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PAPER

How to control the scaling of CaCO_3 : a “fingerprinting technique” to classify additives†

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A titration set-up coupling ion selective electrodes with pH adjustment was used to analyze the effects of additives present during precipitation of calcium carbonate. Besides industrially well-established antiscalants (sodium triphosphate, citrate, polyacrylate and poly(aspartic acid)), also functional polymers being active in morphosynthesis (polystyrene sulfonate and poly(styrene-*alt*-maleic acid)) were analyzed. Interestingly each additive acts in its specific way, suggesting the notation “fingerprinting” for a complex interplay of up to five “solution modes” of influencing CaCO_3 precipitation and crystallisation. The results provide new insights into the modes of additive controlled crystallisation, and in the long run, the insights may facilitate the design of precipitation systems that yield complex and tailor-made crystals.

Introduction

CaCO_3 is the most abundant biomineral and is also prevalent as geological mineral, *e.g.* as marble, chalk, or limestone. It is important for science and industrial applications.^{1,2} CaCO_3 is used *inter alia* as a filler and pigment. A higher economic factor is however the unintentional CaCO_3 formation (incrustation) in water piping systems, heat exchangers or during the desalination process,^{1–4} and its prevention by addition of chemicals. These water softeners that are added to dishwashing or laundry detergents are a necessary part of consumer chemicals. Still, the costs for society related to CaCO_3 scale are estimated to be of the order of some billion Euro per year,⁵ and a sufficient understanding of the underlying processes is, still, far out of reach. Simple pictures of controlling nucleation and growth in the sense of classical nucleation theory turned out to be grossly naïve.

A second motivation is biomineralization, where interference and control of crystallisation have turned out to lead to Darwinistic advantages. Many papers studying the formation of biogenic CaCO_3 ,^{6,7} and the imitation of these processes using artificial polymers like double-hydrophilic block-copolymers^{8–11} or homopolymers^{12,13} have been published. Most of such biomimetic approaches, however, only

empirically study the influences of soluble additives on morphogenesis^{14–16,2} and polymorph control.^{17–22} Just recently, papers studying the process of CaCO_3 crystallisation utilizing *in situ* X-ray-scattering^{23–28} and quasi-time-resolved CryoTEM^{29,30} have tried to address detailed molecular issues. Beyond that, novel aspects of additive/ CaCO_3 interactions and their modes have been published recently.^{31–33} In the field of incrustation research, several studies have been conducted investigating the influence of scale inhibitors on the nucleation rate and the crystal growth rate. In most of these papers, though, two solutions were mixed and the induction time of the nucleation and concentrations after the nucleation were recorded,^{34–37} while in the present work, supersaturation is generated slowly, and the pre-nucleation stage is investigated in more detail. This type of experiment allows detailed insights into a nucleation process starting from the prenucleation stage to the finally nucleated mineral.³⁸ It is therefore well suited to obtain quantitative information about a nucleation process which is valuable for mechanistic studies of crystal nucleation and growth. Furthermore, the quantitative information from these experiments is useful for theoretical descriptions of the nucleation process.³⁹

In a previous contribution, we have tried to classify the complex interplay of additives with a crystallisation process³¹ and found at least nine different categories of action which can be discriminated in experiments:

- type I: binding of calcium ions to the additive by an ion exchange mechanism,
- type II: the additive influences the extent of formation and the structure of soluble calcium carbonate clusters and their equilibria,
- type III: the additive inhibits the nucleation of a secondary phase from these clusters,

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- type IV: the additive adsorbs onto the nucleated particles and stabilizes them,
- type V: the additive influences the local structure of this nucleated secondary phase, *i.e.* its liquid, amorphous, or crystalline character as well as local symmetry,
- type VI: after onset of crystallisation from those secondary phases, the additive influences the nanocrystal shape by face specific adsorption,
- type VII: influence on oriented attachment and vectorial alignment of nanoparticles by modifying the mutual inter-particle interaction potentials,
- type VIII: stabilization of mesocrystals against Ostwald ripening and re-crystallisation, thus stabilizing the hybrid material, and
- type IX: a mechanical reinforcement or toughness increase of the grown superstructure to constitute a beneficial biomaterial hybrid.

The first five categories are active in the very early solution or colloidal dispersion phase analyzed also in this contribution, while types VI to IX occur after crystallisation of a mineral phase. As discussed in detail in our previous short letter, categories I to V can be identified by different features of the time-development of free calcium ions in carbonate buffer during constant addition of calcium ions.³¹ In the present full paper, we expand the earlier work to a systematic comparison of the most relevant technological additives. Low molecular weight compounds like citric acid and sodium triphosphate, which are used as scale inhibitors in commercial products, are analyzed and compared with their polymeric counterparts, such as the mineral dispersing agents Acusol 588G (product name), the scale inhibitor poly(aspartic acid) (PAsp), as well as “academic” polymers like sodium polystyrene sulfonate (PSS)⁴⁰ or poly(styrene-*alt*-maleic acid) (PS-MA),⁴¹ which are known to strongly mediate mesocrystal formation, *i.e.* they are at least active in the later phases of the morphology control.

The classification of the different modes of additive action in designated categories may allow for the future formulation of a first library of CaCO₃ crystallisation additives if it can be based on a large number of different samples. In this library, chemistry, function and modes of additive action can be inter-correlated. That way, unknown functions of crystallisation additives (and mixtures of additives) occurring for example in biomineralization processes may be assigned to their molecular details. Furthermore, tailoring additives for specific functions in the synthesis of advanced CaCO₃ materials may be possible. Even if a chemistry/function correlation is not feasible, such a library facilitates a fast screening of additive effectiveness.

Experimental

All experiments were performed at 24 ± 1 °C. The preparation of the solutions, the titration setup and the experimental procedure including the appropriate calibration procedures are described somewhere else in detail.^{31,38} The following chemicals were purchased and used without further purification: 1 N HCl (No: 1.09057.1000; Merck) and NaOH (No: 1.09137.1000; Merck); sodium triphosphate (STP, No. 393961000; Acros Organics);

NaHCO₃ (99.7%; No. 424270010; Acros Organics); Na₂CO₃ (anhydrous, 99.95%; No. 223484; Sigma-Aldrich), sodium citrate tribasic (dihydrate; 99.5%; No. 32320; Sigma-Aldrich); sodium polystyrene sulfonate (PSS, MW = 1 000 000 g mol⁻¹; No. 464574; Aldrich) and CaCl₂·2H₂O (99.5%; No. 21097; Fluka). Poly(L-aspartic acid sodium salt) (MW = 6800 and 27 000 g mol⁻¹) was bought from Almanda Polymers and poly(styrene-*alt*-maleic acid) (PS-MA; MW ≈ 350 000 g mol⁻¹; No. 662631) from Sigma. Acusol 588G was kindly provided by Rohm & Haas.

Crystallisation experiments

All experiments are performed in a beaker (50 mL) equipped with a stirring bar and filled with a carbonate buffer solution (10 mM) at pH 9.75 and pH 9.00, respectively. Additives were weighed in into the carbonate buffer. CaCl₂ solution (25 mM), preset to the respective pH by addition of 10 mM NaOH-solution (the dilution due to the pH-adjustment is considered), is constantly added at a rate of 0.01 mL min⁻¹ while a calcium ion selective electrode records the Ca²⁺-potential. The pH-value is kept constant during the experiment *via* pH titration. After every experiment, beaker, burette tips and electrodes are washed with acetic acid (10%) and carefully rinsed with distilled water.

As the volumes added to the reaction vessel and the initial concentrations are known, the recorded potentials enable the calculation of the free Ca²⁺-ion concentration and the amount of free, as well as the amount of bound Ca²⁺-ions. Utilizing these values the CaCO₃-ion product can be estimated assuming a 1 : 1 binding of calcium and carbonate ions.

Analytical methods

Optical microscopy in solution and scanning electron microscopy (SEM) were applied to all samples. SEM measurements were performed on a LEO 1550-GEMINI SEM. Light microscopy pictures in solution were taken with an Olympus BX41 microscope equipped with an Olympus Camedia 5060 color camera. Powder wide-angle X-ray (WAXS) patterns were recorded on a PDS 120 diffractometer (Nonius GmbH, Solingen, Germany) with Cu K α -radiation.

Results and discussion

The titration curves discussed in the following depict the time development of free Ca²⁺-ions in carbonate buffer (reference) and in the presence of different amounts of additives. During the experiments, Ca²⁺-solution is added to the buffers at a constant rate. The Ca²⁺-potential is simultaneously recorded by means of a Ca²⁺-ion selective electrode. If not mentioned otherwise, the experiments discussed were performed at a constant pH of 9.75.

We want to briefly recall the different principal features of the titration curves³¹ (see Fig. 1): binding of calcium ions by the additive *via* ion exchange is indicated by a parallel offset of the time development of free Ca²⁺-ions (type I). From the intercept of the extrapolated, linear calcium curve with the time-axis, the Ca²⁺-binding capacity of the additive is accessible (time corresponds to an added amount). The slope of the titration curve prior to nucleation depends on the

Fig. 1 Schematic illustration of the additive interactions detectable by means of the conducted titration experiments. The black and blue curves represent experiments in the absence of additives and in the presence of a solely type II active additive, respectively. The red graph stands for a type I, III and V additive.

equilibrium constant of the pre-nucleation cluster formation. A flattened slope indicates that more Ca^{2+} are continuously bound in those pre-nucleation species when compared to the control experiment in the absence of additives (*i.e.* the equilibrium constant of cluster formation is higher) while a steeper slope points to less calcium hidden in these clusters (type II). The point of nucleation is seen as a peak in this curve, and inhibition of nucleation is the quantity of retardation in comparison to the reference experiment (type III). With nucleation, in the absence of additives, a second phase of amorphous calcium carbonate and subsequently crystalline CaCO_3 forms, usually seen as a plateau in the titration curve related to the ion product of the most soluble species present in the solution.³⁸

It is conceivable that additives, depending on their structure, adsorb on those nucleated droplets/particles, and influence their size and colloidal stability (type IV). This is not directly seen in the titration curve, but can be followed by accompanying light scattering experiments. By this adsorption or even incorporation in the stabilized droplets/particles, the thermodynamics of nucleation have to change likewise. This means, the local structure of the precipitated material changes, reflected also in the solubility of the least stable species present and the resulting plateau height (type V).

Sodium polystyrene sulfonate

Sodium polystyrene sulfonate (PSS) is a negatively charged soluble model polymer bearing sulfonate groups, which has a very strong impact on the CaCO_3 crystal shape and morphology, as described in previous work.⁴⁰ Special interactions with ions in complex media can also be deduced from its medical prescriptions: it is used in the case of lithium intoxication or abnormal high serum potassium levels as an absorbent of the respective alkali metal ion. We start with this curve as it nicely shows that even polymers known as highly active only weakly influence CaCO_3 in the pre-nucleation phase.

The time development of the amount of free Ca^{2+} -ions in the presence of different concentrations of PSS is shown in Fig. 2. It is obvious that in spite of the partly high added

Fig. 2 Time development of the amount of free Ca^{2+} -ions in the presence of varying PSS concentrations. Lines of the same color are repeat experiments under unchanged conditions.

additive amounts, the curves practically do not change. But the minor differences occurring are far smaller than anything shown below. Due to the sulfonate groups it is expected that PSS binds at least calcium ions, what is confirmed by the measurements by a slight shift of the curves. Polymer dissolved in pure water binds about 0.31 Ca^{2+} -ions per sulfonate group, as shown by a magnification of the low concentration data including the carbonate-free case (Fig. 3). In carbonate buffer, however, binding is much less, and thus, only 0.04 calcium ions are bound per sulfonate group in the presence of carbonate buffer. The huge difference in the calcium binding between water and carbonate buffer can be deduced to a reduction of accessible Ca^{2+} -binding sites at the additive through adsorption of pre-nucleation clusters and possibly sodium ions; an effect seen in the presence of all calcium binding additives.

From these data, we can only state that PSS is indeed practically not active in the early stages of CaCO_3 mineralization and therefore does not really classify as a type I–V dispersant. As many of the morphology controlling proteins,⁷ its activity relies on the existence of an already formed crystal nucleus, *i.e.* it classifies as a type VI–IX additive only.

We have chosen this case as an entry case to illustrate that potential influences can indeed be nicely factorized, and that even well-known modifiers can exhibit no action in the early

Fig. 3 Comparison of the calcium binding capacities of PSS (100 mg L^{-1}) in water (red lines) and 10 mM carbonate buffer (blue lines), the dotted lines represent the extrapolation of the calcium curves. Circles in the corresponding color mark the intersection of the dotted lines with the x-axis. The black line refers to the amount of calcium ions added.

pre-crystallisation phases. For further discussion, we like to underline that the supersaturation of Ca^{2+} at the point of nucleation with respect to the phase present after nucleation (the plateau height) is around 4. To summarize, we solely find type VI-type IX action for PSS.

Trisodium citrate

Trisodium citrate is a low-molecular weight additive with 3 carboxylate groups. Due to its biodegradability and calcium binding groups it is used among other applications as a water softener. At the applied carbonate buffer conditions, it is presumably mainly present in the deprotonated form. The effect of citrate on calcium carbonate crystallisation is studied applying a broader range of concentrations of 50, 100, 150, 250 and 500 mg L^{-1} . In Fig. 4, the different time developments of the amount of free Ca^{2+} -ions are presented. In this case, the additive shows a pronounced influence that in addition is proportional to concentrations, and the onset of precipitation can be retarded from 1700 s up to 9300 s, *i.e.* up to a 5-fold concentration of added Ca^{2+} . In the pre-nucleation stage, the graphs can be subdivided into two phases, beginning with a slightly curved part followed by a linear part.

The curved part is due to the slow onset of a rather weak binding of calcium ions to the citrate ions (weak type I). Once saturated, each carboxylate group however binds on the average only slightly more than 0.1 calcium ions, independent of the citrate concentration. This value is unexpectedly low and contradicts the classical view on how citrate indeed influences mineral crystallisation.

Fig. 4 Development of the amount of free Ca^{2+} -ions (top) and of the CaCO_3 -ion product (bottom) in the presence of different concentrations of citrate as indicated. Lines of the same color are repeat experiments under unchanged conditions.

The real binding of Ca^{2+} is however reflected in an altered slope of the linear phase, which in addition depends on the citrate concentration. The higher the citrate content the more calcium ions are bound in solution clusters, and the flatter the slope of the curve characterizing free Ca^{2+} -ions. This behaviour indicates a strong type II additive. Application of a simple Langmuir-adsorption model⁴² depicts that both additive interactions (citrate/calcium ions and citrate/clusters) in the reaction mixture may be regarded to be independent phenomena (for more information see ESI 1†). This allows for a straightforward adoption of the multiple binding equilibrium, which was introduced for pre-nucleation clusters by Gebauer *et al.* in order to determine the stability of the CaCO_3 clusters (for more information see ESI 2†).³⁸ The analysis quantifies that citrate distinctly stabilizes the pre-nucleation clusters.

Interestingly, the concentration of free Ca^{2+} -ions at all curves at the point of nucleation is not really varying and still comparable to the additive free crystallisation. This is better seen in the presentation of the available ion products, Fig. 4 (bottom). Thus, citrate is certainly not a type III additive. Also the plateau stays rather unaffected throughout the experiments, that is in the titration experiments we do not really see an influence on the as-former precipitated species. From XRD, we however know pure-phase calcite is found with citrate, while in the absence of citrate, predominantly vaterite is obtained. We argue that the citrate favors the formation of (more stable) calcitic short-range order in the clusters, which is stabilised and then carried over to the crystals.

Although there are no significant differences in the post-nucleation Ca^{2+} -levels, citrate affects the morphology of the formed crystals with increasing concentration. The crystals yielded in the presence of citrate are shown in Fig. 5. At 50 mg L^{-1} , tiny needles visible on the calcite rhombohedra are found, possibly aragonite which was not detected by WAXS. Already at a concentration of 50 mg L^{-1} citrate, the edges of the rhombohedra seem to be slightly truncated and feature a micro-structured texture. This is more obvious with increasing citrate concentrations. Some crystal faces are now rough and

Fig. 5 SEM images of crystals obtained 3 h after nucleation in the presence of (a) 50 mg L^{-1} citrate (scale bar 1 μm); (b) 100 mg L^{-1} citrate (scale bar 1 μm); (c) 250 mg L^{-1} citrate (scale bar 2 μm) and (d) 500 mg L^{-1} citrate (scale bar 1 μm).

show pores, an effect, which increases at higher citrate concentrations. This indicates a non-classical particle based crystal growth mechanism and higher order additive actions, at least a type VIII action.⁴³

Interestingly, the progression of the CaCO_3 ion product shows that again the maximal supersaturation is about ~ 4.5 : the ion product at the point of nucleation does not rise compared to the reference experiment in the absence of additives. Thus, citrate is a model case for a type II additive, acting mainly as a type VIII additive after formation of the calcite crystals.

Sodium triphosphate

To characterize sodium triphosphate (STP), rather low concentrations from 1 mg L^{-1} to 100 mg L^{-1} had to be employed (Fig. 6). Nucleation inhibition (type III) and Ca^{2+} -binding capability (type I) for concentrations of 10 and 100 mg L^{-1} have been already discussed before.³¹ Interestingly, further decrease of the STP concentration down to 1 mg L^{-1} affects the crystallisation reaction in an unexpected manner. Even an additive content of 1 mg L^{-1} is sufficient to delay the time of nucleation from 30 min in the control experiment to over 2 hours. The STP concentration in these experiments is so low that the additive/bound calcium ratio is around 1 : 100 already 30 min after start of the experiment. Hence, even the presence of very small amounts of STP inhibits nucleation massively. If the additive concentration is increased to 2.5 mg L^{-1} or 5 mg L^{-1} STP, the time of nucleation will prolong to almost

5 hours and about 9 hours, respectively. In the presence of the highest STP concentration examined, nucleation takes place after more than 11 hours. Consequently, STP is a type III additive already at tiny concentrations.

It is clear that such an intense action well beyond stoichiometry cannot be due to molecular binding of Ca^{2+} -ions. Checking the 100 mg L^{-1} -curve, we find indeed a delay of the increase of Ca^{2+} -ion concentration at very short times (also type I activity), but this effect is indeed minor. Interestingly and contrary to citrate, also the starting slope of the curve is not changing, *i.e.* STP is not influencing the pre-nucleation clusters and their equilibria.

Really remarkable is the bending of the curve of the ion product prior to the nucleation drop, *i.e.* more and more Ca^{2+} -ions take part in the clusters at higher binding, the curving represents the onset of an equilibrium with small equilibrium constant. It is unclear if this is due to the addition of STP or just becomes visible because STP suppresses nucleation; due to the unaltered starting slope we prefer the second explanation. This bending is accompanied with a clouding of the solution and before the first drop of the ion product (Fig. 6). After isolation, these aggregates possess a diameter of 70–150 nm and generate an amorphous XRD diffractogram (Fig. 7).

We identify that these species can hardly be a solid phase, and in fact, these aggregates are solutes from a physico-chemical point of view. The CaCO_3 ion product with respect to Ca^{2+} -ions still increases steadily in this part of the titration curve, and there is no onset of a phase transition, *i.e.* we are before the nucleation event (*i.e.* before the sudden drop to the solubility product). This turbidity is only observed at 100 mg L^{-1} STP and in the presence of CaCO_3 clusters. STP solutions in pure water even at higher calcium concentration but without carbonate, in contrast, stay clear. Thus, this effect must be caused by CaCO_3 pre-nucleation clusters, while STP only suppresses nucleation of the new phase. That those aggregates are finally visible to the naked eye documents the presence of structures of enormous size at high concentrations well before the nucleation. This also indicates that the inhibition of nucleation in the presence of STP is not caused by steric shielding of the clusters against each other, *i.e.* STP is presumably only weakly involved in these clusters.

The real influence of STP therefore seems to be modification of the nucleation of the secondary phase. In the presence of 1 mg L^{-1} STP, the CaCO_3 ion product drops only in two out of three experiments directly to the level of the reference

Fig. 6 Time development of the amount of free Ca^{2+} -ions (top) and the CaCO_3 -ion product (bottom) in the presence of STP. The black arrow marks a bend in the violet curve. Lines of the same color are repeat experiments under unchanged conditions.

Fig. 7 SEM image (left; scale bar = 200 nm) of particles isolated in the presence of 100 mg L^{-1} STP 8 h after the start of the experiment and the corresponding WAXS pattern (right).

experiment after the nucleation. In contrast, a shoulder in the course of the graph is observed in the third measurement after the nucleation, indicating the short-term stabilization of an intermediate species. These two curves under identical conditions indicate bifurcation and multistability. This interpretation is supported by the curve of the ion product of the experiment in the presence of 2.5 mg L^{-1} STP, which also features a shoulder in its progression after nucleation. In general, obviously more than one species is able to nucleate, and it depends on the specific run which one prevails. Closely related to that, the curves at STP concentrations of 5 mg L^{-1} are hardly reproducible at all, while the curve set at 10 mg L^{-1} is reproducible again, but even nucleates at earlier times.

We expect such a “re-entry” behaviour when STP acts as a nucleation inhibitor and poisons most of the possible nuclei present, but acts at higher concentrations as a nucleation agent for a previously not accessible new phase. In fact, at all higher concentrations of STP, well soluble phases are stabilized for longer periods after nucleation. The solubility products of these phases are more than 10 times higher than in the control experiment and the particles isolated under these conditions have a spherical morphology with smooth surfaces. In addition, these curves are not plateau-like and even increase with concentration, *i.e.* the new droplet phase formed has a composition dependent solubility and can adjust to the outer thermodynamics. All these points suggest a building path through liquid–liquid phase separation, *i.e.* something similar to a PILP phase¹² (note that there is no polymer present to support a polymer-induced-liquid-phase, but we refrain from introducing an additive-induced-liquid-phase, AILP). It has to be noted that the free Ca^{2+} concentration in the PILP phase as well as the ion product is increasing after PILP formation (Fig. 6) indicating that the PILP droplets change their composition with time as was also found by other groups.⁴⁴ We speculate that subsequently these PILP droplets transform—very slowly—in a secondary nucleation event within the PILP phase into the obtained round crystals. XRD studies of the isolated particles show that in this step, vaterite is formed independent of the STP concentration. In the case of the 5 mg L^{-1} STP measurements, the PILPs are still so weakly stabilized that the crystallisation within the PILPs always occurs on the timescale of the measurements. This is seen as a second hump with the PILP-phase, throughout which indeed the equilibrium concentration of Ca^{2+} is lowered to the values of the crystalline species.

The real action of the STP additive therefore can be summarized as a rather clean type III–V additive, suppressing at lower concentrations all critical nuclei being responsible for the direct formation of a crystalline or solid amorphous CaCO_3 phase and nucleating at higher concentrations a liquid PILP phase, instead. The whole action of STP, contrary to the previously discussed systems, is therefore tightly bound to nucleation. Interestingly, the quantitative supersaturations between the concentration peak and the phase nucleated are again just a factor of 2.5–4, *i.e.* the distinctly higher ion product is due to the higher Ca^{2+} -equilibrium concentrations in the nucleated phase.

Poly(aspartic acid)

Poly(aspartic acid) (PAsp) has already shown interesting effects in the CaCO_3 precipitation as a stabilizer of liquid precursor phases in previous experiments.^{12,45} Due to its biocompatibility it is often used as an alternative to poly(acrylic acid) in water softeners. In Fig. 8, we present the time development of the amount of free Ca^{2+} in the presence of different PAsp concentrations with $\text{MW} = 27\,000 \text{ g mol}^{-1}$ and $6\,800 \text{ g mol}^{-1}$, respectively, as indicated.

Poly(aspartic acid) is an interesting case, as it combines the functionality of citric acid (the carboxylate group) with the action of STP (stabilizing PILPs), and it is a proof of our fingerprinting technique to find the reflections of that behaviour in the titration curves.

As a first simple effect, PAsp is capable of binding Ca^{2+} -ions (type I). The number of calcium ions bound per

Fig. 8 Development of the amount of free Ca^{2+} -ions in the presence of different PAsp concentrations: (top) overall titration curve, (middle) magnification of shorter titration times for evaluation of pre-nucleation stages; arrows in the respective color indicate down bending in the pre-nucleation development of the amount of Ca^{2+} -ions. (bottom) Progression of the CaCO_3 -ion product.

functional group in carbonate buffer is bigger than in the presence of PSS or trisodium citrate. One carboxylate group of the higher-molecular weight PAsp binds 0.16 calcium ions, while the lower-molecular weight polymer adsorbs about 0.21 calcium ions per functional group. PAsp dissolved in pure water binds 0.29 ions ($\text{MW} = 27\,000 \text{ g mol}^{-1}$). It is interesting that the polymer character of the polycarboxylate gives an improved binding behavior, while carboxylate binds stronger than sulfate. Contrary to citric acid but in good agreement with STP, the slope characterizing cluster equilibria and structure is not altered, *i.e.* PAsp does not interfere with cluster formation. As in the case of STP, a weak bending in the curve can be detected again close to the nucleation (see arrows in Fig. 8, middle). Indeed, already here it turns out that citrate and its polymeric counterpart behave very differently, so it is not solely the carboxylate being responsible for the binding.

Poly(aspartic acid) indeed inhibits nucleation very effectively, comparable to STP. Even at a concentration of 10 mg L^{-1} the formation of a new phase is retarded (type III). A significant dependence on the degree of polymerization of the additive cannot be identified, though. After the nucleation in the presence of 100 mg L^{-1} , the ion product drops to a level that is significantly higher than in the control experiment. The maximum supersaturation with respect to this phase is approximately 2.5. About 11 h after this first nucleation, another drop of the ion product can be observed, now down to the level of amorphous calcium carbonate of the control experiment. The exceptionally high solubility product of the best soluble species again points to the formation of a PILP phase, and this was indeed described in the literature.⁴⁵ Opposite to the experiments in the presence of STP, the ion product is slowly decreasing before the second nucleation due to a continuous change of the PILP-phase structure and solidification again confirming that a PILP phase changes its composition with time.⁴⁴ The exclusively spherical habitus of the particles, isolated after the first but before the second drop of the ion product, indicates a crystallisation path through liquid droplets as well (see ESI 3[†]). According to the XRD measurements only vaterite crystals are obtained at the very end of the reaction. These crystals could originate either from the isolation process or from the original reaction mixture, as the determined solubility product solely identifies the best soluble, solid species. The coexistence of PILP and solid phase has been reported already by Gower *et al.*¹²

The reason for the temporary formation of a liquid precursor instead of ACC is the incorporation of sufficient quantities of additives into the nucleated phase. When the additive content in the solution falls below a critical level the formation of ACC is no longer suppressed and the liquid precursor transforms into ACC. The second drop of the CaCO_3 ion product identifies this event.

Even in the presence of 10 mg L^{-1} PAsp only, the solubility product after the nucleation does not fall directly to a level that is comparable with that of the control experiment. In the curves of both analyzed experiments, a shoulder can be found after the nucleation. This indicates the formation of an intermediate phase, which however is not stabilized over a longer period due to the low additive concentration.

Particles, isolated 3 h after nucleation, are not spherical and do not feature vaterite like morphologies.

Therefore, we can identify PAsp as a type I, type III, and type V additive. It is the system coming closest to STP (and therefore can serve as an environmental benign phosphate replacement system, contrary to citrate), but there are still some minor differences, especially concerning the long-term stability of the PILP dispersions.

Acusol 588G

Acusol 588G is a commercially available water softener from Rohm & Haas. According to the manufacturer, it shows both an inhibiting effect on CaCO_3 crystallisation and a dispersing effect on preformed crystals. The scope of applications is not limited to CaCO_3 , but can be expanded to calcium phosphates and silicates.

The molecular weight of this acrylate-sulfonate copolymer is $\text{MW} = 12\,000 \text{ g mol}^{-1}$, according to manufacturer's data. Moreover, elemental analysis reveals that Acusol 588G contains significant amounts of nitrogen besides sulfur. Therefore, the shown polymer structure (Fig. 9, top) comes close to our structural expectations. We analyzed the behaviour of this polymer to see if the extra amount of weakly coordinating

Fig. 9 Assumed chemical structure of Acusol 588G (top). Development of the amount of free Ca^{2+} (middle) and the CaCO_3 -ion product (bottom) in the presence of different concentrations of Acusol 588G. Black arrows indicate the time points at which the respective particles shown in Fig. 10 were isolated.

sulfonate groups can add to the stabilizing performance of the polymer and bring extra features to mineralization control. The developments of the CaCO_3 ion product in experiments in the presence of Acusol resemble those in the presence of poly(aspartic acid). The progression of the amount of free calcium ions illustrates the capability of this additive to bind calcium ions (type I). As the precise structure is unknown, solely binding capacities per mass of additive can be compared. The analysis shows that one gram Acusol 588G dissolved in carbonate buffer bind about 2.7 mM Ca^{2+} . In water, approximately 3.5 mM g^{-1} additives are bound. The cluster equilibrium is unaltered by this additive (not type II). In the presence of this water softener, too, a flattening of the slope of the amount of free calcium ions is found before nucleation (Fig. 9, arrows), supporting our view that this is an inherent feature of clusters rather than polymer induced. Not surprisingly, this polymer actually inhibits the crystallisation of CaCO_3 and is therefore attributable to type III. The effectiveness is comparable to that of PAsp.

At both studied polymer contents, the ion product after nucleation is significantly higher than that of the finally nucleated ACC and the behaviour is again typical for the formation of a PILP phase, as in the presence of PAsp and STP. Even at the lowest studied additive concentration, the CaCO_3 ion product remains 3.5 h at this high level, before secondary nucleation within the PILP phase drops to the value of the control experiment.

At 100 mg L^{-1} polymer content, the second decay of the ion product is not observed within the 17 h overall duration of the experiment. The difference of Acusol 588G to PAsp (and therefore the role of the added stabilizing charges onto the polymer backbone) is found at the later stages of reaction. As found for PAsp, the ion product after PILP formation is decreasing again indicating the continuous change of the composition in a PILP phase.⁴⁴ SEM images document that this polymer influences the morphology of the crystals formed (type VI). Instead of spherical particles, before the second fall of the ion product calcite crystals (WAXS) with a spindle like habitus can be isolated (see Fig. 10). Typical calcite faces ($\{104\}$) can solely be identified at the caps of the rods. The approximately $1.8 \mu\text{m}$ long and $0.6 \mu\text{m}$ wide crystals, which resemble the morphology of *Otoconia*,⁴⁶ are composed by considerably smaller building units, which points to a non-classical growth mechanism and the effective stabilization of intermediate nanoparticles. Conceivable is, for example, that small calcite crystals form directly from the stabilized PILP phase and the additive only adsorbs at distinct faces. In this way, nanoparticles are stabilized from uncontrolled aggregation (type IV) while allowing the assembly of the small crystal-building units (type VII). After the second abrupt decay the ion product reaches a level comparable with that of the control experiment. From that point on, the crystal growth is no longer controlled by the additive, but by classical growth processes and consequently, calcite rhombohedra are formed.

Poly(styrene-*alt*-maleic acid)

A further polymer approach to modify copolymer structure is the increase of hydrophobic character within the backbone. This can be done (under preservation of the functional carboxylate density) by copolymerizing a hydrophobic

Fig. 10 SEM pictures of crystals obtained in the presence of 10 mg L^{-1} Acusol 588G (a) 2 h after nucleation (scale bar $200 \mu\text{m}$); (b) 6 h after nucleation (scale bar $1 \mu\text{m}$) and (c) in the presence of 100 mg L^{-1} Acusol 588G about 12 h after nucleation (scale bar $1 \mu\text{m}$), see also Fig. 9.

monomer with maleic anhydride (which carries two carboxylate moieties in one monomer) in an alternating fashion. The resulting model case poly(styrene-*alt*-maleic acid) is consequently a well-known dispersing agent.

The time development of the amount of free Ca^{2+} in the presence of poly(styrene-*alt*-maleic acid) (PS-MA) is shown in Fig. 11. At first, this polymer has a comparably high affinity to bind calcium ions (type I) at pH 9.75. One PS-MA monomeric unit is capable of binding about 0.7 calcium ions in the presence of carbonate buffer. In addition the polymer inhibits nucleation (type III), however significantly weaker than STP or PAsp. An influence of this additive on the pre-nucleation cluster equilibrium is again not found. The phase formed after nucleation is - as identified by its high equilibrium dissolution and its textural “fingerprints”, presumably a dehydrating PILP phase transforming into amorphous CaCO_3 , which can undergo secondary nucleation towards final crystals. In agreement with the more hydrophobic polymer modifier, a PILP phase and its abrupt transformation into ACC is either completely suppressed (as compared to the related PAsp), or is so dehydrated that it is similar to amorphous phases with their lower equilibrium solubility of Ca^{2+} .

As a sensitivity of amorphous phases on pH was previously identified,²⁸ also precipitation at pH 9.00 (Fig. 11b and d) is analyzed. The relative inhibition of nucleation remains unaltered. After nucleation, however, the curves for 100 mg L^{-1} additive concentration differ distinctly. At pH 9.00, a second bump indicates the restructuring of the PILP precursor, maybe from a weakly hydrated PILP towards an ACC species at lower relative polymer concentrations.

An influence of the precipitated polymorph by PS-MA is confirmed by WAXS. In the presence of 10 mg L^{-1} and at pH 9.75, besides traces of vaterite, calcite is formed, while an increase in the additive concentration leads to pure-phase calcite. SEM images of crystals obtained at pH 9.75 show a morphology very similar to the Acusol case, *i.e.* at 100 mg L^{-1} , rods with calcite (104)-caps are obtained.

Fig. 11 Development of the free amount of Ca^{2+} (a, b) and the CaCO_3 ion product (c, d) in the presence of PS-MA at different concentrations for pH 9.75 (a, c) and pH 9.00 (b, d).

Conclusions

In this study, we showed that scale control additives feature a set of different interactions in a CaCO_3 crystallisation assay.^{31,38} This is evidenced by different time dependent curve progressions in terms of the free Ca^{2+} -ion development as well as the CaCO_3 ion product. In some cases, it is even possible to assign

Fig. 12 Overlay of the developments of the amount of free Ca^{2+} -ions in the presence various studied additives at pH 9.75. All negatively charged additives have a concentration of 100 mg L^{-1} .

each compound to a unique curve development. A comparison of all these curves is shown in Fig. 12. Each additive has its unique curve development, which can serve as fingerprint and allows quantitative assessment of the additive action in CaCO_3 crystallisation. Even additives, which essentially have the same interacting groups but different backbones and geometries (citrate vs. PAsp), show a very different behaviour. Such information is important to understand the action of scale inhibitors and additives in a crystallisation assay.

First of course it is important to repeat that the classical view on the action of a scale inhibitor, the molecular binding of Ca^{2+} -ions onto the additive (type I) does exist, but is clearly the least important one. It is also clear that some additives are orthogonal in their action to each other, *i.e.* citrate rather exclusively just alters the pre-nucleation cluster formation (type II), while STP essentially just acts throughout nucleation and stabilizes a PILP-phase afterwards (types III & V).

PSS finally does not act in these early stages, but relies on a preformed crystalline species to develop its morphology changing influence in the very late stages of crystal growth (type VI–type IX). In these orthogonal cases, we are also allowed to expect synergies in action, as each additive is getting active in a specific step of the cascade. Remarkably, this is well known in biomineralization where specific additives are responsible for the deposition of minerals at different times. Well-known examples from biomineralization are seashells that have an outer prismatic calcite layer, while the inner nacreous layer is constituted of aragonite; it was demonstrated that (mixtures of) macromolecular additives are responsible for the subsequent mineralization and polymorph control in each of the layers with different CaCO_3 polymorphs.⁴⁷

From the joint curves, also common features become visible. In fact, it is clear that a majority of additives (from the ones examined here all except citric acid) does only weakly interfere with pre-nucleation cluster formation so that there is a joint curve of concentration development until the nucleation point. This makes the structural analysis and discussion of such clusters easier, as it is a common, uniform structure which seems to develop. In addition, this structure obviously does not interact with negatively charged additives and polyelectrolytes, *i.e.* is either neutral or also weakly negatively charged.

Another really unexpected but joint feature concerns the point of nucleation. The stepwise drop of Ca^{2+} -concentration

throughout nucleation is always between a factor of 2.5–4, *i.e.* the supersaturation with respect to the next phase formed is only 2.5–4 and therefore rather low. *Vice versa*, this means that an additive cannot really suppress nucleation as such, but only controls the formation of the phase thereafter, poisoning for instance all crystalline species (once formed) and supporting amorphous or liquid phases. This interdependence means that a type III additive is always also a type V additive, with the most effective scale inhibitors being those which stabilize a PILP phase, afterwards. This rather low “effective supersaturation” (with respect to the subsequently formed phase) of course has to be fed back into the theory of nucleation, with the onset of spontaneous nucleation being at much lower supersaturations than previously expected.

Concluding, titration fingerprinting provides a rather detailed look on the manifold phenomena occurring throughout scale formation. The insights obtained for different additive systems may be utilized to design antiscalants with improved effectiveness. Similarly, the approach may render detection of novel correlations between additive chemistry and additive effects possible, which may in turn facilitate the design of crystallisation additives that yield tailor-made crystals.

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