The Multiple Roles of Additives in $CaCO_3$ Crystallization: A Quantitative Case Study

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In the classical picture of crystallization, nucleation is considered to take place in a solution of ions or molecules exceeding a critical supersaturation, leading to the nucleation of the new phase.[1] The growth of nucleated particles and crystals is then considered to take place via addition of single ions or molecules. The role of additives, which modify crystal growth, is restricted in such a view: they can either bind ions or interact with the crystal. Structural complexity found in biominerals,[2–4] and also many synthetic crystallization patterns found in the presence of additives,[5] raised serious doubts on such a simplified view, and meanwhile, a series of novel intermediates and mechanisms have been postulated to occur.[5–7] The role of additives is, however, still hard to attribute, and empirical control of morphology is the rule, not the exception. In our opinion, this is caused by the multiple roles of additives in such processes, which in addition depends on concentrations and other experimental conditions. A possible quantification, at least a classification of all the different interactions, is eagerly needed, but simple tools to do so are not currently known.

This paper aims at opening the pathway to such systematization, here exemplified for calcium carbonate as a model system of complex crystallization. The choice is based on relevance: calcium carbonate is not only of great industrial importance, the major source of water hardness, and the most abundant biomineral, but also one of the most frequently studied minerals, with great scientific relevance in biomineralization and geosciences. Scale formation (incrustation) is also a substantial issue in daily life, industry, and technology, rendering the addition of scale inhibitors to laundry detergents, household cleaners, and also in many industrial applications unavoidable.

Only little is known about the early stages of $CaCO_3$ crystallization, though this mineral has been studied for more than a century now. Identified precursor phases are amorphous calcium carbonate (ACC) in bio-[8] and biomimetic mineralization[9] and liquid precursors (PILP), which have been found in some cases.[6] Directly after ion contact forming precursor species –that is, before nucleation of the novel phase occurs– were postulated,[10,11] and could be revealed.[12,13] Up to then, ACC was the only known species, which occurs after nucleation.[14] It will be shown that a simple titration of Ca^{2+} ions under otherwise carefully moderated conditions is enough to "fingerprint" most of the possible interactions and influences of additives and crystallization conditions. Using different features of the titration curve, it is possible to quantify bound ions, ions stored in neutral equilibrium clusters, the stabilization of such clusters, inhibition of nucleation, and even polymorph control or modification of the structure of amorphous phases.[12,13] Additives can be quantitatively classified according to their action, and a more systematic assignment of structural effects observed in the final crystal or crystalline hybrid material to the molecular interactions throughout the synthetic process should be possible.

The mineralization experiments are performed by adding a calcium solution at a constant rate (10 μL per minute) into an excess carbonate solution while recording the pH value. Changes in the pH are automatically back-titrated, so that the whole reaction is freed from the disturbing influence of pH-induced changes of the carbonate/hydrogen carbonate equilibrium. In parallel, the Ca^{2+} concentration is monitored, thus allowing the determination of ion activity, supersaturation, onset of precipitation, and the different dissolution equilibria.[12,13]

Typical curves (Fig. 1) describe the development of free calcium ions with increasing titration time as a function of the pH.

The reproducibility of measurements is high,[12,13] and essentially deviates only in the onset of nucleation within maximal 500 s, that is, the point in time at which the amount of free calcium ions drops instantaneously (Fig. 1, time 2), as particles are formed. As discussed in earlier work,[12,13] the flattened increase in the amount of free calcium ions as compared to a reference sample without carbonate but same pH (dotted line) can be attributed to the fact that a part, if not the majority, of Ca^{2+} ions does form neutral equilibrium calcium carbonate clusters. Calcium binding in clusters is more distinct at higher pH values, and can be attributed to a higher fraction of carbonate ions in the buffer equilibrium (Fig. 1, stage 1), thus proving that

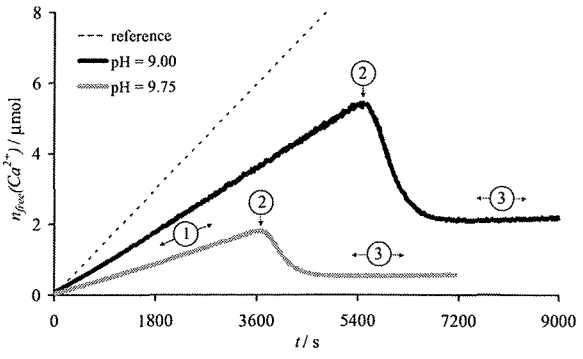

Figure 1. Time development of the amount of free calcium ions measured by a calcium ion-selective electrode (Ca-ISE) in carbonate buffer at pH 9.00 and 9.75. The reference line reflects the amount of calcium ions added. '1' depicts the prenucleation stage, '2' the nucleation event and '3' the particle growth stage.

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the formation of such clusters is based on equilibrium thermodynamics.[12,13] This aspect is visible in calcium binding before and after nucleation (Fig. 1, stage 2). After nucleation, the curves are independent of time, as all Ca^{2+} ions added are incorporated into the growing particles (Fig. 1, stage 3). The constant value is then given by the dissolution equilibrium of calcium carbonate, giving an approximately constant ionic background. The ratio of this value to the peak value before nucleation gives the critical supersaturation of Ca^{2+} ions, which is, in the experiments without additive, only 2–3.

The free-ion product can be calculated with the mean values of the concentration of free calcium ions and the concentration of free carbonate ions. At pH 9.00–9.50, the solubility product of the nucleated amorphous phase is approximately $3.1 \cdot 10^{-8}$ M^2 (defined as ACC I, predominantly nucleating calcite), while the solubility product is approximately $3.8 \cdot 10^{-8}$ M^2 (defined as ACC II, nucleating predominantly vaterite) at pH 9.75–10.0.[12,13] From the curves and a recalculation of the final Ca^{2+} concentration into ion products, it can be directly evidenced that the precipitating particles in all those cases are at first amorphous; complete crystallization towards the different crystalline species takes place at later stages of the reaction, not shown here. Since the solubility product is determined by the most soluble species, the curves are not capable of indicating the onset of amorphous-to-crystalline transitions, as long as amorphous species are present in the system.

Precipitation of calcium carbonate in presence of additives is studied at the minimum of cluster binding energy at pH 9.75.[12,13] In the following, the influence of three different types of additives is analyzed. Poly(acrylic acid) provides a model system for polymeric scale inhibitors, that is, compounds that inhibit nucleation. In addition, we also analyze classical low-molecular-weight scale-inhibiting additives, that is, sodium-triphosphate and citric acid, to evaluate the performance of these systems compared to the polymer. In a last step, we discuss the action of a peptide additive with low charge, which was found to be aragonite binding.[15]

Polycarboxylates, such as poly(acrylic acid) (PAA) or poly-(aspartic acid), are technically added as scale inhibitors to laundry detergents, dishwashers, cooling circuits, etc. Here, the action of PAA as a model compound for scale inhibition is analyzed quantitatively. The PAA utilized has an averaged molecular weight of 5100 g mol^{-1}, that is, one molecule PAA provides approximately 71 carboxylic acid groups.

The calcium titration of two concentrations of PAA (10 mg L^{-1} and 100 mg L^{-1}, as indicated) in the carbonate buffer is shown (Fig. 2). Note that 10 mg L^{-1} is an unusually small, almost not detectable amount of additive, which nevertheless has strong impact on the crystallization scenario.

The parallel offset of the time development at small added Ca^{2+} values is a measure for the binding capacity of PAA for calcium ions. From the intercept with the x-axis, we can calculate a value of approximately 11% (w/w) polymer-bound Ca^{2+}. This relates to the adsorption of 15 calcium ions per PAA molecule, or the binding of one calcium ion to 5 polymeric carboxylic acid groups. This binding capacity for free calcium ions is obviously rather low, but it is classically considered to be the reason for scale inhibition by PAA, apart from growth inhibition of nucleated particles.[16,17] However, as we see from Figure 2, the free concentration of calcium ions is not lowered enough to explain the observed

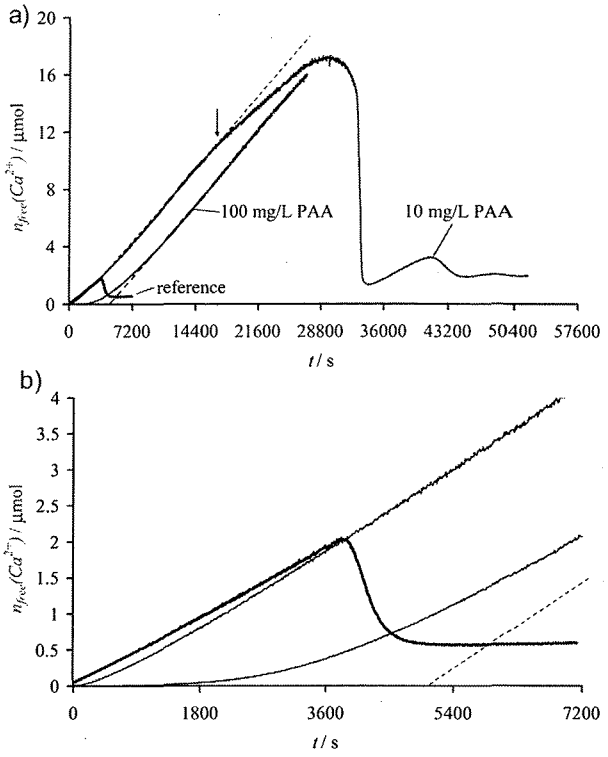

Figure 2. Time development of the amount of free calcium ions in absence of PAA (reference, see also Fig. 1) and in presence of 100 mg L^{-1} and 10 mg L^{-1} PAA at pH 9.75. a) Overall curve. b) Zoom into the early phase clarifying negligible overall adsorption of calcium ions in presence of 10 mg L^{-1} PAA.

effects. The calcium-ion binding capacity plays a secondary role in the inhibition of nucleation.

A second possible role of the additive is interference with the structure of the neutral equilibrium clusters. This is obviously not the case for PAA, as both the reference and the PAA curves have the same slope at intermediate calcium concentrations. Beyond, the initial slope is independent of the PAA concentration, as indicated by the parallel offset in presence of 100 mg L^{-1} PAA and 10 mg L^{-1} PAA. The nucleation is nevertheless distinctly inhibited; already the 10 mg L^{-1} sample allows for a supersaturation eight times higher, while the experiment with 100 mg L^{-1} PAA was aborted after 7.5 h without any visible precipitation at even higher concentrations. Nucleation inhibition in such a case can only be due to the adsorption of the prenucleation-stage equilibrium clusters to PAA, stabilizing the clusters against aggregation and subsequent nucleation. An interesting effect in presence of 10 mg L^{-1} PAA is the decrease in the slope of the time-development of the amount of free calcium ions after approximately 4.5 h, as indicated by the arrow in Figure 2a. This means that an increased amount of calcium ions is bound to soluble cluster species, while the system is still stabilized against nucleation. It is not the purpose of this work to analyze this effect in detail, but both a transition towards a different cluster structure with higher equilibrium constant and the proximity to a spinodal demixing are possible reasons.

A second interesting effect in presence of 10 mg L^{-1} PAA is the new increase in the amount of free calcium ions after the first

nucleation event, which repeatedly tunes in and out. This effect can be ascribed to the difference of adsorption of the polymer on clusters to the adsorption on nucleated particles, thus releasing parts of the stabilizer upon nucleation, which then can turn to be active for the next Ca^{2+} ions added, and keeps them from aggregating onto the preformed particles. New nucleation is distinctly inhibited, indicating that particle growth has a lower activation barrier here than particle nucleation. Moreover, the binding of PAA on precipitated particles reduces the amount of free PAA in solution, thus stabilizing less the system towards novel nucleation of particles. After the third detectable concentration swing, the solubility product drops to the value corresponding to the solubility product of ACC I (see Supporting Information, Fig. S1). In the absence of PAA, ACC I was only observed at lower pH values.[12,13] At first, this means that no ACC II is present 6 h after the first nucleation event, while it was present before. Wide-angle X-ray scattering (WAXS) diffraction patterns (see Supporting Information, Fig. S2) show that pure-phase calcite is obtained in presence of PAA at pH 9.75, while predominantly vaterite, with only traces of calcite, is obtained in absence of additives. Combining the time-development of solubility products and WAXS data, this shows that ACC I indeed relates to an amorphous species, which is the precursor of calcite, and that ACC II also relates to an amorphous species, which is the precursor of vaterite. Pure-phase calcite is obtained because ACC II is stabilized against crystallization, and thus dissolves for the benefit of (more stable) ACC I or crystalline calcite. In this way, the polymer modifier influences the local structure of the precipitating phase.

Due to their technical relevance, we also analyzed the influence of two classical scale inhibitors, sodium-tripolyphosphate (STP) and citric acid, in our crystallization assay (Fig. 3). It is seen that citric acid binds weakly to Ca^{2+} ions: before the time-development becomes linear, it shows a curvature, which gives rise to an intercept with the *x*-axis of the linear time-development, relating to calcium-ion binding affinity. Moreover, citric acid flattens the slope of the linear part of the titration curve, that is, more calcium is moved into the clusters. On the other hand, citric acid only delays, but does not significantly suppress, nucleation, as the reachable supersaturation is similar to the reference experiment. Contrary to simple expectations, it does not effectively bind the nucleated particles. In the end, the influence of citric acid on the prenucleation-stage cluster equilibria results in precipitation of pure-phase calcite (see Supporting Information Fig. S3), while in absence of additives mainly vaterite, with traces of calcite, is obtained (see Supporting Information Fig. S2a). This underlines our previous findings[12,13] that suggested that different structures of amorphous phases are preformed in the cluster equilibria, whereas citric acid promotes the formation of clusters with structures that relate to calcitic preorder.

Opposed to the action of citric acid, STP not only binds Ca^{2+} ions in a stronger fashion than citric acid, but a manifold of material as stabilized clusters. In contrast to citric acid, STP promotes the formation of pure-phase vaterite (see Supporting Information, Fig. S4). The accessible supersaturation reaches values of 30, which is the highest value within this set of additives. There are many consequences of this observation worth discussion, but, most importantly, it means that particles do nucleate from the equilibrium clusters, and not from the free ions. Stabilization of clusters and particles after a first nucleation

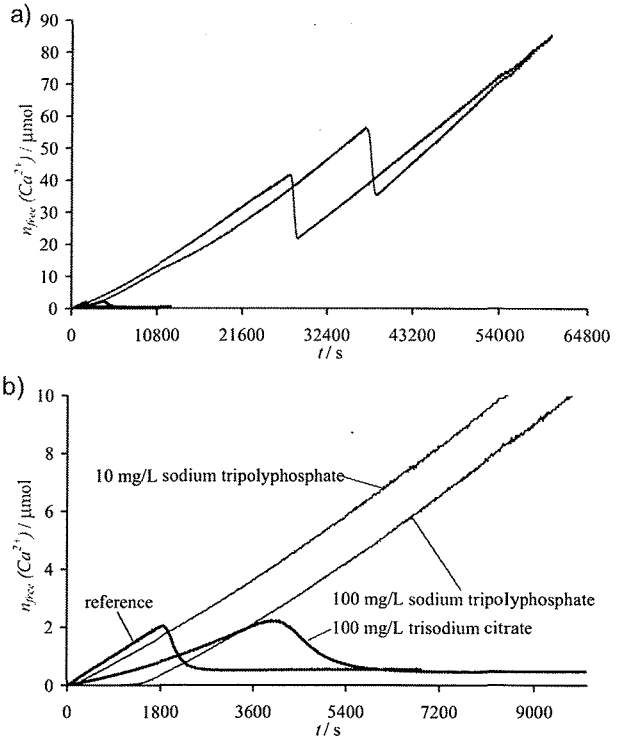

Figure 3. Time development of the amount of free calcium ions at pH 9.75 in presence of citric acid and two concentrations of sodium tripolyphosphate (10 mg L^{-1} resp. 100 mg L^{-1}), as indicated. This is compared to the reference experiment. a) Overall titration curve characterizing STP and b) magnification of shorter titration times for citric acid evaluation.

event is even improved compared to PAA; a fact which currently remains unclear to us. The fact that a 10 mg L^{-1} solution performs only slightly weaker than a 100 mg L^{-1} solution of STP underlines that the colloidal stability of the intermediate cluster structures, and not stoichiometric binding events (as expected by an ion-binding like mechanism), are most relevant in suppressing nucleation of a new phase.

There is a vast, almost unmanageable amount of scientific literature dealing with the investigation of the action of biopolymer or peptide additives on crystallization.[18-24] For the purpose of an expanded view on $CaCO_3$ crystallization in presence of additives, we analyzed the action of a peptide additive in addition to the scale inhibitors studied above. The peptide only shortly discussed in this work is a 12-mer described in Ref. [15], exhibiting binding affinity to geological aragonite crystals as derived in a phage assay. The sequence according to the three-character-code of amino acids is Ile-His-Ile-Lys-Phe-Lys-Gln-His-Gln-Asn-His-Asn. The peptide is nonacidic, while most proteins, which are supposed to play important roles in biomineralization processes, are highly acidic.[25,26] Nevertheless, we chose this peptide because it exhibits affinity to calcium carbonate, which is the prerequisite for any action on its precipitation.

The time-development of the amount of free calcium ions without additive (reference) and in presence of 0.1 g L^{-1} of the peptide is shown in Figure 4. It is obvious that the peptide does not bind calcium ions even at the applied concentration. It also

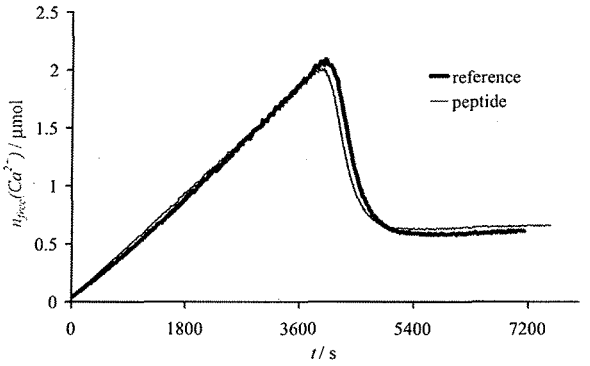

Figure 4. Time development of the amount of free calcium ions at pH 9.75. 'Reference' indicates the time development in absence of additives. Within experimental accuracy, no effect of the peptide additive (0.1 g L^{-1}) can be detected.

does not interfere with soluble cluster formation (the slope of the curve), and does not retard nucleation. In the classical sense, the peptide is definitely not a scale inhibitor. However, the precipitated powders show that the peptide effectively suppresses calcite formation. This effect is to be discussed in more detail in a following publication, additionally analyzing other peptide sequences. In the present context, it is important to state that the peptide does not interfere with the early phases of crystallization, that is, does not bind to Ca^{2+} ions or Ca clusters and does not suppress nucleation, but modifies the crystallizing species, which is therefore a further possible action of an additive. The peptide is capable of binding clusters and ACC, and influences the polymorph formed in this way. It is assumed that such additives also stick onto the final crystal superstructures, stabilizing them against Ostwald ripening and textural rearrangements. This is presumably also the most important function of such peptides in biological systems.

The influence of additives/polymers on the precipitation of calcium carbonate and the resulting structures formed can, to a large extent, already be categorized by means of the time-development of the amount of free calcium ions in a crystallization assay, as shown above. We can clearly differentiate between different types of independent actions.

- type I: adsorption of calcium ions,
- type II: influence on soluble-cluster formation and equilibria,
- type III: inhibition of nucleation of a precipitated nanoparticle phase,
- type IV: adsorption on nucleated particles and their stabilization,
- type V: influence on the local structure of nucleated particles, that is, type of amorphous phase or crystalline polymorph.

Not directly illustrated by the present experiments, but already well known from our previous publications,[27-32] these categories have to be completed to include some more obvious roles of additives on the final crystal texture and its properties:

- type VI: influence on the nanocrystal shape by face-specific adsorption,
- type VII: influence on the oriented attachment and vectorial alignment of nanoparticles by modifying the mutual interparticular interaction potentials,
- type VIII: stabilization of the resulting mesocrystals[5,33] against Ostwald ripening and re-crystallization, thus stabilizing the hybrid material,
- type IX: mechanical reinforcement or toughness increase of the crystal and modifier phase, to constitute a beneficial biomaterial hybrid.[27].

Interestingly, additives can act very differently, and sometimes in an orthogonal fashion. As discussed above, PAA is actually a type I/III/IV/V additive, citric acid a type I/II/V additive, STP a type I/III/IV/V additive, and the peptide a type V additive. The case of traditional crystallization-inhibition additives as STP indicates that substances that are very effective in the early phases of crystallization do not necessarily create well-ordered crystal textures with beneficial mechanical properties (see Supporting Information, Fig. S4). It is a nearby assumption that, in such cases, a mixture of additives or biopolymers has to be considered to control the structure formation over its different stages, while, ideally, having a "specialist" for each relevant step – a feature that is also discussed in biomineralization mechanisms. However, it is, for instance, definitely wrong to use a single "expert molecule" to control a crystallization reaction throughout the entire process: the negative morphological observations found in many reports indicate a too-simplified view on crystallization control, starting from ion binding, to control all further steps in a similar manner.

The quantitative analysis of calcium carbonate precipitation in a carefully designed titration device showed that nucleation of calcium carbonate occurs in a complicated, multistep process, where not only free ions, but also neutral soluble clusters, play a key role.[12,13] The impact of additives on calcium carbonate crystallization is correspondingly complex. Additives can already act in the prenucleation stage, by adsorption of ions and clusters. The adsorption of clusters stabilizes the system against nucleation, that is, against aggregation of clusters. Here, the additives may preferentially adsorb certain structures, and preferential adsorption would lead to a change of local coordination structure, simplifying the nucleation of different amorphous structures and polymorphs. The developed experimental procedure allows the quantitative analysis of different precursor species and of the action of additives on the particular precursor species. It is now possible to classify and quantitatively describe the action of additives. It can be shown in which stage of precipitation the additives act, and different actions on the precipitation can be detected, which is an important aid in understanding complex crystallization processes.

PAA turned out to be a classical nucleation inhibitor that, however, does not work by the binding of Ca^{2+} ions or growth inhibition of nucleated particles, as classically assumed, but by interaction with soluble neutral $CaCO_3$ clusters. On the other hand, STP binds a large amount of ions as well as clusters, reaching supersaturations of up to 30, whereas citric acid only binds Ca^{2+} ions, modifies cluster structures and thus the polymorph formed, but does not significantly suppress nucleation.

On the base of these experiments, we propose to categorize the action of additives into at least nine different types: I) calcium-ion binding capacity (PAA, citric acid, STP), II) impact on prenucleation-stage cluster equilibrium (citric acid), III) adsorption on prenucleation-stage clusters causing nucleation inhibition (PAA, STP), IV) adsorption on nucleated particles and stabilization thereof (PAA, STP), and V) influence on the local

structure of nucleated particles, such as type of amorphous phase or crystalline polymorph (PAA, STP, citric acid, Peptide).

Beyond the early influence described here, there is in addition VI) the influence on the nanocrystal shape by face-specific adsorption, VII) the influence on the oriented attachment and vectorial alignment of nanoparticles by modifying the mutual interparticular interaction potentials, VIII) the stabilization of the resulting mesocrystals against Ostwald ripening and recrystallization, and finally, IX) the mechanical reinforcement or toughness increase of the crystal and modifier phase to constitute the biomaterial hybrid.

The quantitative analyses of the action of numerous other additives, which are already known to facilitate mesocrystal formation or polymorph switch within this scheme, are part of ongoing work. It is now also quantitatively possible to relate the additive action to its concentration, which is known to have a distinct influence, as well as to other experimental variables.

Such experiments are, in our opinion, highly relevant for the fundamental understanding of crystallization and nucleation inhibition, with impact on the generation of biomimetic materials of huge scientific and industrial importance.

Experimental

All experiments were performed at 24 ± 1 °C. The preparation of solutions, the commercial setup and the principal experimental procedure including the appropriate calibration procedure were already discussed in detail elsewhere [12,13]. Additives were used without further purification. Polyacrylic acid (PAA, molecular weight 5,100 g mol^{-1}) was bought from Fluka (No. 81132), sodium-triphosphate and citric acid (technical grade) were kindly provided by Reckitt Benckiser. Peptide synthesis was performed using standard solid-phase supported peptide synthesis protocols [34,35] on an Applied Biosystems ABI 433a peptide synthesizer and was described in more detail in [12].

Crystallization Experiments: The experiments were performed in a beaker (50 mL) that was filled with carbonate buffer (25 mL, 10 mM, with or without the particular additive), pH 9.75. Calcium chloride solution (10 mM) set to pH 9.75 (volume dilution due to pH-adjustment is considered) was dosed at a rate of 10 μL per minute for experiments in presence of PAA and the peptide and calcium chloride (25 mM) solution set to pH 9.75 at a rate of 10 μL per minute for experiments in presence of trisodium citrate and sodium tripolyphosphate. The pH value was kept constant via titration utilizing sodium hydroxide solution (10 mM), and the calcium potential was monitored. Electrodes, beaker and burette tips were cleaned with acetic acid (10%) and carefully rinsed with distilled water after every experiment.

Acknowledgements

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