

A Straightforward Treatment of Activity in Aqueous CaCO_3 Solutions and the Consequences for Nucleation Theory

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The problem of activity in salt solutions is as old as Arrhenius' theory of electrolyte dissociation itself,^[1] since it is directly connected to a primary caveat that was addressed by Planck and van't Hoff:^[2] the measured and calculated conductivities of electrolyte solutions did not agree, especially at high concentrations. Hence, Arrhenius introduced the notion of "inactive" and "active" molecules with respect to their ability to conduct electricity.^[2] Today, we generally use activity coefficients to describe deviations from ideal behavior in real systems. In electrolyte solutions, any such deviation is essentially supposed to originate from Coulomb interactions. The first quantitative theory to account for this was developed by Debye and Hückel.^[3] It applies for sufficiently dilute solutions,^[4] although numerous advancements and extensions for the treatment of higher concentrations have been reported.^[5] For strong electrolytes, the Debye–Hückel theory predicts complete dissociation in the dilute regime,^[6] so that the role of "inactive" species can be neglected. In other words, there are no undissociated ions in this case, while the dissociated ones exhibit a decreased activity. However, the situation becomes more complicated when ion pair formation occurs.^[7] The concept of calcium carbonate ion pairing has been continuously proposed and refuted,^[8] but indeed, any associated CaCO_3 complexes would represent an "inactive" species that will make up an additional contribution to activity beyond the Debye–Hückel term. Clearly, ion association in solution has important consequences for the properties of an electrolyte system. For instance, it is evident that bound ions will not contribute to conductivity, and hence remain "inactive". Similar conclusions can be drawn regarding the nucleation of a solid phase from such salt solutions, at least in the framework of classical theories,^[9,10] which assume that critical nuclei are generated via attachment of single ions in a pseudo-equilibrium; conceptually, these nuclei correspond to the activated complexes considered in fundamental models of chemical kinetics.^[11] Thereby, ion pairs and complexes must be regarded as inactive, because they do not explicitly participate in the formal equilibria underlying the formation of initially unstable, or at critical size metastable, nuclei.

Recently, Gebauer et al.^[12] demonstrated that association in CaCO_3 solutions is not limited to simple ion pairs and that larger neutral entities, so-called "pre-nucleation clusters", exist in equilibrium with the free ions prior to nucleation.^[13] Similar to ion pairs, these clusters must be considered thermodynamically stable, within the boundary of the single-phase solution system.^[12,13] The presence of associates consisting of up to some tens of ions has been corroborated by means of analytical ultracentrifugation,^[12] and they were later directly imaged in solution utilizing cryo-electron microscopy.^[14,15] Furthermore, the experimentally determined ion binding profiles as well as corresponding thermodynamic parameters almost quantitatively comply with theoretical predictions made for such clusters on the basis of computer simulations,^[16] which showed that Ca^{2+} and CO_3^{2-} ions assemble themselves into highly dynamic chains of alternating cations and anions that are stable in the concentration range of typical experimental settings. This structural form of pre-nucleation clusters has been labeled "dynamically ordered liquid-like oxyanion polymers" (DOLLOPs),^[16] and may be regarded as "living ionomers" resulting from polycondensation reactions^[17] of hydrated calcium and carbonate ions as well as CaCO_3 ion pairs.

In the original work,^[12] cluster formation was evaluated under the assumption that the investigated dilute systems behaved like ideal solutions. Here, these previous analyses are extended to also take into account the role of ionic activity. We demonstrate that ideal treatment is a sustainable approximation for the commonly investigated range of concentrations, and rationalize seeming contradictions in the solubility values reported for amorphous calcium carbonate (ACC) intermediates in the literature. Last but not least, we show that calcium carbonate associates formed in the pre-nucleation stage (that is, ion clusters with a DOLLOP-like structural motif) are by no means "inactive" when it comes to phase separation, but rather represent the truly active and relevant species in this process.

Figure 1 shows the development of Ca^{2+} detected by an ion-selective electrode (ISE) upon continuous addition of CaCl_2 solution into carbonate buffer at pH 9.5 (see Section S1 in the Supporting Information for experimental details). Generally, the amount of free calcium increases linearly until a critical stage is reached (maximum of the curve), where nucleation occurs; subsequently, the Ca^{2+} level drops to a constant value that corresponds to the solubility of the initially formed solid phase.^[16] The different profiles in Figure 1 were obtained by calibrating the Ca^{2+} -ISE using either water (blue curves) or 13 mM sodium chloride (red curve), which emulates the ionic strength of the carbonate buffer at the beginning of the experiments. Under real conditions, measurements based on calibrations in water give calcium activities, while calibrations with

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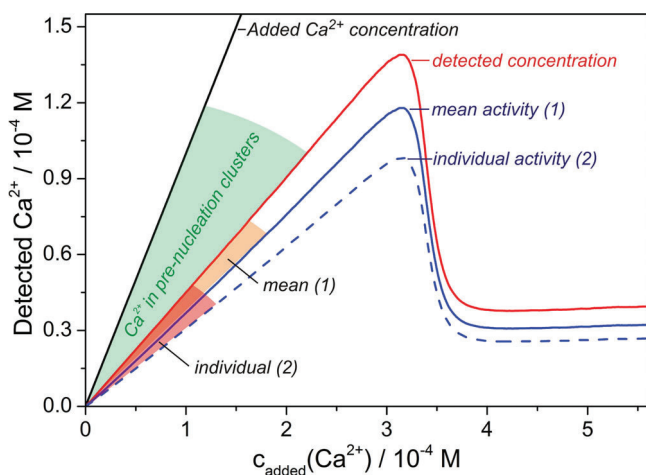


Figure 1. Profiles of the detected Ca^{2+} concentration (red curve), mean Ca^{2+} activity (blue curve), and individual Ca^{2+} activity (dashed blue curve) as a function of the added concentration in 10 mM carbonate buffer at pH 9.5. The difference between added and detected Ca^{2+} can be separated into two distinct contributions, originating from pre-nucleation cluster formation (green wedge) and electrostatic effects (light orange and red areas). The latter can be described by either mean or individual activity coefficients. For explanations see text.

NaCl solutions lead to actual concentrations (see Section 2.1 in the Supporting Information). In both cases, significantly less calcium is detected than added (Figure 1, black line). This confirms that the apparent decrease in the amount of free Ca^{2+} in the carbonate buffer is not simply due to reduced ionic activity, but mainly derives from ion association, i.e. binding of Ca^{2+} in pre-nucleation clusters (green wedge in Figure 1).

Based thereon, we can now quantitatively assess activity effects due to electrostatic interactions in the experiments, following two distinct strategies. Approach 1 relies on the axiom that only average activities are accessible in any physicochemical measurement, owing to the requirement of electroneutrality. In this case, the ratio of concentration (as detected by calibration in NaCl) and mean activity (obtained when calibrating in water) directly gives the mean activity coefficient, γ_{\pm} (see Section S2.1 in the Supporting Information). The resulting fraction of seemingly “inactive” calcium is indicated by the orange wedge in Figure 1. Contrarily, in approach 2, it is assumed that individual calcium activity can indeed be measured by ion-selective techniques, as suggested by some authors,^[18,19] but debated by others.^[20] Here, it is necessary to consider the diffusion potential across the diaphragm of the reference electrode.^[18,19] While the values estimated for this potential are similar in the carbonate buffer and suitable NaCl solutions (cf. Table S1 in the Supporting Information), there are significant differences for the calibration in water, and the measured potentials have to be corrected accordingly (see Section S2.2 in the Supporting Information). As a consequence, the apparent calcium activity is lowered (Figure 1, dashed blue line) and the quotient of the corrected values with the measured Ca^{2+} concentration (red curve in Figure 1) gives the individual activity coefficient for the calcium ions, $\gamma(\text{Ca}^{2+})$. The red wedge in Figure 1 represents the fraction of supposedly “inactive” calcium resulting from this approach.

Experiments at pH 9, 9.25, 9.75 and 10 show analogical behavior (Figure S1 in the Supporting Information), while calibration data corroborate that activity coefficients are included in the electrode intercepts (Section S1.1 and Figure S2 in the Supporting Information). The values obtained for γ_{\pm} and $\gamma(\text{Ca}^{2+})$ at the different pH levels vary within the limits of error (Figure S3 in the Supporting Information), and may thus be regarded pH-independent within experimental accuracy over the studied range. Following approach 1 and 2, we obtain average apparent activity coefficients of $\gamma_{\pm} = 0.84 \pm 0.06$ (mean value \pm maximum deviation) and $\gamma(\text{Ca}^{2+}) = 0.68 \pm 0.05$, respectively. The former value is close to what is expected by general electrolyte theory^[21] for γ_{\pm} of a 2:1 salt (as a rough approximation for the carbonate buffer) at the given ionic strengths ($\gamma_{\pm} = 0.80$ – 0.83 , see Table S2 in the Supporting Information), while the latter agrees well with single-ion activity coefficients calculated using either the Debye–Hückel limiting law ($\gamma(\text{Ca}^{2+}) = 0.64$ – 0.69 ; see Table S3 in the Supporting Information) or conventional solution speciation software ($\gamma(\text{Ca}^{2+}) = 0.60$ – 0.66 ; see Table S4 in the Supporting Information). Thus, both approaches lead to apparent activity coefficients that comply with respective theoretical predictions.

The observation of linear ion binding behavior prior to nucleation (Figure 1) has been used to argue that association is limited to simple ion pairs.^[22,23] This notion was inferred by considering a chemical equilibrium between multiple ions (here $n\text{Ca}^{2+} + n\text{CO}_3^{2-} \rightleftharpoons [\text{CaCO}_3]_n$), for which it can easily be shown that at a large excess of one of the involved ions (CO_3^{2-} in the present experiments), the other (Ca^{2+}) can only be bound with $n = 1$ for linear profiles to be obtained (i.e. the clusters contain only one single calcium). This, however, is not necessarily true if the $[\text{CaCO}_3]_n$ clusters are formed in a sequence of consecutive equilibria, rather than in a single step as assumed above. In a previous study, these multiple equilibria were described quantitatively by introducing the assumption that all binding events are equal and independent.^[12] At first sight, this might seem to be a drastic simplification, which nonetheless is immediately supported by computer simulations that predict linear binding profiles for the formation of DOLLOPs.^[16,24] Indeed, when the equilibrium constant of association is independent of cluster size, binding of ions in ion pairs and higher associates can *per se* not be distinguished from a macroscopic point of view, as the equilibrium $n\text{Ca}^{2+} + n\text{CO}_3^{2-} = [\text{CaCO}_3]_n$ will then reduce to $\text{Ca}^{2+} + \text{CO}_3^{2-} = [\text{CaCO}_3]_{\text{cluster}}$, whereby $[\text{CaCO}_3]_{\text{cluster}}$ represents an ion pair in any of the possible associated states (i.e. single ion pairs and clusters with various aggregation numbers). This complex system of interdependent equilibria can still be analyzed in depth utilizing a model that was initially developed for the quantitative assessment of protein-ligand interactions.^[25] Corresponding evaluations confirm that linear binding profiles are perfectly consistent with the formation of larger clusters,^[12,16] and do not inevitably imply that the associates are limited to include merely one single calcium ion.

Using the above-mentioned model of multiple-binding equilibria, we have evaluated the pre-nucleation regime of the titration curves, in order to derive the equilibrium constants for the formation of an ion pair within a cluster (K_{IP}) and the corresponding free energies (ΔG_{IP}). To that end, we neglect

Table 1. Ion association constants (K_{IP}) for CaCO_3 pre-nucleation clusters derived on the basis of a multiple-binding equilibrium^[12], corresponding free energies of reaction at standard conditions (ΔG_{IP}), and thermodynamic solubility products of the initially precipitated phase (K_{sp}) obtained for different pH levels. Values for pH 9 and 9.5 represent conditions under which pc-ACC is nucleated, whereas the remaining values are associated with the formation of pv-ACC. The given absolute ranges of error were obtained by propagation of the experimental uncertainty in the electrode slopes and intercepts.

pH	K_{IP} [M^{-1}]			ΔG_{IP} [kJ mol^{-1}]			K_{sp} [$\times 10^{-8} \text{ M}^2$]		
	ideal ^{a)}	real (1) ^{b)}	real (2) ^{c)}	ideal ^{a)}	real (1) ^{b)}	real (2) ^{c)}	ideal ^{a)}	real (1) ^{b)}	real (2) ^{c)}
9	1570 ± 200	1190 ± 300	1480 ± 360	-18.2 ± 0.3	-17.6 ± 0.6	-18.1 ± 0.6	2.95 ± 0.19	2.90 ± 0.53	2.32 ± 0.41
9.25	1320 ± 80	1150 ± 240	1450 ± 280	-17.8 ± 0.2	-17.5 ± 0.5	-18.0 ± 0.5	2.97 ± 0.16	2.95 ± 0.52	2.33 ± 0.39
9.5	1200 ± 100	1120 ± 210	1420 ± 250	-17.6 ± 0.2	-17.4 ± 0.5	-18.0 ± 0.4	3.31 ± 0.21	3.37 ± 0.56	2.64 ± 0.42
9.75	1070 ± 100	980 ± 170	1270 ± 220	-17.3 ± 0.2	-17.1 ± 0.4	-17.7 ± 0.4	3.34 ± 0.29	3.32 ± 0.55	2.60 ± 0.41
10	1180 ± 80	1040 ± 190	1380 ± 240	-17.5 ± 0.2	-17.2 ± 0.5	-17.9 ± 0.4	3.32 ± 0.20	3.31 ± 0.60	2.60 ± 0.44

^{a)}Calculated assuming ideality; ^{b)}Calculated according to approach 1 (mean activity); ^{c)}Calculated according to approach 2 (individual activity).

calcium-bicarbonate association, which is reasonable at the given pH levels and concentrations owing to the very weak $\text{Ca}^{2+}/\text{HCO}_3^-$ interaction^[26] (see also Table S4 in the Supporting Information), and then calculate the actual amount of free carbonate ions on the basis of 1:1 binding via mass balance considerations.^[12,16] The values resulting from ideal or non-ideal treatment, according to either approach 1 or approach 2, are compiled in **Table 1**. It is evident that any deviations in K_{IP} and ΔG_{IP} from previously published values, which were obtained under the assumption of ideal solutions,^[12] do not exceed the limits of experimental error. Certainly, the error made when taking the measured mean activity as concentration (ca. 15%, cf. Figure 1) is larger than the typical variance of the titration experiments (<10%, cf. error bars in Figure S1 in the Supporting Information). However, subsequent derivation of thermodynamic parameters almost quantitatively corrects for these deviations, as activities must be used in calculations of non-ideal scenarios ($K_{IP,real} = K_{IP,ideal}/\gamma_{app}^2$, with $\gamma([\text{CaCO}_3]_{cluster}) \approx 1$). This demonstrates that ideal treatment of the titration data is acceptable and gives sound results. Furthermore, it is worth emphasizing that the K_{IP} values listed in Table 1 agree quite well with CaCO_3 ion pairing constants reported in the literature,^[8] and that the experimentally determined fractions of bound Ca^{2+} almost coincide with those predicted by speciation software for $[\text{CaCO}_3]^0$ (see Table S4 in the Supporting Information). Indeed, this is exactly what is expected, as previously the existence of larger clusters has not been considered and all observed binding was simply ascribed the formation of ion pairs. Thus, their stability was systematically overestimated in earlier work, since the corresponding equilibrium constants contain unidentified contributions from higher associates.^[16] In essence, the K_{IP} values obtained herein and elsewhere^[8,27] therefore represent average equilibrium constants for CaCO_3 ion pairs in the different complexed species occurring, weighted according to their relative abundance under the respective conditions. Indeed, by comparing binding data obtained from the titration experiments with estimations of ion distributions in free and bound states based on analytical ultracentrifugation (AUC) measurements, it can be shown that, on time average, the associated fractions of Ca^{2+} and CO_3^{2-} ions reside in entities that are significantly larger than simple ion pairs (see Section 2.3 and Table S5 in the Supporting Information).

Apart from insight into ion association phenomena in solution before nucleation, our titration experiments further allow for a quantitative determination of the solubility of the initially precipitated solid particles. Free ion products ($c_{free}(\text{Ca}^{2+})c_{free}(\text{CO}_3^{2-})$) resulting from the distinct approaches show generally similar developments (i.e. they increase continuously up to the point of nucleation and subsequently drop to a constant level), but differ in terms of absolute values (see Figure S4 and Table S6 in the Supporting Information). Corresponding thermodynamic solubility products for the nucleated phase, calculated from the post-nucleation plateau according to $K_{sp} = \gamma_{app}c_{free}(\text{Ca}^{2+})\gamma_{app}c_{free}(\text{CO}_3^{2-})$, with $\gamma_{app} = 1$ (ideal), $\gamma_{app} = \gamma_{\pm}$ (approach 1), or $\gamma_{app} = \gamma(\text{Ca}^{2+})$ (approach 2), are given in Table 1. In line with earlier studies,^[12] we find that amorphous calcium carbonate is formed initially, with proto-calcite (pc-ACC) and proto-vaterite (pv-ACC) short-range order being observed in the low (9–9.25) and high pH regimes (9.5–10), respectively. Moreover, the correlation between the stability of the pre-nucleation species and the solubilities of the distinct ACCs^[28,29] is seen in all data, regardless of which evaluation method is used. We note that the ionic strength of the solutions does not change markedly during titration and after nucleation (Figure S5 in the Supporting Information), that is, the activity coefficients determined for the initial conditions are applicable over the entire experiment. The solubility products obtained for ACC are virtually identical for ideal and non-ideal evaluation according to approach 1 (cf. Table 1). In turn, approach 2 yields slightly lower values, some of which are just outside the error range with respect to the results from ideal treatment. Nevertheless, the data are fairly consistent and, importantly, the measured solubilities are all much lower than what is documented for ACC in the literature. The most commonly employed values are probably those reported by Brečević and Nielsen^[30] and Clarkson et al.,^[27] who determined $K_{sp}(\text{ACC})$ to $4.0 \times 10^{-7} \text{ M}^2$ and $9.1 \times 10^{-7} \text{ M}^2$, respectively (at 25 °C), both being more than an order of magnitude higher than the solubility products resulting from the titration assays. In fact, phases with such high solubility can generally not be formed in the present experiments, because the free ion products do not reach values $\geq 4.0 \times 10^{-7} \text{ M}^2$ and the solutions thus are undersaturated with respect to these ACC particles at all stages (this is also true when ion activity products are considered). To understand these

discrepancies, it is necessary to compare the methods by which the ACC materials were prepared in the different studies. As pointed out previously,^[31] Clarkson et al. added sodium triphosphate (STP) as a stabilizer for ACC in their solubility measurements.^[27] However, recent work has demonstrated that STP can have a dramatic influence on the early stages of CaCO₃ crystallization even when present at concentrations in the ppm range.^[32] Indeed, solubility products obtained by using the same technique as applied here for ACC generated in the presence of 5 ppm triphosphate are almost identical to the value of Clarkson et al.^[27] This confirms the reliability of the titration data and shows that a K_{sp} of $4 \times 10^{-7} \text{ M}^2$ is not valid for neat ACC, but rather corresponds to a more soluble amorphous form that is stabilized by the triphosphate ions (possibly via incorporation of the additive into the structure of ACC). The solubility product given by Brečević and Nielsen, on the other hand, was determined in the absence of additives in a procedure where ACC was formed at high supersaturation (although actual concentrations are not explicitly quoted in the original work).^[30] Here, we suspect that the resulting high solubility can be rationalized within the notion of polyamorphism in ACC.^[29] That is, when precipitated from concentrated solutions, ACC may occur in a structural modification that is less stable and exhibits a lower degree of short-range order^[33,34] than the proto-structured ACCs^[28] obtained from the dilute systems investigated in our experiments.

We now turn to discuss whether or not the ion associates identified in solution before precipitation can be regarded “inactive” during the actual nucleation process, as envisaged by classical theories. A direct negative answer may be given when considering previous studies, which have evidenced that nucleation occurs through aggregation of pre-nucleation clusters (that contain the bound ions).^[12–15] In the following, we provide further compelling experimental support for the notion that these clusters are active in the nucleation process, based on titrations in the presence of excess salt. **Figure 2** shows the temporal evolution of free Ca²⁺ detected in carbonate buffers that contained different amounts of NaCl. In all cases, the Ca²⁺-ISE was

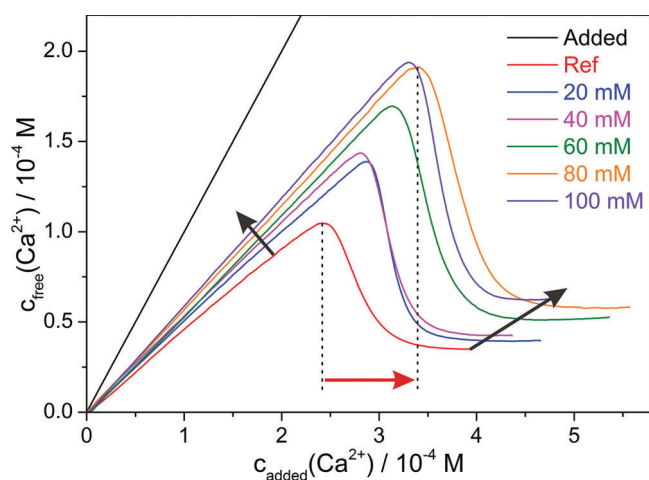


Figure 2. Development of the free Ca²⁺ concentration upon continuous addition of 10 mM CaCl₂ into 10 mM carbonate buffers (pH 9.5) containing different amounts of sodium chloride as indicated.

calibrated in ionic strength-adjusted environments and thus, all curves represent actual calcium concentrations. Obviously, less and less calcium is bound throughout the different stages with increasing salt concentration (Figure 2, black arrows). At the same time, ionic activity is continuously lowered, as demonstrated by the resulting activity coefficients, which again agree well with theoretical values (see Figure S6 in the Supporting Information). The free concentration of Ca²⁺ (and CO₃²⁻) increases with growing ionic strength (and decreasing γ), because $c(\text{Ca}^{2+})c(\text{CO}_3^{2-})\gamma_{\text{app}}^2 = c(\text{CaCO}_3)/K_{\text{IP}}$ – that is, ion dissociation is favored in the presence of excess salt. In order to rule out any other possible reasons for the higher observed levels of free Ca²⁺, we have measured the concentration of free Na⁺ by means of an ISE, showing that sodium does not compete with calcium for the binding of carbonate ions (Figure S7 in the Supporting Information). Comparative studies with a number of different anions further confirm that the nature of the used counter-ion does not affect the outcome of the experiments to any considerable extent (Figure S8 in the Supporting Information). On the other hand, addition of NaCl to the buffer leads to a progressive delay of nucleation (red arrow in Figure 2), which is well reproducible and becomes more pronounced when even higher amounts of salt are added (Figure 3a). Importantly, the degree of nucleation inhibition scales inversely with the fraction of Ca²⁺ bound prior to nucleation (see Figure S9 in the Supporting Information) and furthermore, nucleation occurs at about the same absolute concentration of bound Ca²⁺ at all NaCl contents (Figure 3b). This suggests strongly that a critical concentration of bound CaCO₃ is required for nucleation to take place and, hence, that bound Ca²⁺ is relevant for the process. Otherwise, it would be difficult to explain why nucleation is delayed by excess salt although the actual supersaturation (based on the free ions) is higher than at lower ionic strengths. We note that the presence of NaCl may affect the CO₃²⁻/HCO₃⁻ equilibrium, shifting it towards the side of the carbonate ions^[35] This, however, should lead to a higher fraction of bound calcium, owing to the Ca²⁺/CO₃²⁻ association equilibria, and thus cannot explain the observed effects. Addition of NaCl rather causes enhanced dissociation of CaCO₃ units bound within pre-nucleation clusters, simply due to the reduction of activity with increasing ionic strength. Therefore, more calcium ions are needed at higher NaCl contents to reach the same critical level of bound calcium carbonate as at lower salinity, and hence allow for nucleation to occur. Inhibition of nucleation in the presence of excess salt thus is a purely thermodynamic effect that arises from a decrease in ionic activity, and its influence on pre-nucleation cluster equilibria.

In summary, we have shown that treating aqueous calcium carbonate solutions as ideal systems is a good approximation for the investigated range of concentrations, which does not cause significant errors. Indeed, thermodynamic parameters derived for CaCO₃ pre-nucleation species on the basis of non-ideal evaluations are identical, within experimental accuracy, to those published in previous work,^[12] where data were processed under the assumption of ideality. Indeed, our results are consistent with ion pairing constants reported in the literature;^[8,27] however, we consider the occurrence of clusters significantly larger than ion pairs, since the ionic interaction is strong enough for association to proceed beyond the dimer,

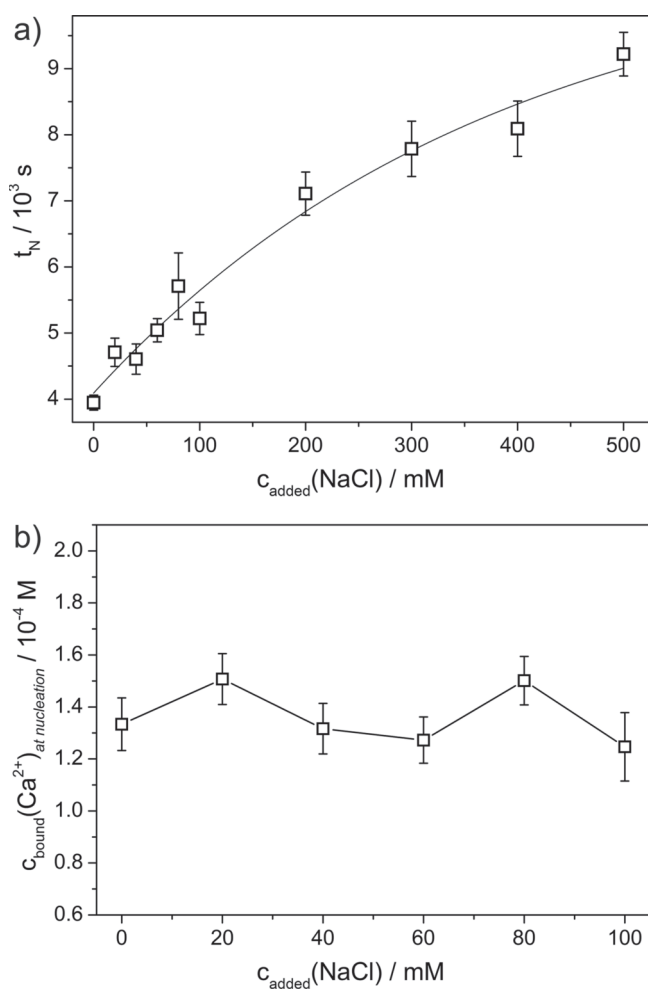


Figure 3. a) Average nucleation times (t_N , $\pm 1 - \sigma$ standard deviations, $N \geq 3$) observed for CaCO_3 crystallization in the presence of distinct concentrations of excess NaCl at pH 9.5. It is evident that additional salt can considerably delay nucleation. Note that the curve connecting the data points is only a guide for the eye and does not represent any physicochemical model. b) The concentration of bound Ca^{2+} at the point of nucleation in NaCl-containing carbonate buffers. The obtained values are constant within the limits of experimental error, indicating that there is a critical amount of bound calcium ions for nucleation to occur in solution. This is clear evidence that bound Ca^{2+} and, thus, pre-nucleation clusters are relevant for the nucleation process.

leading to the formation of dynamic ionic polymers (DOLLOPs).^[16] This notion has direct relevance for basic electrolyte theories, and moreover bears fundamental analogy to the early work of Tanford on surfactant equilibria and self-assembly (micellization) in aqueous solutions.^[36] In this respect, the phenomenon of higher association appears to be general for ionic solutes that maintain sufficiently strong interactions – with important consequences for industrial applications, including the design of novel antiscaling strategies as well as bottom-up approaches for the controlled synthesis of nanoparticles in such systems.

Regarding the initially nucleated amorphous phase, we find that the ACC particles formed at the moderate supersaturation

levels prevailing in our titration assays exhibit thermodynamic solubility products that are about one order of magnitude lower than those determined in earlier studies, where ACC was produced in presence of additives^[27] or at very high supersaturation.^[30] This supports the idea of polyamorphism in amorphous calcium carbonate, i.e. that ACC can exhibit different structures with distinct degrees of (meta)stability and hence also variable solubilities. An important consequence of these circumstances is that one must be careful when excluding the initial nucleation of ACC in systems that are nominally undersaturated with respect to the amorphous phases described in the literature. ACC can obviously be formed at much lower ion activity products than previously thought, because more stable types of ACC exist that have drastically lower solubility. This finding has essential implications for the fields of bio- and biomimetic mineralization, where the role of ACC as a precursor to crystalline calcium carbonate is extensively discussed.^[37–39] Finally, our experiments have demonstrated that ions bound in the pre-nucleation stage should not be regarded as “inactive” during the onset of precipitation. While this conclusion has already been drawn in previous studies,^[12,13] the present work provides direct evidence that a decrease in ionic activity leads to effects that cannot be explained within the classical picture of nucleation, but are very well consistent with the concept of stable pre-nucleation clusters. Under the given conditions, these clusters appear to play a key role in the nucleation process and therefore must be accounted for when formulating advanced nucleation theories.

Acknowledgements

The authors thank Dr. Roland Neueder (University of Regensburg) and Dr. Luc Nicoleau (BASF Construction Chemicals) for valuable discussions and suggestions. Rose Rosenberg (University of Konstanz) and Antje Völkel (MPI Gollm) are acknowledged for help with AUC experiments and data evaluation. M.K. is grateful to BASF SE for funding of a postdoc position. D.G. is a fellow of the Zukunftscolleg of the University of Konstanz and supported by the Fonds der Chemischen Industrie.

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