

Myo-inositol hexakisphosphate (phytate) inhibits calcium carbonate crystallisation in hard water

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Abstract

A batch system and a flow system with synthetic water were used to study calcium carbonate precipitation and phytate crystallisation inhibitory effects. Afterwards, phytate inhibitory effects on calcium carbonate crystallisation were tested in a real system, working with a cistern filled with hard water. Finally, the effects of phytate on calcium carbonate crystallisation were compared with another phosphate derivative and with a chelating agent.

The results obtained with the batch system demonstrated that 1.52 μM of phytate completely avoided calcium carbonate crystallisation when the calcium concentration was less than 7.9 mM and the carbonate concentration was 5.83 mM. The results corresponding to the flow system showed that 3.03 μM of phytate led to a 95% reduction of calcium carbonate scale formation on a copper pipe after 48 h, operating at 30°C. In addition, in a full-scale system, the dissolved calcium and bicarbonate concentration was increased for a given time when adding phytate to the cistern. Finally, using a batch system, it was demonstrated that phytate effects on calcium carbonate crystallisation reduction were superior to those shown by triphosphate and EDTA.

The results presented demonstrate that phytate at very low concentrations can be used to prevent calcium carbonate scale formation without changing the mineral composition of water due to its capacity as a crystallisation inhibitor.

Keywords: phytate, inhibition, calcium carbonate, scale formation, water hardness

Introduction

The driving force for crystallisation is supersaturation. Thermodynamically, a supersaturated system is unstable, and depending on kinetic factors (promoters or crystallisation inhibitors) solids formation will be enhanced or delayed. Water hardness can lead to scale formation, consisting primarily of CaCO_3 , but including other salts such as CaSO_4 , resulting from high supersaturation. This problem is critical in systems working at high temperatures, such as boilers. In these systems hard water cannot be used, because of the risks of breakages and explosions.

Calcium carbonate is the most abundant mineral in nature (Sikes, 1988) and is present as three polymorphic varieties, calcite, aragonite and vaterite. Morphological control of calcium carbonate can be achieved using different additives (Kang, 2005; Kim, 2005; Hou, 2005). Among the water-softening methods proposed to avoid calcium carbonate scale formation are ionic exchangers, consisting of zeolites (Malik, 2001; Semenov, 2004) and ion-exchange resins (Gwon, 2002); reverse osmosis systems (Hirayama, 1990); and the formation of stable complexes with EDTA (Westin, 2005) and phosphates such as tripolyphosphate (Arai 1968). Recently, electrodialysis has been used to remove calcium and magnesium from solutions (Toti, 2000; Kabay, 2002).

Calcium carbonate precipitation can also be reduced or delayed by using so-called crystallisation inhibitors. These substances prevent or disturb precipitation by adsorbing onto the surfaces of nuclei as they form or onto the faces of growing

crystals, respectively, thereby modifying the kinetics of crystallisation. Among the agents described as calcium carbonate crystallisation inhibitors are ions such as Fe^{2+} , Fe^{3+} , Mg^{2+} and Zn^{2+} (Reddy, 1980; Macadam, 2004; Hasson, 2004; Coetzee, 1998).

Calcium carbonate crystallisation has also been widely studied using the inhibitory properties of other compounds, among them phosphates and phosphonates (Reddy (1972; 1973); Nancollas, 1975), aminophosphonates (Guo, 2004), polyacrylic acid (Klepetsanis (1999; 2000)), and copolymers containing maleic acid (Klepetsanis, 1998).

Myo-inositol hexakisphosphate (phytate) is a powerful inhibitor of the crystallisation of calcium salts and has been shown to prevent calcium renal lithiasis (Grases (1999; 2000a)). Phytate levels found in tissues reflect dietary intake (Grases (2001a; 2001b)). Previous work has found that phytate only manifested toxic effects at very high doses (the LD_{50} value for male rats was 1.3 $\text{mmol}\cdot\text{kg}^{-1}$ administered orally as sodium phytate (Ichikawa, 1987). Administration of phytate as the natural food salt (phytin: calcium magnesium phytate) at amounts that provide maximum absorption equivalent to doses that correspond to the consumption of the so-called 'Mediterranean diet' (1 to 2 g phytate/d) were not found to have any chronic effect (Grases, 2004). Furthermore, in the field of renal lithiasis and cardiovascular calcifications it is the most effective crystallisation inhibitor of calcium oxalate (Grases (1994; 1996a; 1996b)), its efficacy on brushite (calcium phosphate) even being superior to other phosphate derivatives such as pyrophosphate, hexametaphosphate, etidronate or triphosphate (Grases (2000b; 2007)). All these data led us to believe that phytate could be used as an alternative to scale prevention and we therefore assayed the crystallisation inhibition properties of phytate on calcium carbonate solids formation by adding it to supersaturated water.

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Solution A (μM)		Solution B (μM)	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	162.3	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	162.3
KNO_3	29.7	KNO_3	29.7
$\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$	400	$\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$	400
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.802	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.802
ZnCl_2	14.66	ZnCl_2	14.66
CaCl_2	0-12 500	NaHCO_3	11 670

Methods

Preparation of synthetic hard water

Synthetic hard water was prepared by mixing equal volumes of Solutions A and B, as shown in Table 1. Solution A was stored for a maximum of 1 week at 4°C, and Solution B was always prepared immediately before use. Chemicals of reagent-grade purity were dissolved in deionised, redistilled water. All solutions were filtered through a 0.45 μm pore filter before use.

Calcium carbonate precipitation using a batch system and study of phytate crystallisation inhibitory effects

To study calcium carbonate crystal formation in synthetic hard water, turbidimetric measurements were performed by means of a photometer (Metrohm 662) equipped with a fibre-optic light guide measuring cell with an attached light path 2 x 10 mm reflector, using monochromatic light (550 nm). Crystallisation processes were performed at 25°C using cylindrical tubes (height 100 mm, inner diameter 150 mm). Five ml of Solution A were added to the crystallisation tube, followed by 5 ml of freshly prepared Solution B; the pH of each solution was adjusted to 8.3 with diluted HCl or NaOH. The tube was immediately stoppered and the solution was stirred to homogeneously distribute the precipitates. The absorbance was measured after 15 s and 1 h later. Final calcium concentration ranged between 0.4 to 12.9 mM. Precipitations were carried out at phytate concentrations of 0 to 3.03 μM .

The solids formed under these experimental conditions were filtered and dissolved in 1 M HCl and total dissolved calcium and magnesium were determined by atomic emission spectrometry, using an inductively coupled plasma (Perkin-Elmer SL, Optima 5300DV) spectrometer. Calcium carbonate crystals formed after 1 h were filtered, dried in a desiccator and analysed using a scanning electron microscope equipped with an energy dispersive X-ray analyser (EDS).

Calcium carbonate precipitation using a flow system and study of phytate crystallisation inhibitory effects

Synthetic hard water was recirculated by a multi-channel peristaltic pump for 48 h at a flow rate of 4 ml/min through a copper pipe of 200 mm length, 4 mm inner diameter and 6 mm outer diameter (Fig. 1). Synthetic hard water was changed after 24 h to reduce the effects of supersaturation changes due to crystal deposition on the pipe walls. Crystallisation was performed at a temperature of 30 and 65°C.

Final calcium concentration ranged from 2.9 to 5.4 mM and the pH of both solutions was adjusted to 8.0. Precipitation was carried out at phytate concentrations of 0 to 4.55 μM .

After 48 h the precipitate on the pipe walls was dissolved

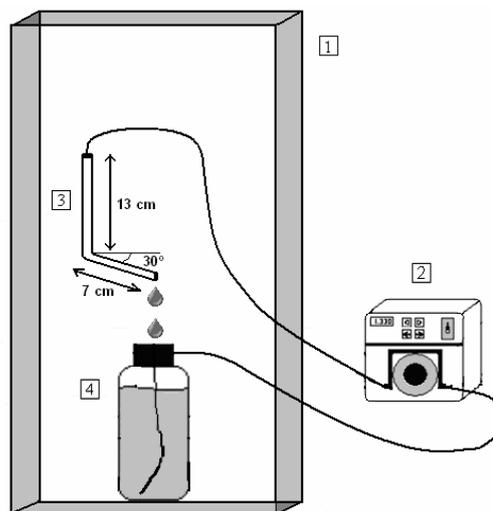


Figure 1

Diagram of the experimental flow system used to study calcium carbonate crystallisation. 1. Temperature-controlled chamber; 2. Peristaltic pump; 3. Copper pipe; 4. Synthetic hard water

in 1 M HCl and the calcium concentration was determined by atomic emission spectrometry using an inductively coupled plasma (Perkin Elmer Optima 5300 DV) spectrometer.

Full-scale system to study phytate crystallisation inhibitory effects on calcium carbonate precipitation

A cistern with a capacity of 10 000 l (supplying water to three families) was used to study the inhibitory capacity of phytate in a real system. The cistern was refilled weekly from a natural well filled with hard water. Upon filling the cistern, phytate (as a potassium salt) was added to a final concentration of 0 to 1.52 μM . After 2 d of filling, a water sample was collected from a tap of one of the families, and its pH and calcium and bicarbonate concentrations were measured.

Comparison of the effects of phytate, triphosphate and EDTA on calcium carbonate precipitation reduction using a batch system

To compare the effects of phytate, triphosphate (as sodium salt) and EDTA (as sodium salt) on calcium carbonate crystallisation, kinetic turbidimetric measurements were performed by means of a photometer (Metrohm 662) equipped with a fibre-optic light guide measuring cell with an attached light path 2 x 10 mm reflector, using monochromatic light (550 nm). Crystallisation processes were carried out in a thermostated (25°C) and magnetically stirred cylindrical glass flask (height 12.5 cm, diameter 9 cm). 250 ml of a 6 mM CaCl_2 solution were added to the crystallisation flask, the measuring cell was immersed in the solution and the magnetic stirrer was switched on. Then 250 ml of a solution of 11.67 mM NaHCO_3 were added. Final calcium and carbonate concentrations were 3 mM and 5.82 mM respectively. The pH of both solutions was adjusted to 8.5 with diluted HCl or NaOH. The range of additives concentrations assayed were 0 to 3.03 μM for phytate, 0 to 408 μM for triphosphate and 0 to 2 000 μM for EDTA, the chart recorder was immediately switched on in order to print the absorbance-time curve. A diagram of the experimental device used is shown in Fig. 2.

Figure 2
Diagram of the experimental device used to compare the effects of phytate, triphosphate and EDTA on calcium carbonate crystallisation. 1. Recorder; 2. Altern tension stabiliser; 3. Photometer equipped with a fibre-optic light-guide measuring cell; 4. Thermostatic bath; 5. Magnetic stirrer.

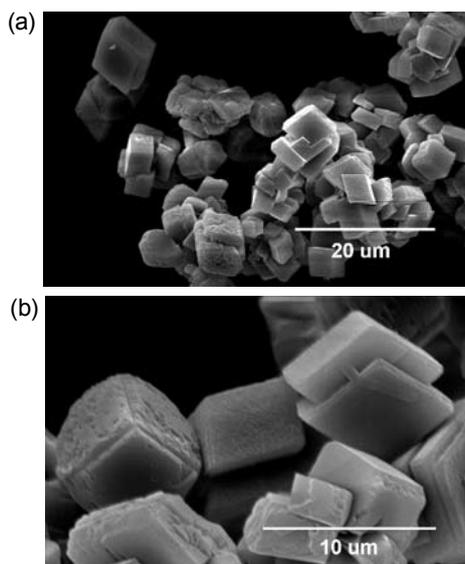
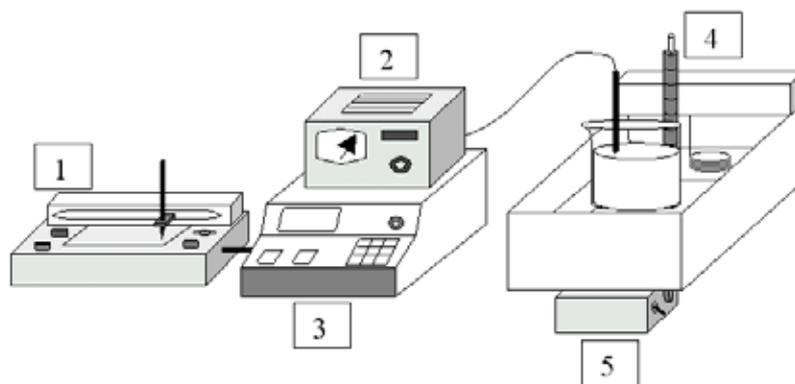
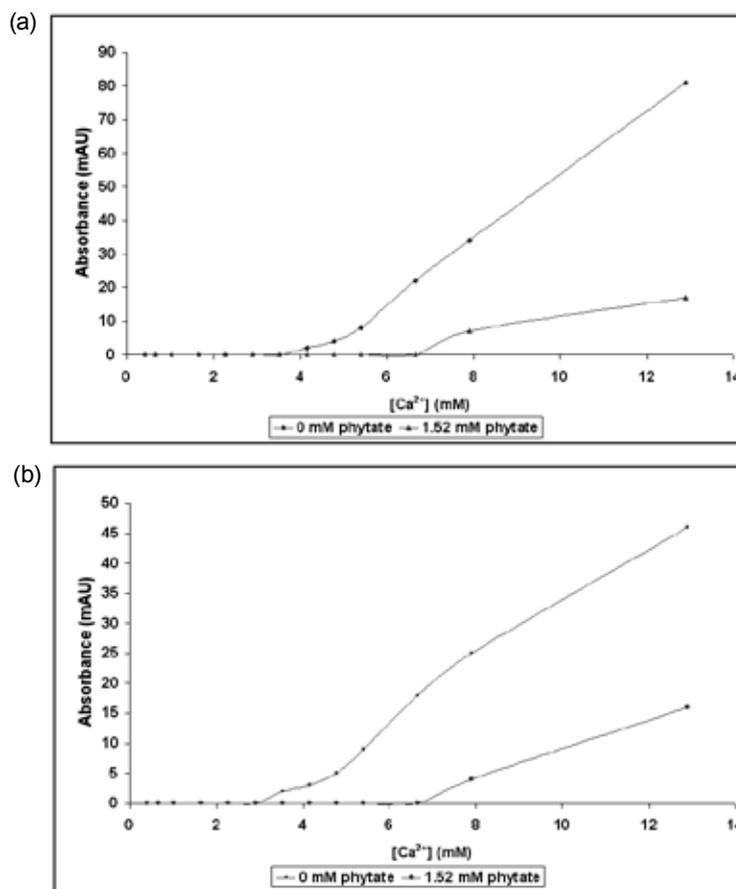


Figure 3 (top)
Calcite crystals formed using a batch system.
Experimental conditions: pH = 8.3, [Ca²⁺] = 12.9 mM, t = 1 h

Figure 4 (right)
Batch system of calcium carbonate precipitation and the inhibitory effects of phytate.
Experimental conditions: a. pH = 8.3, t = 15 s. b. pH = 8.3, t = 1 h



Results and discussion

Throughout these experiments, controls were utilised, consisting of inhibitor concentration 0 µM, with each water matrix to ensure that the reduction of calcium carbonate crystallisation was due only to the effects of phytate and not to other compounds.

Using the batch system, it was found that only calcium carbonate was formed, with no precipitation of mixed magnesium salts. Scanning electron microscopy (SEM) of the calcium carbonate crystals formed after 1 h revealed that the crystallographic structure corresponded to calcite (Fig. 3), with no magnesium present in the precipitated samples. Addition of 1.52 µM phytate to the synthetic hard water completely inhibited calcium carbonate precipitation when calcium concentrations were below 6.65 mM and when the carbonate concentration was 5.83 mM (Fig. 4). Drinkable hard water contains 2.5 to 3 mM cal-

cium and 3.33–8.33 mM carbonate, and has a pH of 6.5 to 8.5. Thus, 1.52 µM phytate can inhibit crystallisation of calcium carbonate in drinkable hard water. The reduction in crystallisation is probably not due to the formation of Ca-phytate complexes, since phytate is present at a 1 000-fold lower concentration than calcium.

The results obtained using the flow system are shown in Tables 2 and 3 and Fig. 5. This experimental device was designed using conditions as close as possible to actual concentrations of calcium (2.9 to 5.4 mM) and pH (8.0). The amount of CaCO₃ precipitated was directly related to the calcium concentration in the synthetic water. In the presence of 2.9, 3.4 and 5.4 mM of calcium, 3.03 µM phytate reduced the degree of CaCO₃ scale formation on the copper pipe after 48 h by 82.8%, 94.5% and 95.0%, respectively, at 30°C. The degree of precipitation was higher at 65°C than at 30°C, due to an increase in the kinetics of crystallisation and the decrease of calcium carbonate solubility

TABLE 2
Percent inhibition of calcium carbonate precipitation using the flow system at 30°C (n = 3)

[Ca ²⁺] = 2.9 mM		[Ca ²⁺] = 3.4 mM		[Ca ²⁺] = 5.4 mM	
[phytate] (μM)	% inhibition	[phytate] (μM)	% inhibition	[phytate] (μM)	% inhibition
0	-	0	-	0	-
1.52	67.9 ± 9.2	1.52	89.5 ± 1.9	1.52	82.8 ± 3.9
3.03	82.8 ± 0.7	3.03	94.50 ± 0.04	3.03	95.0 ± 2.1
4.55	82.5 ± 1.0	4.55	92.5 ± 1.3	4.55	93.1 ± 2.6

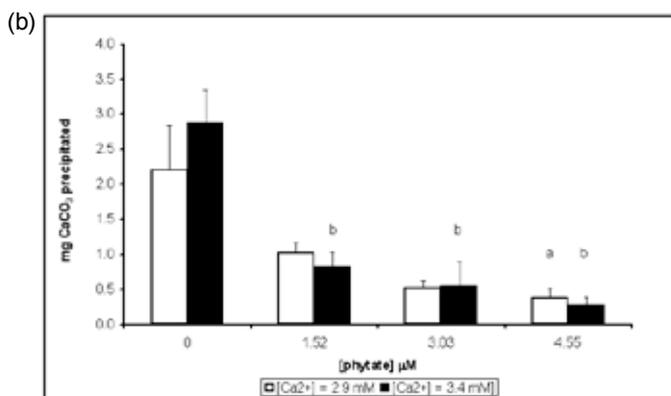
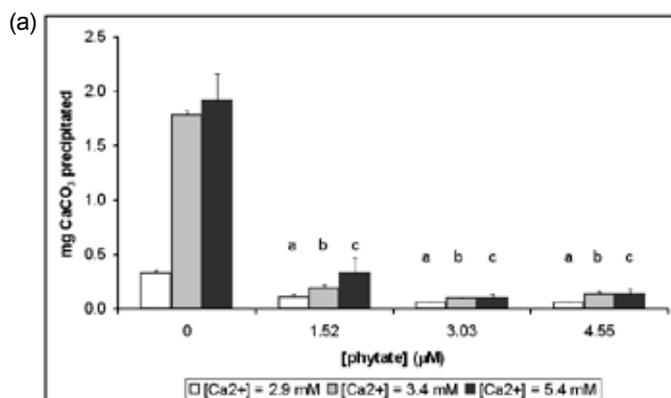


Figure 5

Flow system of calcium carbonate precipitation and the inhibitory effects of phytate. Experimental conditions: pH = 8.0, t = 48 h.

- a. T = 30°C ^a p < 0.05 vs [phytate] = 0 μM, [Ca²⁺] = 2.9 mM
 ^b p < 0.05 vs [phytate] = 0 μM, [Ca²⁺] = 3.4 mM
 ^c p < 0.05 vs [phytate] = 0 μM, [Ca²⁺] = 5.4 mM
- b. T = 65°C ^a p < 0.05 vs [phytate] = 0 μM, [Ca²⁺] = 2.9 mM
 ^b p < 0.05 vs [phytate] = 0 μM, [Ca²⁺] = 3.4 mM

at the higher temperature (Table 3). A maximum diminution of 90.8 % was attained at a calcium concentration of 3.4 mM and a phytate concentration of 4.55 μM, again showing that very low concentrations of phytate can reduce calcium carbonate formation in a flowing system.

The results obtained with a full-scale system, at high calcium and bicarbonate concentrations, are summarised in Table 4. Calcium carbonate precipitation was indirectly evaluated by determining the concentrations of dissolved calcium and carbonate ions. When calcium carbonate is precipitated, the calcium and carbonate concentrations in the cistern should decrease. Thus, if precipitation is inhibited by the addition of a crystallisation inhibitor, the calcium and bicarbonate concentrations should be higher. We found that increasing the phytate concentration in the cistern increased the concentrations of dissolved

TABLE 3
Percent inhibition of calcium carbonate precipitation using the flow system at 65°C (n = 3)

[Ca ²⁺] = 2.9 mM		[Ca ²⁺] = 3.4 mM	
[phytate] (μM)	% inhibition	[phytate] (μM)	% inhibition
0	-	0	-
1.52	53.2 ± 6.4	1.52	71.3 ± 7.2
3.03	76.2 ± 4.8	3.03	81.0 ± 12.5
4.55	82.7 ± 6.2	4.55	90.8 ± 4.5

TABLE 4
Water parameters determined with the full-scale system

[Phytate] (μM)	pH	[Ca ²⁺] (mM) ± SE	[HCO ₃ ⁻] (mM) ± SE
0	7.92	2.69 ± 0.02	5.10 ± 0.03
0.15	8.00	2.70 ± 0.01	5.10 ± 0.03
0.38	7.71	2.86 ± 0.09	5.40 ± 0.03*
0.76	7.40	2.84 ± 0.03*	5.70 ± 0.03*
1.52	7.43	3.02 ± 0.01*	5.90 ± 0.03*

* p < 0.05 vs [phytate] = 0 μM

calcium and bicarbonate, showing that phytate can inhibit formation of calcium carbonate solids due to its ability to inhibit crystallisation.

Comparing the effects of phytate, triphosphate and EDTA on calcium carbonate crystallisation (Fig. 6) it can be observed that phytate provoked a maximum delay on calcium carbonate crystallisation at very reduced concentrations, reaching a time of nucleation of 24.3 h with 3.03 μM of phytate. Working with EDTA, a concentration of 2 000 μM was necessary to obtain the same effect as phytate. This is due to the fact that phytate acts as a crystallisation inhibitor whereas the action of EDTA is as a chelating agent. The maximum inhibitory effects caused by triphosphate were observed with a concentration of 54 μM. When increasing the concentration to get a major delay of crystallisation it was found that the nucleation time decreased, probably due to the crystallisation of calcium phosphate salts. This comparison demonstrates that phytate clearly delays the crystallisation of calcium carbonate at concentrations in the micromolar range, therefore being superior to triphosphate and EDTA (chelating agent) in this regard.

Crystallisation inhibitory properties of phytate had been previously studied using other calcium salts. It was demonstrated that phytate is a powerful inhibitor of heterogeneous nucleation of calcium oxalate (Grases (1991; 1994)), homogeneous nucleation of calcium phosphates (Grases, 1996a) and crystal growth of calcium oxalate (Grases, 1989) due to its self-adsorption on nuclei in formation or on the faces of a growing crystal. The results with the batch systems presented in

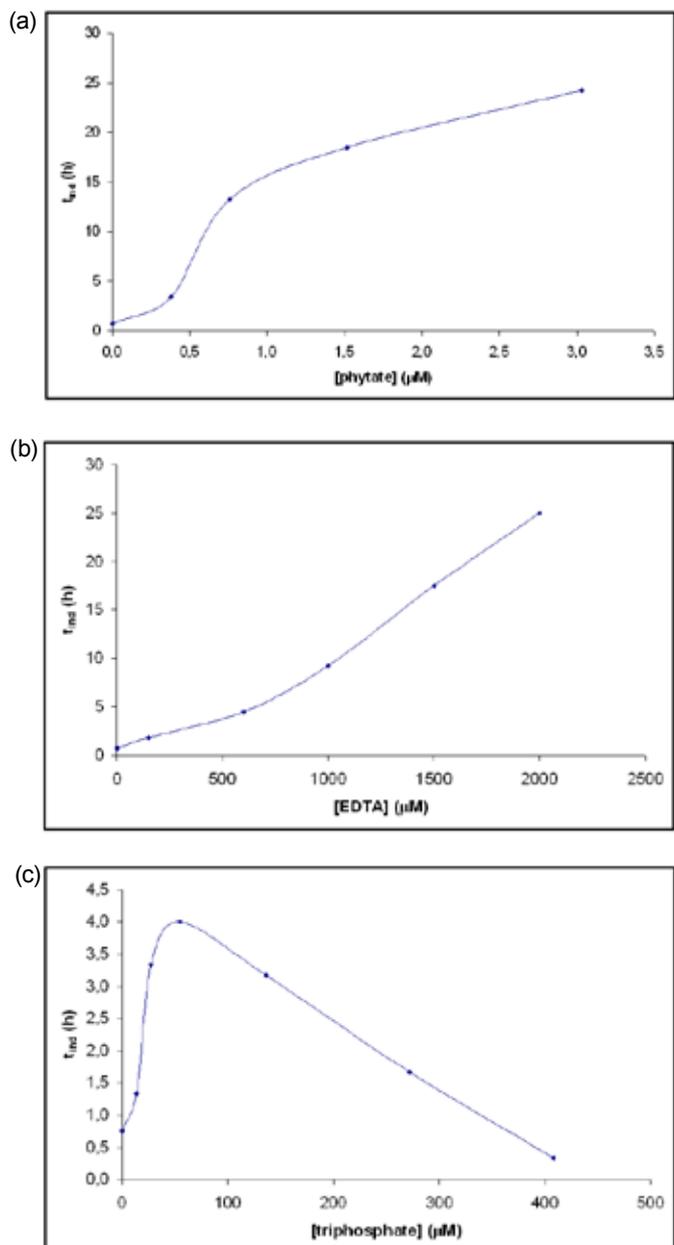


Figure 6
 Compared effects of different additives on calcium carbonate crystallisation
 a. Phytate
 b. EDTA
 c. Triphosphate

this paper, demonstrate that phytate can inhibit homogeneous nucleation of calcium carbonate, whereas the results obtained working with the flow system show that phytate at very reduced concentrations can inhibit heterogeneous nucleation of calcium carbonate.

Phytate is a natural compound that, at these concentrations, is non-toxic. Moreover, it can be beneficial to human health, due to its antioxidant, anti-neoplastic properties (Shamsuddin, 1997; Owen, 1996; Vucenik, 1998) and its prevention of calcium renal lithiasis (Grases (1999; 2000a) and cardiovascular calcifications (Grases 2006). Focusing the discussion on water treatment, the effect of phytate on inhibiting calcium carbonate scale formation is directly related to its effect on calcium renal

lithiasis and cardiovascular calcifications. In the three cases phytate acts as a crystallisation inhibitor by self-adsorption on the calcium crystal in formation or growth and, although they represent very different research fields they are a consequence of the same chemical effect. Conventional hard water treatment methods, including reverse osmosis, precipitation or ionic exchangers, are based on reducing supersaturation, eliminating the ions responsible for water hardness. Few domestic or industrial systems incorporate crystallisation inhibitors to reduce the degree of scale formation. Other methods, such as the addition of EDTA, may have negative effects on human health and the environment. Although phosphorus-containing compounds have been used as detergent additives to prevent calcium carbonate scale formation, due to the ability of these compounds to chelate calcium and magnesium, they can lead to eutrophication by increasing phosphate concentration in the receiving water bodies. In this paper, we present a methodology for hard water treatment based on the incorporation of phytate, a crystallisation inhibitor that does not change water supersaturation with respect to calcium carbonate. Phytate does not have a negative impact on the environment (i.e. eutrophication) since it inhibits crystallisation rather than acting as a chelating agent. Thus, the phytate concentrations required to significantly reduce the degree of calcium carbonate crystallisation are in the micro-molar range.

Conclusions

These results show that very low concentrations of phytate may prevent calcium carbonate scale formation due to its ability to inhibit crystallisation. At these concentrations, phytate may be beneficial for human health, mainly in the field of calcium renal lithiasis (Grases, 2000c) and cardiovascular calcifications (Grau, 2005) and will not have deleterious environmental effects.

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