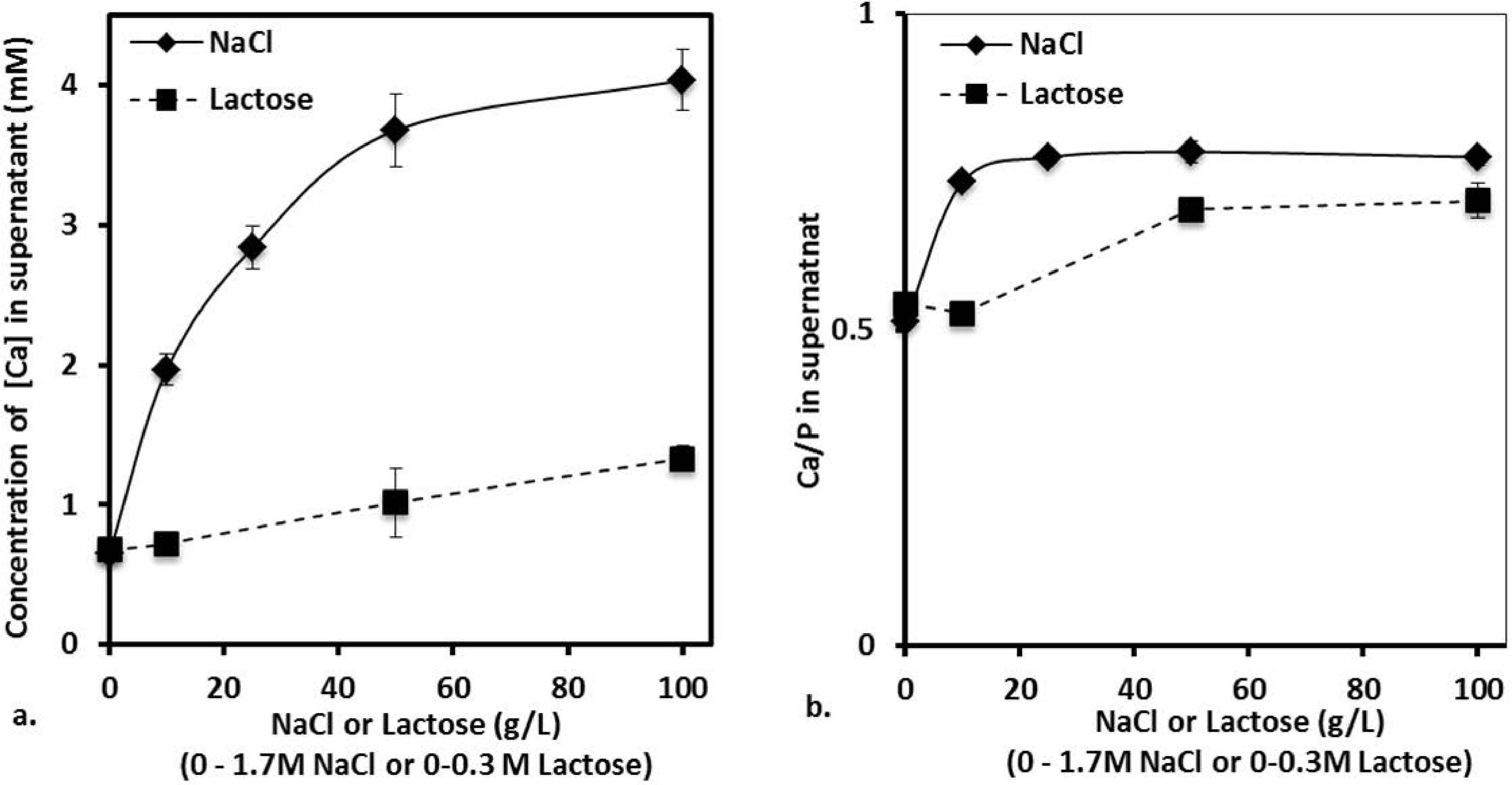
Table 3

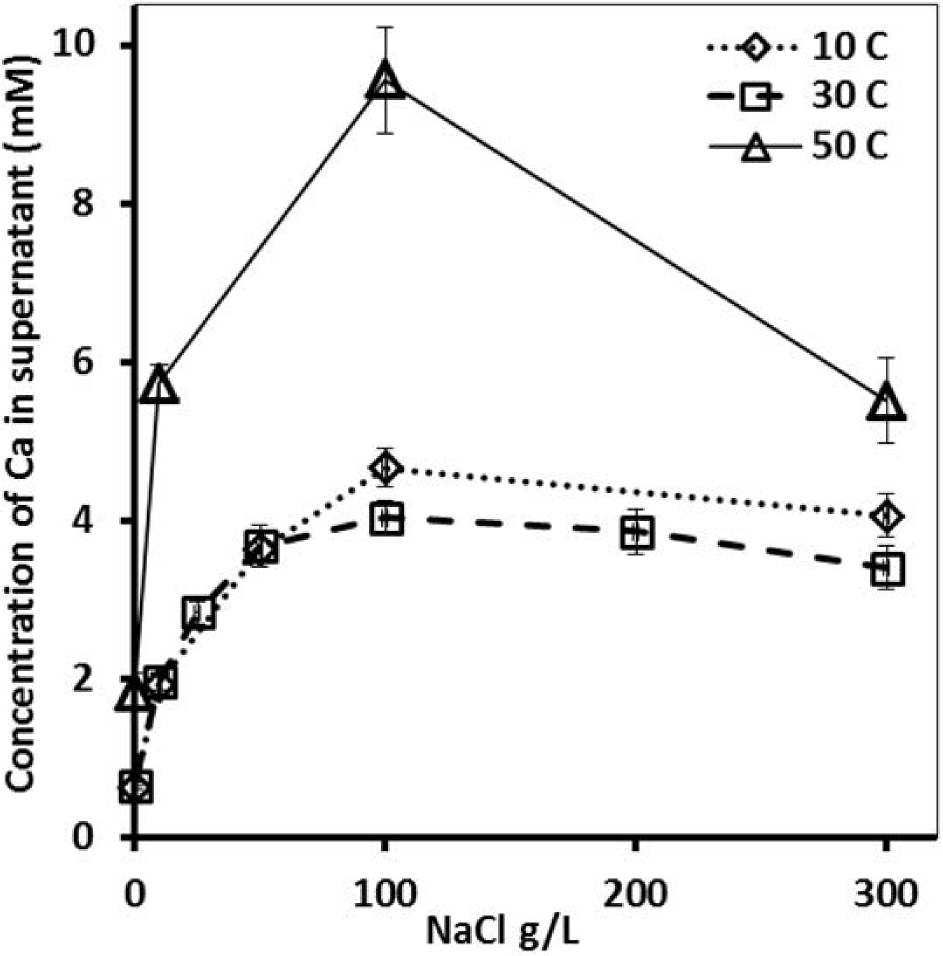
	Calcium (mM)		Phosphorus (mM)	
	170 mM NaCl	**100 mM NaLactate**	**170 mM NaCl**	**100 mM NaLactate**
10ºC	1.97 ± 0.13	1.99 ± 0.22	2.65 ± 0.15	2.46 ± 0.05
30ºC	1.96 ± 0.11	1.87 ± 0.10	2.67 ± 0.06	2.71 ± 0.03

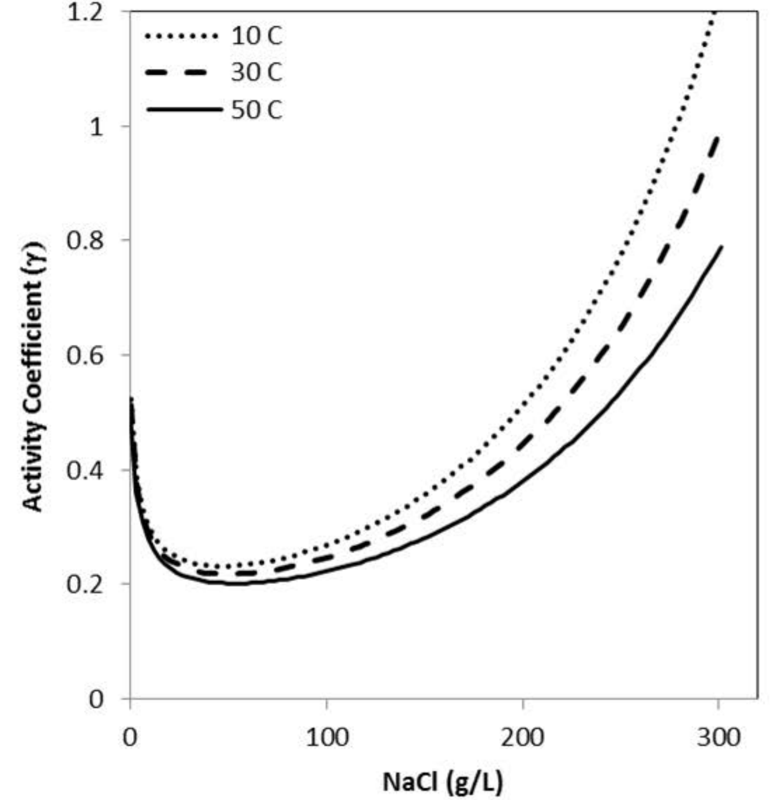
Table 4

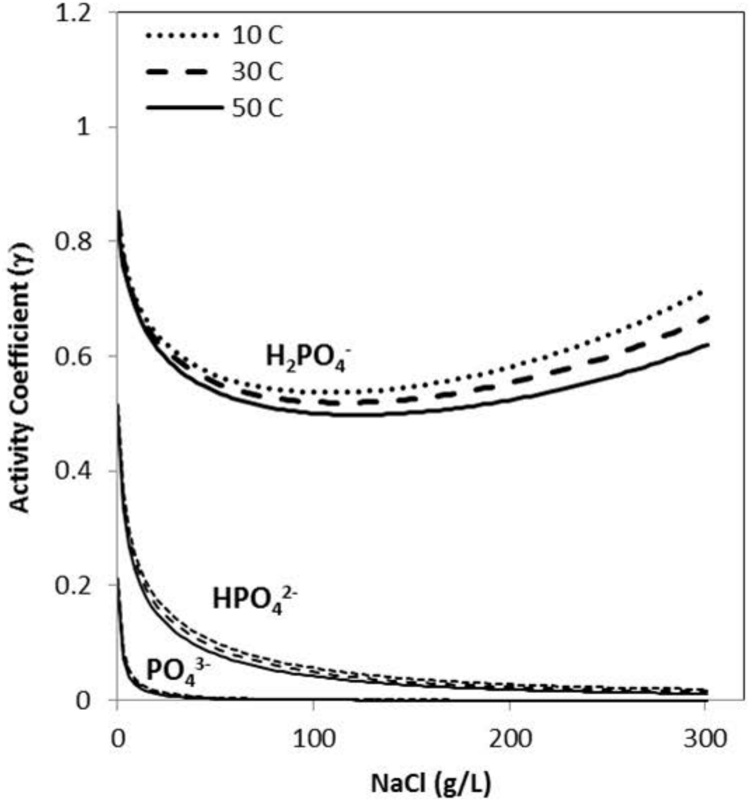
10°C	30°C	50°C	10°C	30°C	50°C
Sodium Lactate			**Trisodium Citrate**		
$\mathbf{0.66 \pm 0.04^{a}}$	0.61 ± 0.04^{a}	0.45 ± 0.01^{b}	0.84 ± 0.06^{c}	0.81 ± 0.03^{c}	0.27 ± 0.04^{d}
Lactic Acid			**Citric Acid**		
$\mathbf{0.96 \pm 0.08^{e}}$	1.04 ± 0.04^{f}	0.92 ± 0.03^{e}	0.88 ± 0.07^{g}	0.78 ± 0.04^{c}	0.64 ± 0.03^{a}

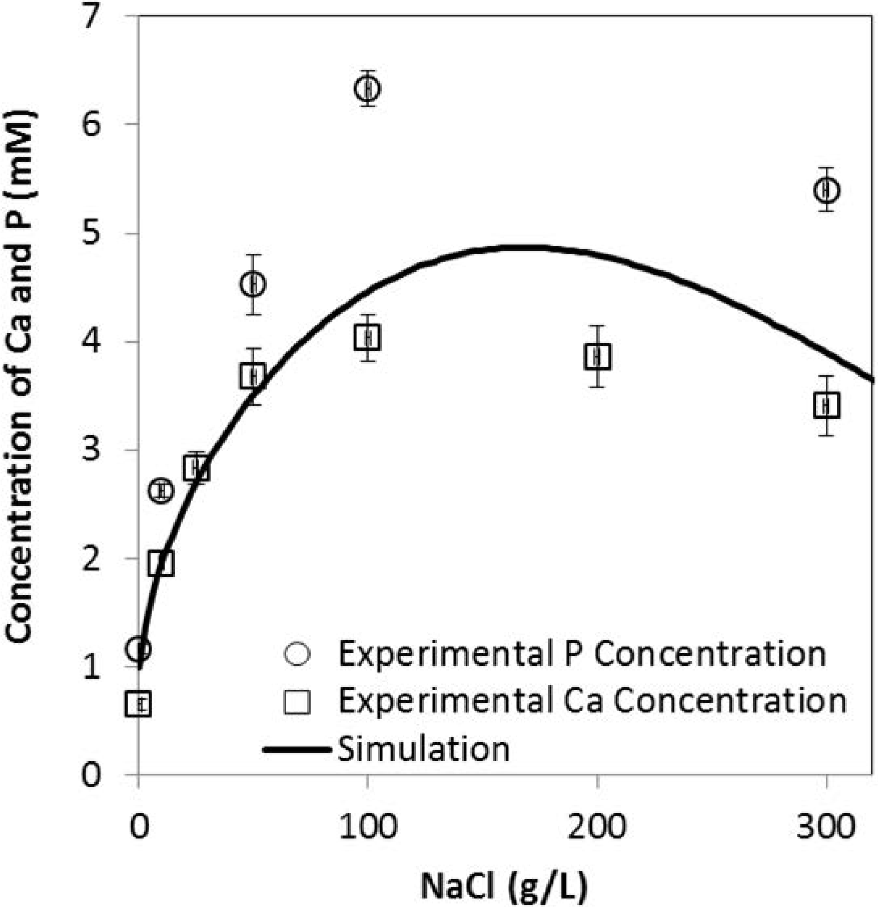

DCPD
NaCl (0 – 300 g/L)
Lactose (0 – 100 g/L)
Organic anion/acid (0 – 100mM)
Water Jacket
Stirrer Plate (250 rpm)

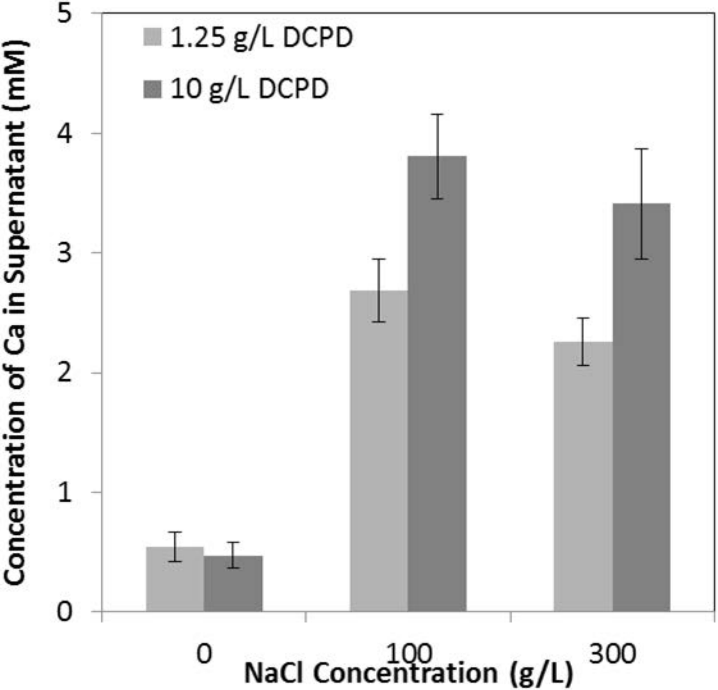

a.
Concentration of [Ca] in supernatant (mM)
0
1
2
3
4
NaCl
Lactose
0
20
40
60
80
100
NaCl or Lactose (g/L)
(0 - 1.7M NaCl or 0-0.3 M Lactose)
b.
Ca/P in supernatnat
0
0.5
1
NaCl
Lactose
0
20
40
60
80
100
NaCl or Lactose (g/L)
(0 - 1.7M NaCl or 0-0.3M Lactose)


Concentration of Ca in supernatant (mM)
0
2
4
6
8
10
0
100
200
300
NaCl g/L
10 C
30 C
50 C

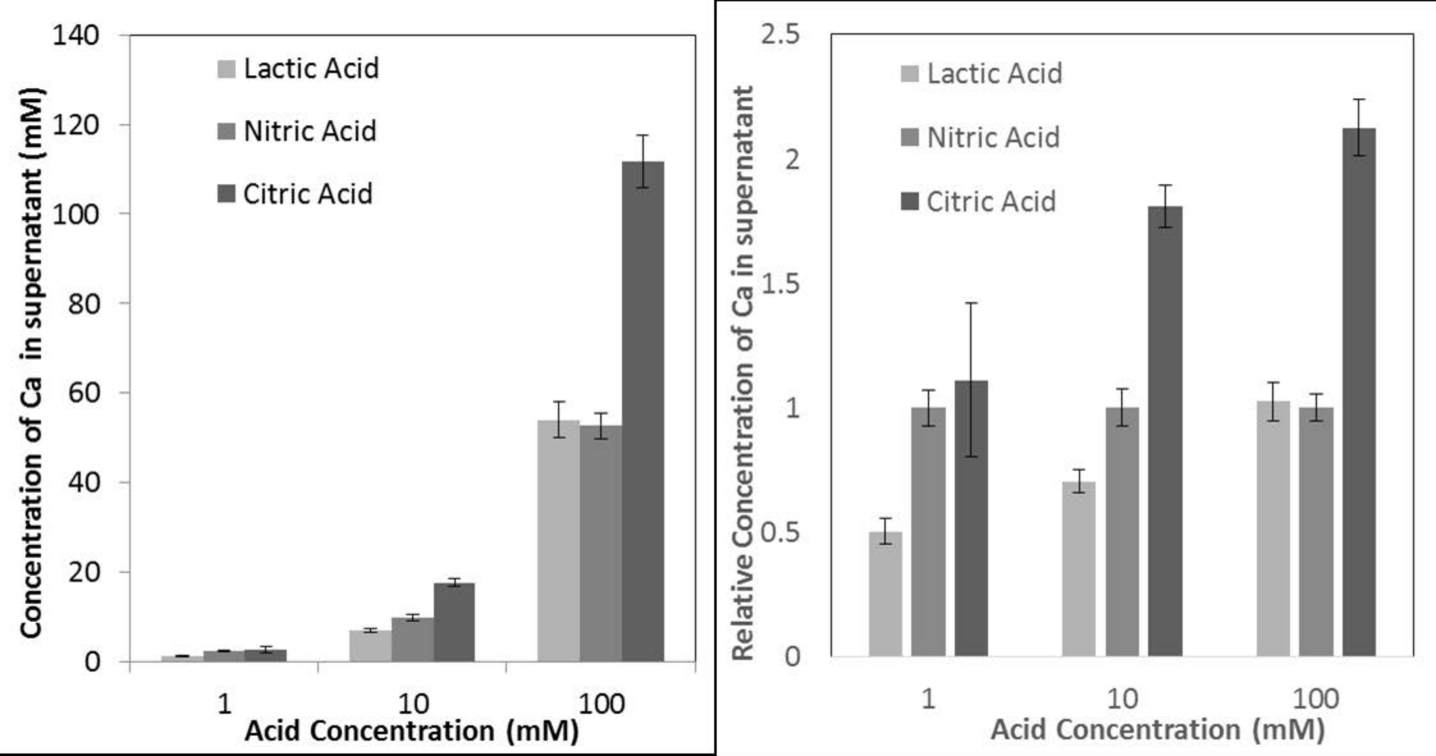

10 C
30 C
50 C
Activity Coefficient (γ)
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
NaCl (g/L)

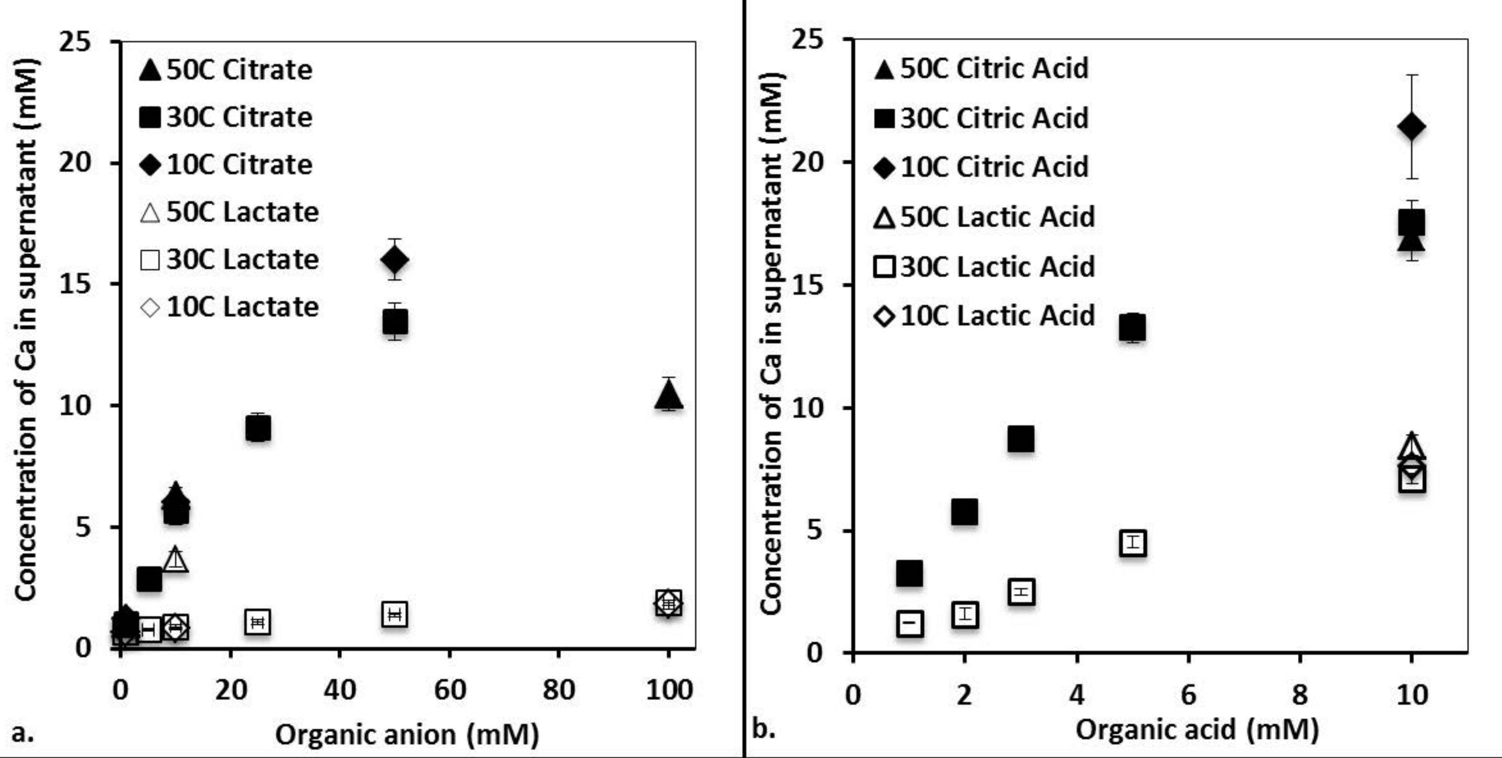

10 C
30 C
50 C
$H_2PO_4^-$
HPO_4^{2-}
PO_4^{3-}
Activity Coefficient (γ)
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
NaCl (g/L)

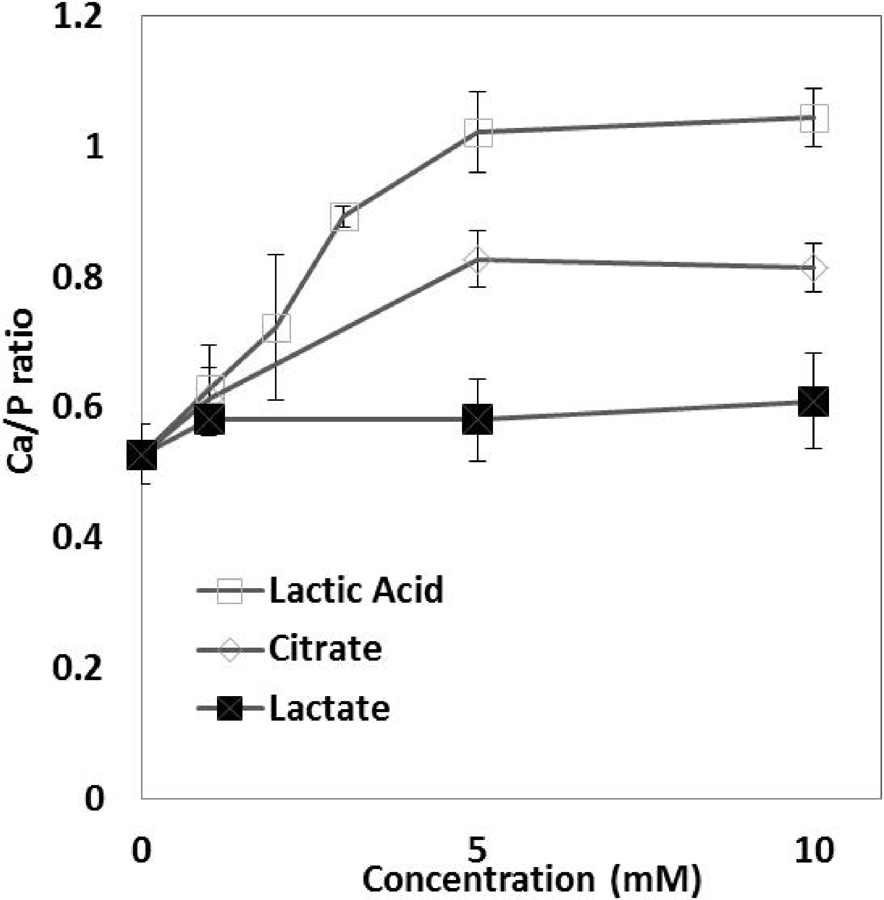

Concentration of Ca and P (mM)
0
1
2
3
4
5
6
7
0
100
200
300
NaCl (g/L)
○ Experimental P Concentration
□ Experimental Ca Concentration
Simulation

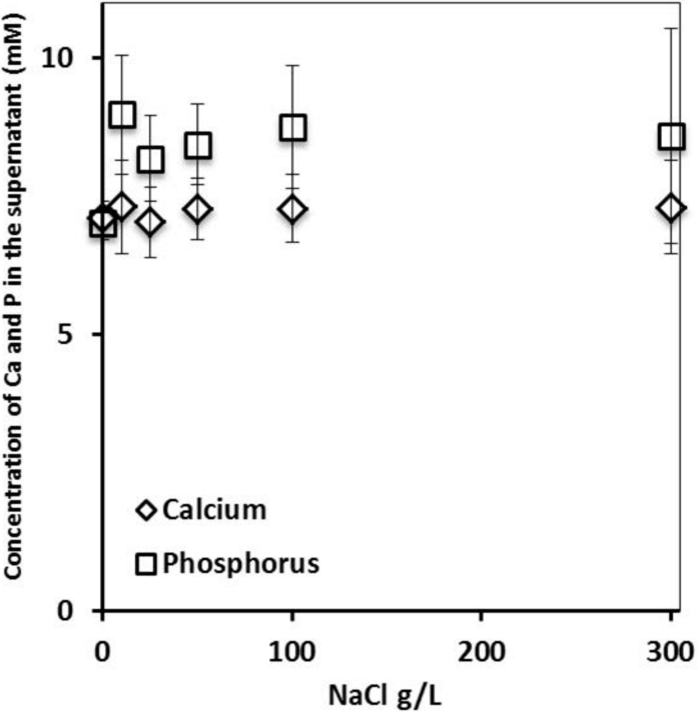


1.25 g/L DCPD
10 g/L DCPD
Concentration of Ca in Supernatant (mM)
5
4
3
2
1
0
0
100
300
NaCl Concentration (g/L)


Concentration of Ca in supernatant (mM)
140
120
100
80
60
40
20
0
Lactic Acid
Nitric Acid
Citric Acid
1
10
100
Acid Concentration (mM)
Relative Concentration of Ca in supernatant
2.5
2
1.5
1
0.5
0
Lactic Acid
Nitric Acid
Citric Acid
1
10
100
Acid Concentration (mM)


a.
Concentration of Ca in supernatant (mM)
0
5
10
15
20
25
▲ 50C Citrate
■ 30C Citrate
◆ 10C Citrate
△ 50C Lactate
□ 30C Lactate
◇ 10C Lactate
0
20
40
60
80
100
Organic anion (mM)
b.
Concentration of Ca in supernatant (mM)
0
5
10
15
20
25
▲ 50C Citric Acid
■ 30C Citric Acid
◆ 10C Citric Acid
△ 50C Lactic Acid
□ 30C Lactic Acid
◇ 10C Lactic Acid
0
2
4
6
8
10
Organic acid (mM)


1.2
1
0.8
0.6
0.4
0.2
0
Ca/P ratio
0
5
10
Concentration (mM)
Lactic Acid
Citrate
Lactate


Concentration of Ca and P in the supernatant (mM)
10
5
0
0
100
200
300
NaCl g/L
◇ Calcium
□ Phosphorus