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Chapter 8

INHIBITORY EFFECTS OF "GREEN" ADDITIVES ON THE CRYSTAL GROWTH OF SPARINGLY SOLUBLE SALTS

Konstantinos D. Demadis[1*] and Mualla Öner[2]

[1] Crystal Engineering, Growth & Design Laboratory, Department of Chemistry, University of Crete, Heraklion, Crete, GR-71003, Greece
[2] Department of Chemical Engineering, Yildiz Technical University, Davutpasa, Istanbul, 34210, Turkey

ABSTRACT

In this chapter the effects of environmentally friendly additives on formation and growth of sparingly soluble salts are presented in a concise way. Specifically, the influence of a biodegradable, environmentally friendly polysaccharide-based polycarboxylate, carboxymethyl inulin (CMI), on the crystal growth kinetics of calcium oxalate has been studied. The spontaneous crystallization method was used to delineate the crystallization kinetics of calcium oxalate (CaC_2O_4, CaOx). The results demonstrate that the retardation in crystal growth is controlled by the carboxylation degree of the CMI and its concentration. These studies also show that CMI additives direct calcium oxalate crystallization from calcium oxalate monohydrate (COM) to calcium oxalate dihydrate (COD). Colloidal silica is known to be a substantial problem in silica-laden process waters. Although silica "technically" is not a salt, it is often categorized with them, as it brings about the same problems as mineral scale deposits (reduced heat transfer, etc.). We also report a strategy to retard silicic acid condensation in supersaturated aqueous solutions by using non-toxic, "green", zwitter-ionic phosphonomethylayed chitosan (PCH). An overview of the use of green additives in water treatment is also presented.

Keywords: green chemistry, environmentally friendly, scale inhibition, calcium oxalate, additives, water treatment, polymers, silica

* Email: demadis@chemistry.uoc.gr, Web Page: http://www.chemistry.uoc.gr/demadis

INTRODUCTION

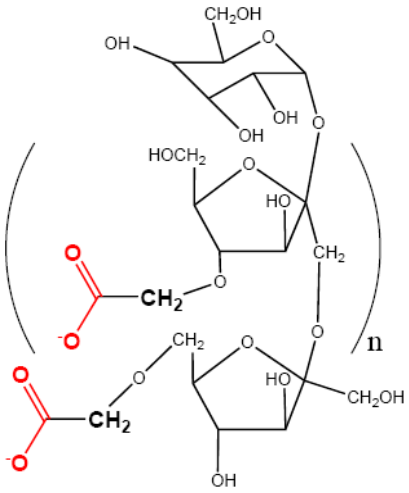
The crystal growth of calcium oxalate (CaC_2O_4, CaOx) and its chemical control have been at the epicenter of intense study by engineers and urologists for several decades [1]. Its formation is of particular importance not only to researchers working in the biomineralization field [2], but also to engineers/chemists who are active in the industrial crystallization applications' field [3]. Calcium oxalate (CaC_2O_4, CaOx) is a naturally occurring mineral found in fossils, plants, mammal urinary calculi [4] and is a by-product in some processes such as paper, food and beverage processing [5]. Calcium oxalate crystallization yields different hydrates. The thermodynamically most stable is the monoclinic monohydrate COM ($CaC_2O_4.H_2O$, whewellite [6]). Under certain conditions, the metastable tetragonal dihydrate ($CaC_2O_4.(2+x)H_2O$, $x<0.5$), weddelite [7]) and triclinic trihydrate ($CaC_2O_4.xH_2O$, $3>x>2.5$; COT [8]) can form. An understanding of CaOx crystallization processes is essential to urologists for possible development of new therapeutic agents, but also to water chemists who seek its inhibition as an undesirable salt in the process industries.

Among water-formed deposits colloidal silica ($SiO_2 \cdot nH_2O$, *n* is variable and dependent on hydration) is especially troublesome because it can cause serious materials failure and operational shut-downs. Silica scale prevention, in principle, can be achieved by use of scale inhibitors, key components of any chemical water treatment [9]. Unfortunately, traditional scale control methods (inhibition and crystal modification) applied to crystalline mineral salt precipitates, do not apply to silica because of its amorphous state. Therefore, much more well-designed inhibition approaches have to be applied for controlling silica deposition. Increasing environmental concerns and discharge limitations have imposed additional challenges in treating process waters. Therefore, the discovery and successful application of chemical additives that have mild environmental impact has been the focus of several researchers [10, 11]. In this paper we also focus on use of "green" inhibitors silica scale inhibition. This research is part of our on-going investigation on the discovery and application of scale inhibitors, with an emphasis on "green" additives, in industrial process waters [12-20].

OVERVIEW OF TWO "GREEN" SCALE INHIBITORS: CMI AND PCH

In this investigation we present a study of the effect of carboxymethylinulin (CMI, figure 1) on the crystallization kinetics and phase transformation of CaC_2O_4 using a plethora of experimental techniques (FTIR, XRD and SEM). CMI is produced from a chemical reaction of the biopolymer inulin and select reagents [21]. Inulin [22] is extracted from the roots of the chicory plant (dry matter content: 20-25%, inulin content: 14.9-18.3 %). Inulin is a polydisperse polysaccharide consisting mainly, if not exclusively, of $\beta(2\rightarrow1)$ fructosyl fructose units with normally, but not necessarily, one glucopyranose unit at the reducing end. It is also known that the fructose molecules are all present in the furanose form. Inulin is used as dietary fiber, fat substitute, sweetener (fructose syrups). Carboxymethylinulin (CMI) has been investigated in a series of acute toxicity (oral rat, > 2000 mg/kg B.W.), sub-acute toxicity (28 days, rat 1000 mg/kg B.W.), mutagenicity (Ames Test, *in vitro* cytogenetics, no

effect) and dermal sensitization studies (guinea pigs, no effect) to evaluate its toxicological profile [23]. All studies followed accepted testing guidelines as recommended by international regulatory agencies (OECD, EEC and US EPA). No significant toxicological findings were evident. Results of the present toxicity studies with CMI, all conforming to internationally-accepted testing standards, show that the toxicological profile of CMI is consistent with other polycarboxylates used in foods. Among other attractive attributes of CMI, its inherent biodegradability and non-toxicity are most prominent. Data that support these conclusions include: toxicity (ppm) $EC_{10} > 10000$, bacteria (ppm) $EC_0 = 2000$, Daphnia (ppm) $EC_{50(24h)} = 5500$, fish (ppm) $LC_0 > 10000$. These results should be contrasted to those obtained for a polyacrylate polymer (M.W. = 1500) commonly used as precipitation inhibitor in industrial waters: toxicity (ppm) $EC_{10} = 180$, bacteria (ppm) $EC_0 = 200$, Daphnia (ppm) $EC_{50(24h)} = 240$, fish (ppm) $LC_0 = 200$.



CMI

Figure 1. The building blocks in the CMI polymer backbone. The carboxymethyl groups (highlighted in red) are introduced by the reaction of monochloroacetic acid with the ring –OH groups. Up to three –CH_2COOH groups can be introduced per ring. The presence of acidic –CH_2COOH groups on the CMI backbone makes it an anionic polyelectrolyte (at appropriate pH regions).

We have selected to utilize chitosan-based biopolymers, figures 2 and 3, as additives that may enhance silicic acid solubility. More specifically, in the context of this chapter we studied the polymer chitosan on which animomethylenephosphonate groups have been grafted by a Mannich-type reaction. We selected this particular polymer because it possesses attractive similarities to naturally-found, silica controlling proteins called silaffins [24], among other reasons outlined below. In particular: (a) PCH possesses cationic charge by virtue of its protonated (at pH 7) $–NH_2$ groups, (b) PCH possesses anionic phosphonate groups (deprotonated $-PO_3H_2$ moieties) that resemble the phosphate groups in silaffins, (c) PCH contains tertiary and secondary, protonated amine groups (from the aminomethylenephosphonate moiety), also present in silaffins. Furthermore, PCH is synthesized from chitosan (a degradation product of chitin, a renewable material) in an efficient and low-cost manner. PCH also has low aquatic toxicity (*vide infra*). An additional

reason that prompted us to study the effect of PCH on silicic acid condensation is the report that the diatom cell wall (frustulum) is made of nanostructured amorphous silica that is associated with polysaccharides and proteins. The link that may exist between polysaccharides and the formation of biosilica seems to be an interesting area to explore.

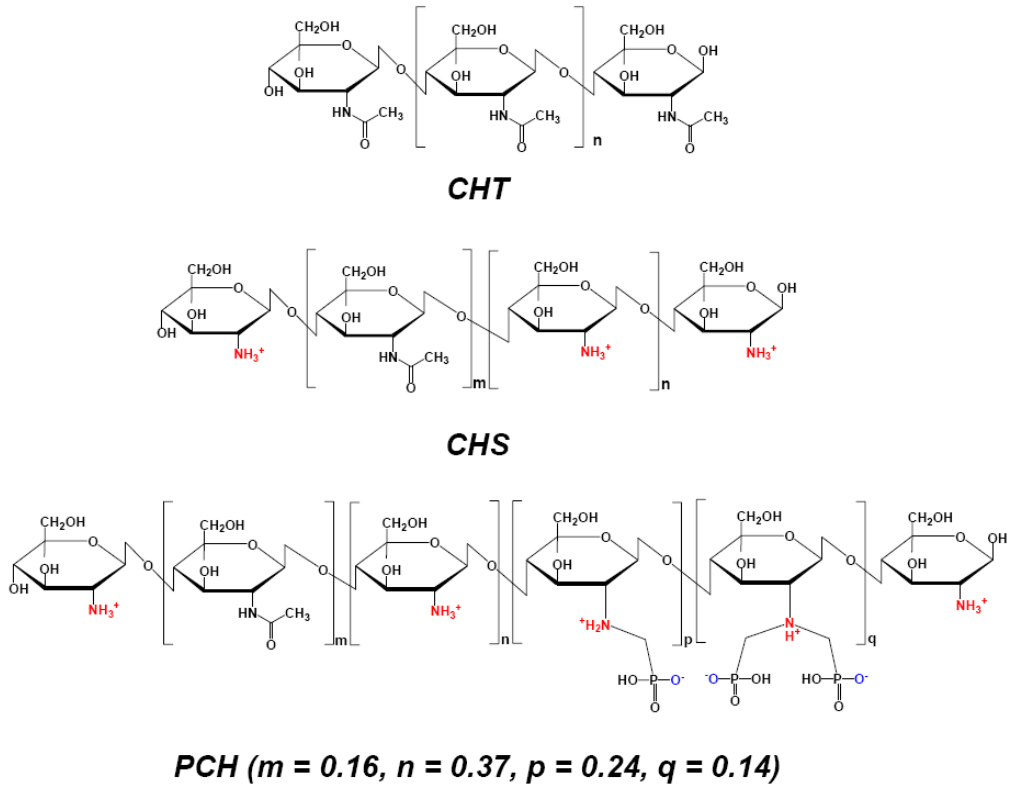


Figure 2. Schematic structures of the polymers chitin (CHT), chitosan (CHS), phosphonomethylated chitosan (PCH). Added groups are clearly shown on the PCH polymer backbone.

A facile model system to study human disease and drug responses are zebrafish, *Danio rerio*. They are particularly suited for this purpose because they represent a vertebrate species, their genome is sequenced, and a large number of synchronously developing, transparent embryos can be produced. Zebrafish embryos are permeable to drugs and can easily be manipulated using well-established genetic and molecular approaches. LC_{50} values (50 % lethal concentration) is the concentration at which 50% of test organisms are killed. Adult wild-type zebrafishes were maintained at 26 ºC and fed with ZF Biofood. Fertilized zebrafish embryos were obtained by hormonally induced treatment to spawners and *in vitro* fertilization. Three assays with a variable number (4-7) of water soluble-N-methylene phosphonic chitosan concentrations between 99 and 1000 mg/l and a control without N-methylene phosphonic chitosan were conducted in 96-well multiplates at 26ºC. Spawns and eggs were selected at 3-4 hpf (hours post-fertilization) according to standard criteria. Lethal effects were observed in 48 hpf embryos, based on four morphological and functional endpoints, specific for lethality studies. The LC_{50} of N-methylene phosphonic chitosan was determined in our laboratory using the Probit 1.5 software as 279.6 ± 29.7 mg/L. The LC_{50} of

chitosan was reported as 300±18 mg/l (1) and in our case the phosphonomethylated chitosan derivative (PCH) 280 ± 30 mg/l. The PCH polymer tested presents a LC_{50} value similar to chitosan (CHT), indicating that phosphonomethylation does not enhance toxicity.

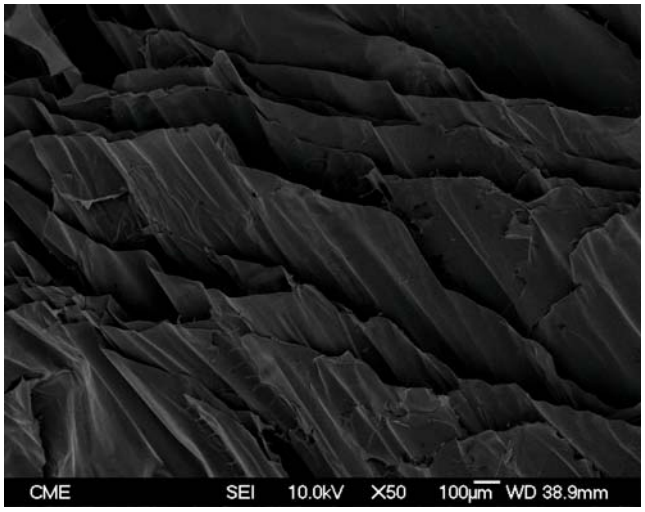


Figure 3. An SEM image of PCH in the solid state.

The goal, therefore, of this research was dual: (a) to provide insights into the effects of non-toxic polymers on CaOx crystallization and inhibition efficiency and to provide a perspective for possible use of CMI in pathological stone therapy, and (b) to provide versatile and "green" inhibitors of undesirable scale deposits for possible applications in the process industries. Lately, the research area of "green additives" has evolved dramatically [12-20].

EFFECTS OF CMI POLYMERS ON CALCIUM OXALATE CRYSTALLIZATION

Three CMI polymers with an average molecular weight of 2-3 KDa were investigated under identical crystal growth conditions. These polymers are CMI-15, CMI-20, and CMI-25. The code numbers indicate the degree of substitution (DS). DS is defined as the average number of carboxylate moieties *per* fructose unit. For example, for CMI-15, DS = 1.5; for CMI-20, DS = 2.0; for CMI-25, DS = 2.5. Carboxymethylation of inulin in laboratory scale is carried out in aqueous alkaline medium with monochloroacetic acid as the reagent [21]. The effect of an additive can be quantified as the ratio of the rate of crystallization of the pure solution (R_o, mg/L·min) to the rate of crystallization in the presence of additive (R_i, mg/L·min) at the same concentration and temperature. This methodology has been described in detail elsewhere [25]. The induction period (t_{ind}) was determined by monitoring variations in $[Ca^{2+}]$, accompanied by atomic absorption spectrometric measurements. The time between the generation of a supersaturated state and the first observed change in calcium concentration was defined as t_{ind}. The time periods were determined from the recordings of the time evolution of $[Ca^{2+}]$ in solution, which is directly related to the volume of the precipitated calcium oxalate, and averaged from at least three separate experiments. The "induction period" is defined as the "time lag" during

which there is negligible change in the bulk solute concentration. The presence of a trace amount of polymers resulted in an increase in induction period, followed by precipitation at a rate comparable to the rate of crystallization from pure solutions. All polymers at 1 ppm concentration are reasonably good growth inhibitors, but CMI-25 appears to be more effective than CMI-20 and CMI-15 at the same concentration. CMI-25 at 1 ppm concentration can effectively block all the active growth sites and hence bring the calcium oxalate growth rate to a complete stop over a 5 h period. Figure 4 shows the effect of RR on induction time for CMI-20.

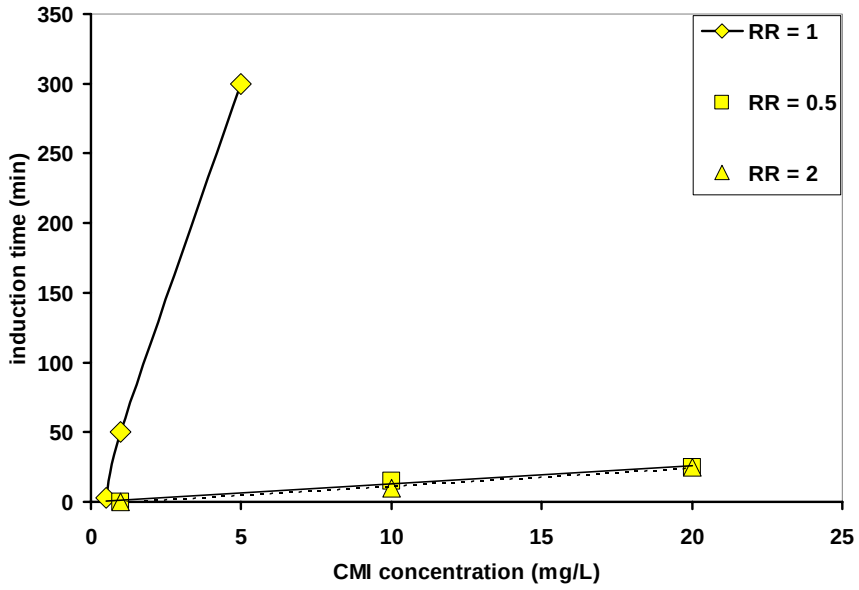


Figure 4. Effect of CMI-20 concentrations on induction time at three different reactant ratios (RR). RR is defined as the value of $[Ca^{2+}]/[C_2O_4^{2-}]$. Reproduced with permission from reference 1a, © American Chemical Society.

Figure 5 shows the effect of carboxymethylation degree of the polymer on calcium oxalate inhibition. The effectiveness of polymers increases with increasing carboxyl content on the polymer backbone. Induction time increases from 20 min to 300 min as the carboxyl content increases from 1.5 to 2.5. It was observed that products with a higher degree of carboxyl group prolong the induction period for crystallization because of the ability of the anionic carboxylate groups to adsorb on the calcium oxalate crystal surfaces. Several investigations have indicated that polymers which exhibit an inhibitory effect on crystallization kinetics of soluble salts are highly substituted with carboxyl groups [26]. Apparently the larger number of carboxylate functional groups increases the ionic attractive interactions between the adsorbate ($-COO^-$) and the positive sites (Ca^{2+}) at the solution interface. If the inhibitor ions are rapidly adsorbed, the nuclei remain subcritical and eventually disappear through dissolution. The inhibitor polyanions are then available for repeated adsorption at the edges of newly developing nuclei. This eventually leads to breakdown and disintegration of a number of the available embryos before further growth can take place.

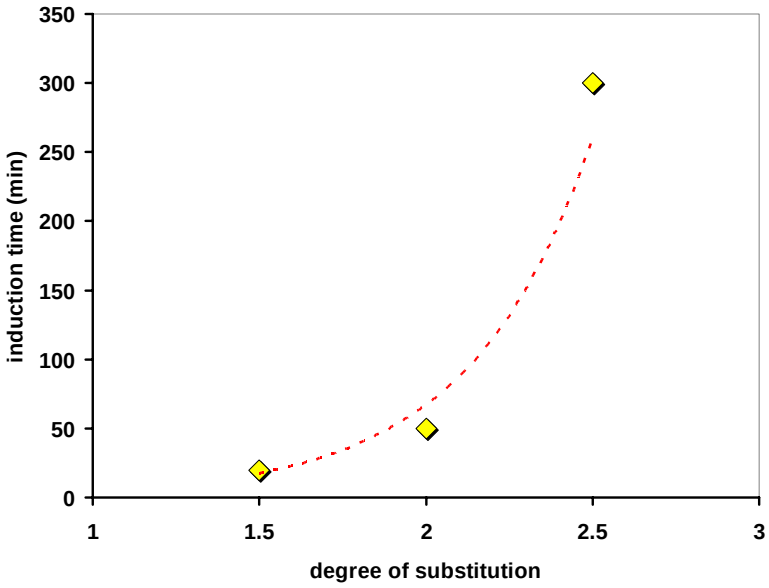


Figure 5. The effect of DS increase on induction time. The line is added to aid the reader. Reproduced with permission from reference 1a, © American Chemical Society.

In this way outgrowth of the nuclei beyond their critical value/size is hampered; in due course, because of their thermodynamic instability, most nuclei will re-dissolve, thus freeing the polymer for interaction with other embryos. The polymers, by effectively reducing the number of active growth sites through adsorption on the crystal surface, will prolong induction periods. During the induction period, most of the active growth sites are "poisoned" by the adsorbed macromolecule additives. However, some of the growth sites of lower energy may still be free to grow, and thus, the crystallization proceeds at a very slow rate. The rate of precipitation/deposition of calcium oxalate following the induction period is dependent on polymer concentration.

The marked effect of polymers on the crystal growth of calcium oxalate from supersaturated solutions have been explained in terms of the following factors: (a) Polymers may change the ionic strength of the solution; (b) Polymers may form stable complexes with calcium ion; (c) Adsorption of the polymer on the crystal surfaces either indiscriminately or at specific growth sites (d) polymer incorporation into the calcium oxalate lattice. Under the experimental conditions employed herein, the presence of the induction period is believed to originate from inhibitor surface adsorption. The polymer concentration is too low to modify the ionic strength of the solution and the calcium ion binding is small enough to be ignored. Polymer insertion/incorporation into the lattice can be ruled out because these macromolecules are much larger in size compared to the oxalate ion.

Since the amounts of additive in solution are small, the growth inhibition is most likely caused by biopolymer adsorption of the active growth sites on crystal surfaces rather than binding to solution Ca^{2+} ions. This assumption was tested by fitting the kinetic results in a Langmuir type kinetic isotherm. When a polyelectrolyte poisons active growth sites on the crystal faces, the coverage by polymers, θ, for this adsorption model is described by Equation 1.

$$\theta = KC_i / (1+KC_i) \quad (1)$$

where K is the adsorption or affinity constant which is the ratio of the rate constants for adsorption and desorption, k_{ads}/k_{des}, and can be considered as a measure for the adsorption affinity of the polymer onto the crystal surface and C_i is the total equilibrium concentration of the polymer. The rate in the absence of polymer, R_0, is reduced to a slower rate, R_i, according to the relationship

$$R_i = R_0(1-\theta) \quad (2)$$

Combination of Equations 1 and 2 gives

$$\frac{R_0}{R_0 - R_i} = 1 + \frac{k_{des}}{k_{ads}} \frac{1}{C_i} \quad (3)$$

Equation 3 shows that this model predicts a linear relationship between $R_0/(R_0-R_i)$ and $1/C_i$. The linearity of the plots of Equation 3 for calcium oxalate crystal growth in the presence of polymers (figure 6) suggests that the inhibitory effect of CMI polymers is due to adsorption at active growth sites. k_{ads}/k_{des} can be evaluated from the slope of the resulting straight line. The values of the affinity constant as calculated for CMI-25, CMI-20 and CMI-15 are 23.86, 15.55 and 6.09 L/mg respectively. The high value of the affinity constant for CMI-25 may reflect stronger equilibrium adsorption of CMI-25 on the crystal surface, compared to that of CMI-20 and CMI-15.

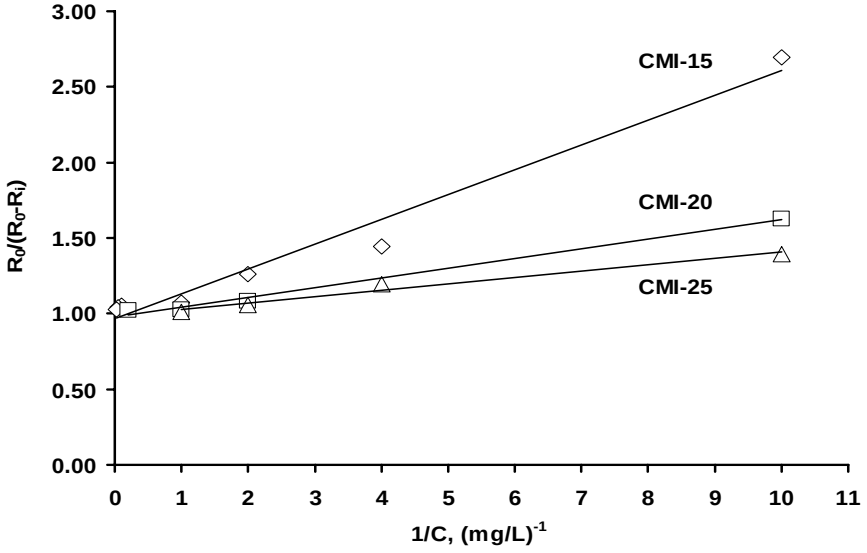


Figure 6. Langmuir-type adsorption isotherm for the effect of polymers. Reproduced with permission from reference 1a, © American Chemical Society.

MORPHOLOGICAL CHANGES OF CALCIUM OXALATE CRYSTAL SURFACES DUE TO CMI POLYMERS

It has been known that very low concentrations of additives can greatly affect rates of crystallization and the habit of the growing crystals. Even if the additives are present in such low concentrations their effect may still be significant. It is almost always the case that the presence of additives reduces the growth rate of a crystal, and various index faces may be affected differently and leads to a modification of habit. The shape or morphology of a crystal is determined by the relative growth rate along different crystallographic directions. The crystal face that is perpendicular to the fast growing direction is usually not expressed in the final crystal form while the face that is parallel to the fast growing direction is usually expressed. Thus modification of the crystal morphology can be achieved by altering the relative crystal growth rate with inhibitor capable of specific inhibition [27].

SEM images were taken in order to study the morphological and phase transformation effect of CMI polymers on calcium oxalate crystals. The presence of polymers in supersaturated solutions affects not only the kinetics of crystal growth but crystal morphology and phase transformation of calcium oxalate crystals as well. Calcium oxalate can form three different hydrated structures in aqueous solution: the least soluble and thermodynamically the most stable monohydrate phase as well as metastable di- and tri-hydrates. The experimental work has been done to study the effect of CMI polymers on spontaneous nucleation and growth of the metastable hydrates. SEM images were collected for subsequent visual analysis in order to assess the effects of CMI polymers on crystal shape, size as well as phase transformation. In figures 7-8, SEM micrographs are presented of the various crystal forms observed in this study. In all control experiments without additives, COM was the dominant phase in all concentrations (figure 7a).

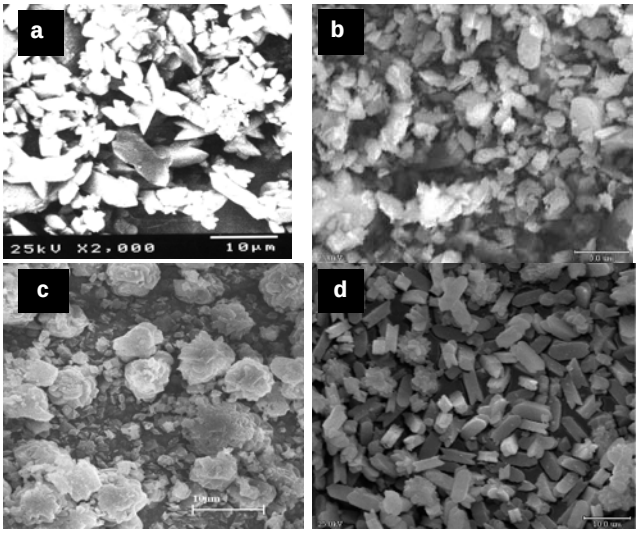


Figure 7. SEM images of CaOx crystals grown for 5 h from solution at 37 °C and reactant ratio of 1. Crystals grown from a solution containing (a) no CMI polymer, (b) 0.5 ppm CMI-15, (c) 0.5 ppm CMI-20, and (d) 0.5 ppm CMI-25. Reproduced with permission from reference 1a, © American Chemical Society.

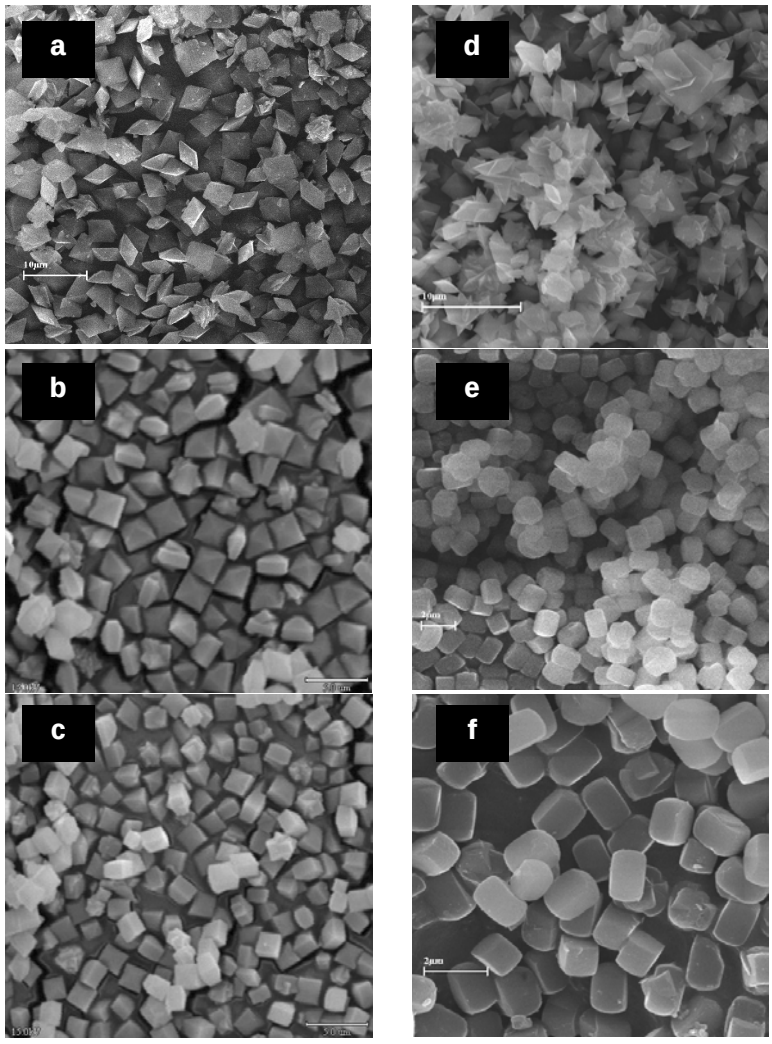


Figure 8. SEM images of CaOx crystals grown for 5 h from a solution at 37 °C and reactant ratio of 2. Crystals grown from a solution containing (a) 1 ppm CMI-15, (b) 10 ppm CMI-15, (c) 20 ppm CMI-15, (d) 1 ppm CMI-20, (e) 10 ppm CMI-20, and (f) 20 ppm CMI-20.

The individual crystals of platelets and a few flowerlike agglomerates were observed during crystallization of calcium oxalate in the absence of polymers. The agglomerates were about 6 μm in size. The grown prismatic crystals were identified as calcium oxalate monohydrate by XRD, see figure 9, and compared with that of the powder diffraction File 9-432 JCPDS 2000.

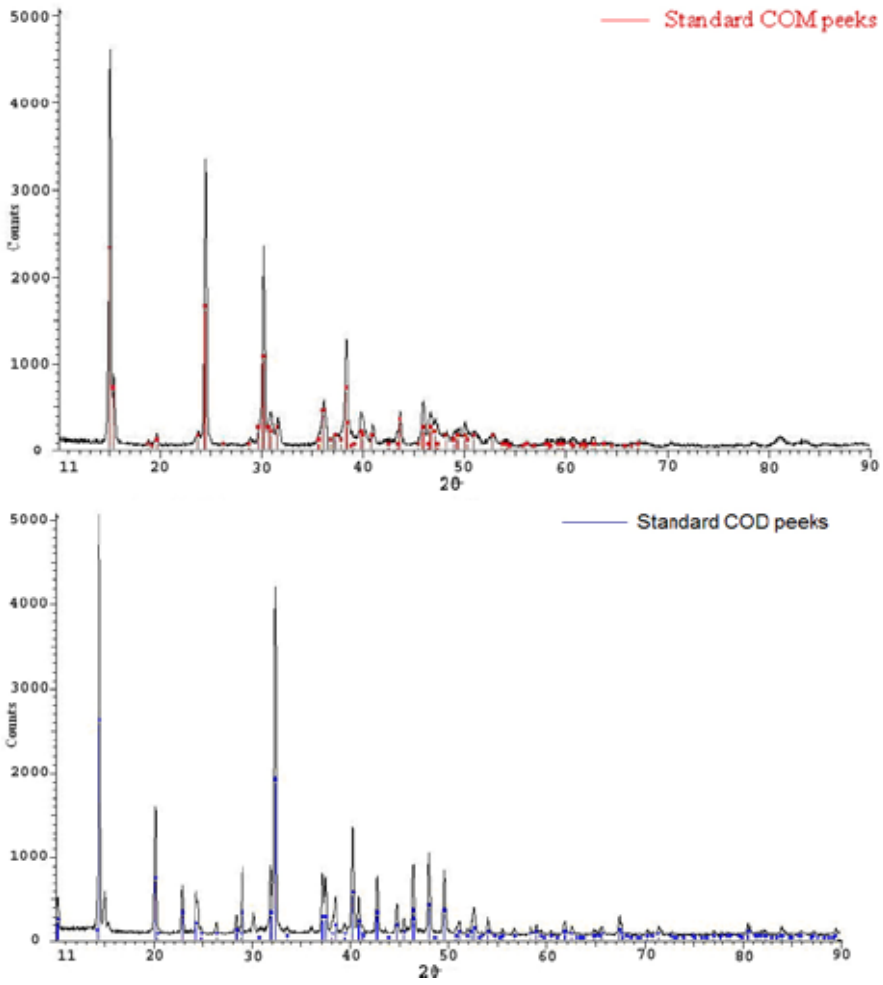


Figure 9. XRD powder patterns: (upper) no polymer additive, and (lower) presence of CMI 20. Reproduced with permission from reference 1a, © American Chemical Society.

When using the polymeric additives during the crystallization, the crystal morphology and the structure of the calcium oxalate were modified. This modification was dependent on both biopolymer concentration and reactant ratio. When the polymer concentration is 0.5 ppm and the reactant ratio maintained at 1/2, 1 and 2, aggregates of monoclinic COM crystals with contact twining are obtained in the presence of all CMI polymers (figure 7b-d). COM is the most commonly occurring CaOx and is the thermodynamically most stable phase and crystallizes in a monoclinic structure. The shape of COM is ranging from needles to plates, then to prisms, based on the crystallization parameters.

Addition of 1.0 ppm CMI-15 causes a distinct morphological and phase change to calcium oxalate crystals. As shown in figure 8a, the prismatic COM crystals totally disappeared and the product was tetragonal prisms of the COD exhibiting the prismatic habit with pyramidal endcaps with sizes of 4.2 × 3.7 μm. With an additive concentration of 10 mg/L, there was an outgrowth of the COD crystals along the [001] direction, resulting in elongated crystals and the development of {100} crystal faces. The side length perpendicular

to the tetragonal axis increases from 0.80 μm to 0.93 μm when the CMI-15 concentration increases from 10 to 20 ppm. The same behavior was observed with the addition of CMI-20 (figure 8d-f). COD has a tetragonal structure with a space group of I4/m and a crystal habit that can be best described as tetragonal bipyramidal [28]. The calcium surface concentration of the COD on the (100) face (0.0439 ions/A^2) is greater than that on the (101) face (0.0225 ions/A^2). This result implies that the (100) face can adsorb a higher number of carboxylate moieties from the polymers. Therefore the interactions between polymer and the COD (100) faces resulted in a gradual morphological transition of COD crystals from tetragonal bipyramids to elongated tetragonal prisms with increasing polymer concentration.

A large number of reports have appeared in the literature on the effects of various additives on calcium oxalate crystallization. These include polyphosphate [29], sodium cholate [30], maleic acid copolymers [31], polyaspartic acids [32] poly-(styrene-alt-maleic acid) [33], various polyhydroxycarboxylic acids [34], acrylic polymers [35], tartarates [36], diisooctyl sulfosuccinate [37], mucin [38], Tamm-Horsfall proteins [39], herbal extracts [40], uric acid [41], poly(sodium 4-styrene-sulfonate) [42], liposome solutions of different carboxylates [43], fluorescent molecules [44], algae-derived sulfated polysaccharides [45], osteopontin [46], aspartic-rich synthetic peptides [47], synthetic osteopontin phosphopeptides [48], uropontin [49], pyrophosphate [50], citrate and isocitrate [51], tryptophan [52], dipalmitoylphosphatidylcholine [53], inositol hexaphosphate (phytate) [54], glycosaminoglycans [55], fibronectin [56], glycoproteins [57], unidentified macromolecules from whole human urine [58], α-ketoglutaric acid [59] and adenosine phosphates [60].

A number of important observations point to the way carboxylate and phosphate containing macromolecules may effect CaOx crystallization. The precipitation of calcium oxalate hydrates was investigated in the presence of polyphosphate by Nancollas and Tomazic [61]. It was reported that there is a greater adsorption of polyelectrolyte by COM than COD or COT. The greater adsorption of polymer by COM results in greater inhibition of COM nucleation and growth than for COD and COT. The effect of anionic polyelectrolytes on the crystallization of calcium oxalate hydrates was studied by Manne *et al*. [62]. It was found that only the monohydrate was formed in the presence of low concentrations of polyelectrolyte. At intermediate concentration (1-50 ppm) trihydrate was the predominant product and at high concentrations (above 50 ppm) the dihydrate was the dominant product. Brecevic and Kralj [63] were studied the influence of some amino acids on calcium oxalate dihydrate transformation. They found that tryptophan and histidine promote the formation of COD over COM.

The studies report habit changes, aggregations and attachment in calcium oxalate crystals, under the influence of glucosaminoglycans [64], polyglutamic [65] and polyaspartic acid [32] and anionic lipids [66]. Each of these macromolecular structures contains carboxyl groups, which are ionized at normal physiological pH values.

Recently, real time in situ Atomic Force Microscopy (AFM) was used to determine the activity and selectivity of macromolecular binding profiles to specific calcium oxalate crystal faces (monohydrate and dihydrate). AFM can visualize the crystal growth and directly measure the adhesion force between molecules and crystal surfaces. Elegant work by Ward *et al.* (with AFM) [67] and by Jung *et al*. (with SEM and XRD) [68] has focused on studying the interactions of polymers with pendant carboxylate groups (polyacrylate, polyaspartate and polyglutamate) with various crystallographic planes of the CaOx monohydrate crystals. It was found that polyglutamic acid inhibited growth more strongly along [010] direction than along

[001], consistent with preferential binding of polyglutamic acid to the (010) planes. In contrast, polyaspartic acid was more effective in inhibiting growth along the [001] direction. As a result it was concluded that CaOx formation was regulated by anionic molecules-crystal interaction that depend on molecular level recognition between anionic subunits of the macromolecules and lattice ions in the CaOx crystal.

Negative "charge density" (the number of bonds separating the negative charge located on the carboxylate groups) must be invoked when examining inhibitory activity. Bonds separating the deprotonated oxygen in the $-COO^-$ groups in the polymers' backbone are: polyacrylate 6, polyaspartate 12, polyglutamate 12, CMI 16 (for CMI-15, containing 1 carboxylate group *per D*-fructofuranose ring), or 9 (for CMI-20, containing 2 carboxylate groups *per D*-fructofuranose ring) see figure 10. Based on the work of Ward *et al*. [67] there is a inversely proportional relationship between inhibitory activity and the number of bonds separating the carboxylate units. Our results confirm this significant observation, as the inhibitory activity ranking is CMI-15 < CMI-20 < CMI-25 ~ PAA. The effects of charge density on inhibitory activity may be a wider theme in inhibition chemistry.

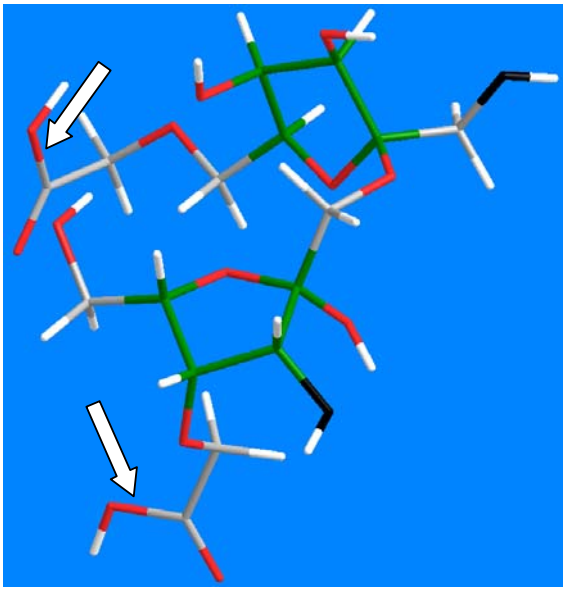


Figure 10. Energy-minimized fragment of the CMI polymeric backbone. Carboxylic acid groups that can be deprotonated at appropriate pH regions are shown by arrows.

EFFECTS OF PCH POLYMER ON SILICIC ACID CONDENSATION

Phosphonated chitosan (PCH) was tested for its ability to enhance silicate solubility and affect silica formation in "short-term" experiments (8 h) at dosages 40, 150 and 200 ppm. The results are presented in figure 11. Silicate condensation appears to be independent of additive dosage within the first 8 h. Soluble silicate reaches a value of ~ 350 ppm 8 h. Compared to control solutions not containing this results in ~ 150 ppm additional silicate stabilization.

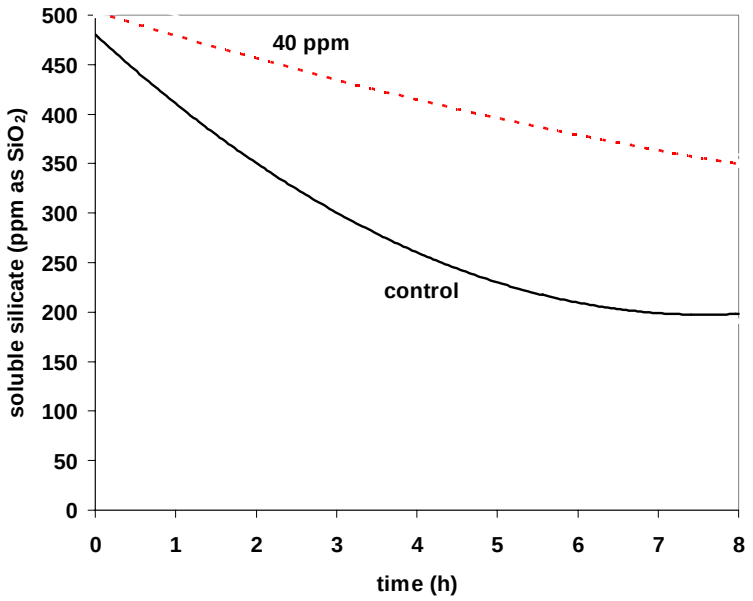


Figure 11. Colloidal silica formation demonstrated by monitoring soluble silicic acid level decrease. In the absence of PCH additives rapid reduction of soluble silicate is observed (control). In the presence of 40 ppm PCH substantial retardation of this process is observed.

The long-term silicate stabilization by PCH was studied over a 72 h time period. The effect of various dosages of PCH (10, 20, 40, 60, 100, 150 and 200 ppm) on silica formation is shown in figure 12.

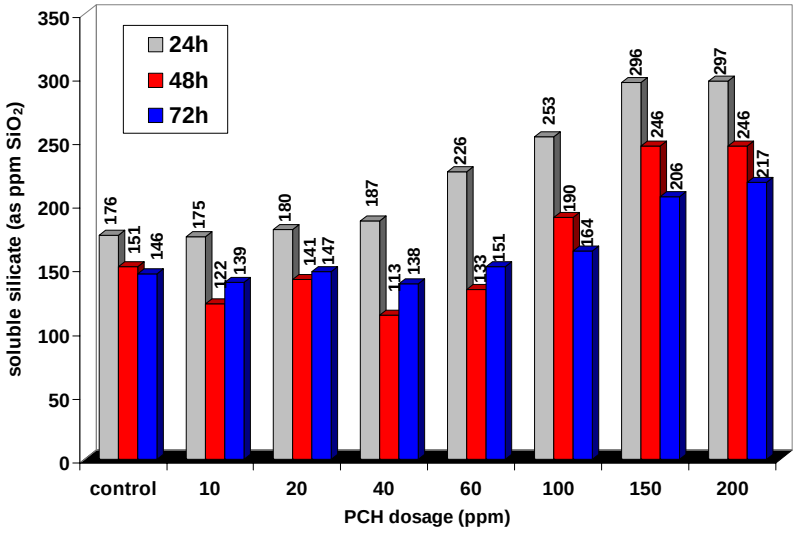


Figure 12. Long-term (24 h – 48 h – 72 h) enhancement of silicic acid solubility in the presence of various levels of PCH (from 10 ppm to 200 ppm).

An immediate observation is that PCH can only delay silicate condensation over the course of 72 h, thus only partially inhibiting silica formation. These experiments reveal that there is a dosage dependence on silicate stabilization that reaches a maximum at 150 ppm PCH (296 ppm silica compared to 176 ppm of the "control"). This translates to 120 ppm silicate stabilization (over the control) for the first 24 h. Low dosages (10 and 20 ppm) exhibit virtually no inhibitory effects. There seems to be a sharp reduction in inhibitory action after 24 h. This has been observed numerous times in our experiments with a variety of additives. Specifically, for the 150 ppm dosage, there is a 50 ppm drop in silicate levels between 24 h and 48 h. The same is true for the 200 ppm dosage.

Figure 13 presents silica precipitates after 24 h, 48 h and 72 h of silicate condensation time in the presence of 150 ppm PCH or combinations with CMI. The first row of images clearly shows that silica precipitation continues beyond 24 h, as indicated by the accumulation of additional amounts of deposit. This demonstrates that PCH cannot effectively inhibit silica formation. As proven before in our laboratories cationic inhibitors tend to get entrapped in the amorphous silica matrix [11, 13, 15, 16]. This is due to the presence of local regions of positive charge. To mitigate this problem an anionic polymer can be added. We tested blends of PCH and CMI. Rows 2, 3, and 4 in figure 13 show silica precipitates that form in the presence of combinations of PCH and CMI. The silica precipitates appear to get reduced as CMI dosage increases.

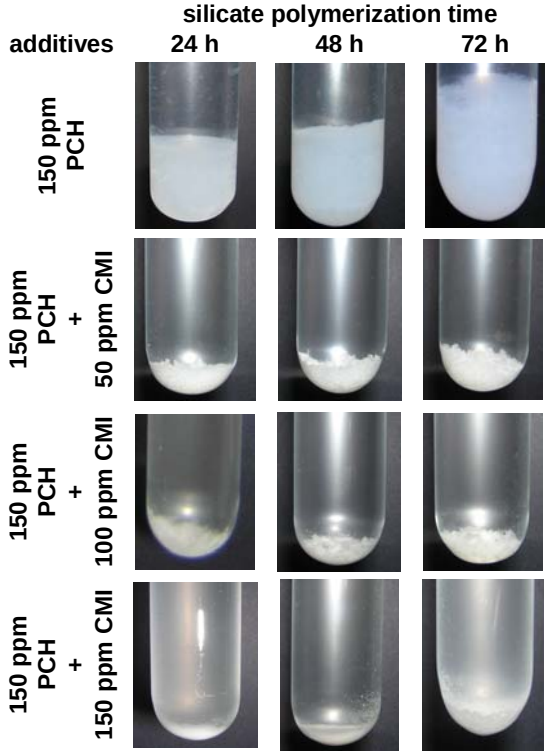


Figure 13. Optical images of silica precipitates in the presence of PCH and CMI synergistic combinations.

Silica precipitates were also studied by SEM, and the results are shown in figure 14. Generally, silica particles are spherical in shape. Upon PCH dosage increase they tend to agglomerate and form larger aggregates. In addition, these agglomerates grow larger as silicic polymerization time increases. After 600 h of polymerization the formation of large particles (> 2 μm) is obvious, see figure 14.

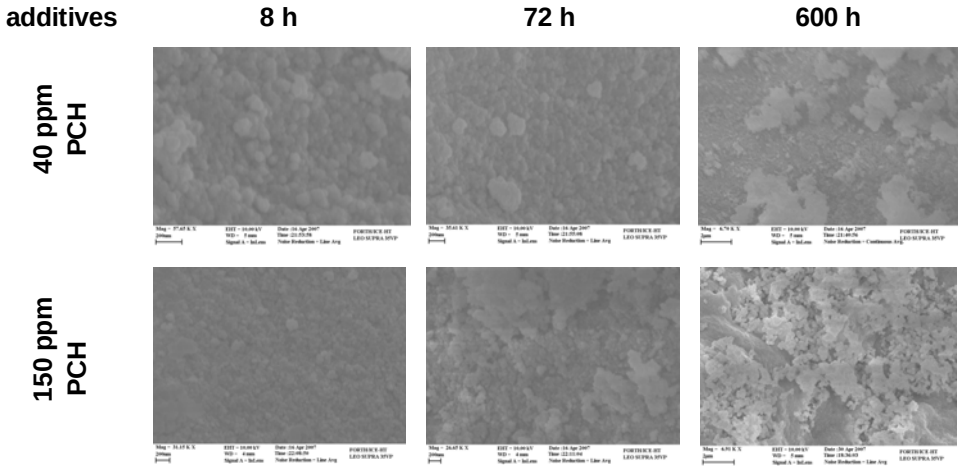


Figure 14. SEM images of silica deposits in the presence of 40 ppm (upper row) and 150 ppm (lower row) PCH, after silicic acid polymerization times of 8 h (1st column), 72 h (2nd column) and 600 h (3rd column). All scale bars are 200 nm, except the 3rd column, 2 μm.

POSSIBLE MECHANISMS OF SILICA INHIBITION BY THE PCH POLYMER

Silicic acid condensation is a complex process whose mechanistic details have been the subject of a number of literature reports [69]. Silicic acid condensation in the presence of threshold amounts of scale inhibitor is even more complicated because of added reaction pathways. When silicate polymerization occurs in the presence of a cationic polymeric additive, the following competing events occur:

1) Polymerization of silicic acid/silicate anions. This occurs through an S_N2-like mechanism that involves attack of a monodeprotonated silicic acid molecule on a fully protonated silicic acid molecule. This pathway generates at first short-lived silicate dimers, which in turn continue to polymerize in a random way to eventually yield colloidal silica particles.
2) Silicic acid/silicate ion stabilization by the cationic additive. This is the actual inhibition step and occurs presumably through cation-anion and/or hydrogen bonding interactions.
3) Flocculation between the polycationic inhibitor and the produced negatively charged colloidal silica particles (at pH 7) that are formed by the uninhibited silicic acid polymerization.

Cationic inhibitor is trapped within the colloidal silica matrix, based on reaction (3). This is demonstrated by the appearance of a light flocculent precipitates (or stable dispersions at times). Inhibitor entrapment causes its depletion from solution and its deactivation. Therefore, only a portion of the inhibitor is available to continue inhibition, albeit at much lower levels than initially added to the polymerization medium. Thus, soluble silicate levels continue to decrease because eventually there is insufficient inhibitor to perform inhibition. Inhibitor entrapment is directly proportional to cationic charge density.

An energy-minimized fragment of PCH is shown in figure 15. At pH of silicic acid condensation (~ 7) the cationic groups are the protonated primary amine group on the ring and the tertiary amine group of the amino-*bis*(methylenephosphonate) moiety, both indicated in blue. The anionic charge is located on the phosphonate moieties, indicated in magenta. The –OH groups remain protonated. Although PCH can be seen as a zwitter-ionic polymer, it is obvious that the positive charge is in excess because only 38 % of the primary amine groups of chitosan have been phosphonomethylated (see figure 2). This "excess" of cationic charge is responsible for silica growth inhibition. We have previously demonstrated that anionic additives have no effect on silicic acid polymerization.

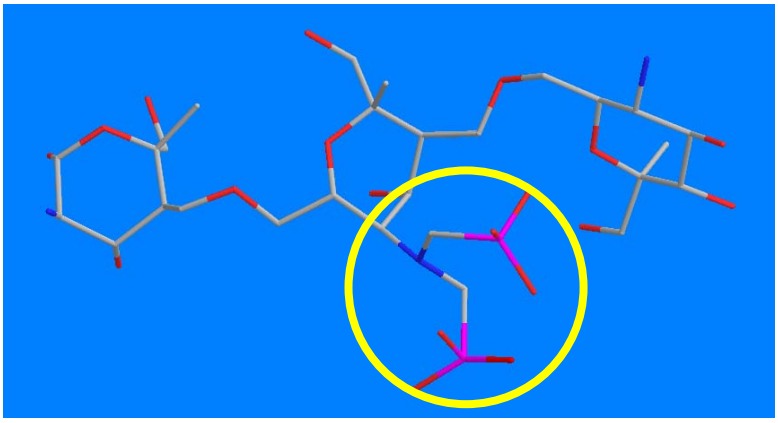


Figure 15. Energy-minimized fragment of the PCH polymer. The central ring contains the amino-*bis*(methylenephosphonate) moiety (circled). Color codes: C grey; O red; N blue; P magenta. H atoms are omitted for clarity.

CONCLUSIONS/PERSPECTIVES

Research on inhibitor chemistries is intense and lately focused on additives that are not only efficient and cost-effective, but also environmentally friendly. With governments and environmental groups becoming increasingly aware of the positive impact that can be brought on the environment by use of safe, non-toxic and green inhibitors, intense research in this field is a "one-way street".

Biopolymers tested in this study are effective as growth inhibitors under the described experimental conditions. The higher affinity of CMI-25 for CaOx crystal surfaces is reflected on the more profound effect of CMI-25 on the CaOx crystal growth rate. CMI-25 polymeric chains have a higher anionic charge density because they contain a higher number of carboxyl groups compared to CMI-15 or CMI-20. Therefore, they mitigate the interaction ability of the

appended anionic $–COO^-$ groups with the solid phase. The polymer backbone can then act as a "fence" on the crystal surface, thus forming an obstacle for propagating steps that lead to further crystal growth. The results described herein indicate that anionic biopolymers can inhibit calcium oxalate crystal growth and that such inhibition is directly linked to fractional coverage of adsorption sites. The degree of inhibition of calcium oxalate crystallization by CMI biopolymers is related to the maximum surface charge density due to adsorbed polymer. The polymer concentration and the $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio are found to be important parameters for the control of morphologies and phase transformation of CaOx crystals. Agglomerated and twinned COM crystals formed in control experiments. Presence of CMI polymers favors the transition of COM to COD crystals. The high binding affinity of the CMI polymer molecules resulted in morphological transition of COD crystals from tetragonal bipyramids dominated by the (101) faces to elongated tetragonal prisms dominated by the (100) faces.

Zwitter-ionic polymers based on the chitosan backbone can be colloidal silica growth inhibitors. PCH, with phosphonate groups chemically introduced, can substantially retard silicic acid condensation and maintain high levels of soluble silicate for an extended period of time. Silicic acid/silicate is thought to be stabilized by a combination of hydrogen bonding and ionic interactions that perturb the dimerization/polymerization process.

Inhibition of sparingly soluble salts is an active area of research and intense industrial interest [70]. Progress in this field of technology is fueled by the continuous need for inhibitors in a plethora of disciplines, from medicine to water treatment. An added need for non-toxic, green, biodegradable and eco-friendly inhibitors is expected to drive research forward.

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