Impact of Sodium Polyacrylate on the Amorphous Calcium Carbonate Formation from Supersaturated Solution

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Supporting Information

ABSTRACT: A detailed in situ scattering study has been carried out on the formation of amorphous calcium carbonate (ACC) particles modulated by the presence of small amounts of sodium polyacrylate chains. The work is aiming at an insight into the modulation of ACC formation by means of two polyacrylate samples differing in their molecular weight by a factor of 50. The ACC formation process was initiated by an in situ generation of CO₃²⁻ ions via hydrolysis of 10 mM dimethylcarbonate in the presence of 10 mM CaCl₂. Analysis of the formation process by means of time-resolved small-angle X-ray and light scattering in the absence of any additives provided evidence for a monomer addition mechanism for the growth of ACC particles. ACC formation under these conditions sets in after a lag-period of some 350 s. In the presence of sodium polyacrylate chains, calcium polyacrylate aggregates are formed during the lag-period, succeeded by a modulated ACC growth in a second step. The presence of anionic polyacrylate chains changed the shape of the growing particles toward loose and less homogeneous entities. In the case of low amounts (1.5−7.5 mg/L) of the long chain additive with 97 kDa, the size of the aggregates is comparable to the size of the successively formed hybrid particles. No variation of the lag-period has been observed in this case. Use of the short chain additive with 2 kDa enabled increase of the additive concentration up to 100 mg/L and resulted in a significant increase of the lag-period. This fact, together with the finding that the resulting hybrid particles remained stable in the latter case, identified short chain sodium polyacrylates as more efficient modulators than long chain polyacrylates.

INTRODUCTION

Formation of inorganic solids from supersaturated solutions is a widespread phenomenon in industry, household, and nature. The field includes topics as diverse as scale formation in pipes for hot water and CaCO₃ deposition on textiles during laundry or the formation of protective shells for living organisms. In the first two examples, undesired deposits are formed, which may be avoided by using water-soluble polymeric additives.¹−³ The latter example is a typical biomineralization process.⁴ Solid formation in biomineralization is usually controlled by biopolymers, which generate structural textures on nanometer and micrometer scales.⁵−⁷ These specific textures are responsible for the final performance of the resulting natural materials. Hence, the prospect of control of such solid formation is a constant source of motivation in research.

One of the most thoroughly investigated systems is the precipitation of CaCO₃. As in many other cases, formation of solid CaCO₃ from supersaturated aqueous solution usually does not lead directly to a thermodynamically stable, crystalline solid but follows Ostwald’s step rule.⁸ Amorphous CaCO₃ (ACC) as the metastable phase with the highest solubility is formed first.⁹,¹⁰ Rieger et al. could provide evidence that the transformation into more stable phases takes place via a redissolution step of ACC.¹¹ The redissolving ions successively form calcite crystals whereby the disappearing ACC exhibits a granular structure with constituent ACC particles smaller than 100 nm.

Further details on the mechanism of ACC formation were provided by SAXS¹²,¹³ and by electrochemical experiments,¹⁴ which suggest the following mechanism for CaCO₃ formation. In a prenucleation stage, the calcium and carbonate ions are in thermodynamic equilibrium with small clusters. Once the overall CaCO₃ concentration exceeds a critical concentration, nucleation sets in, whereby, most likely, clusters turn into nuclei.¹⁴ Further evidence for the occurrence of clusters could be provided by ESI-MS experiments, which indicated multitudes of CaCO₃ units.¹⁵ Nucleation induces the growth of sphere-like ACC particles. In a concentration regime of 3.5 mM ≤ [CaCO₃] ≤ 4.5 mM, these growing particles are extremely monodisperse in size and approach a distinct finite size.¹² The
final size value as well as its extent of polydispersity increases with increasing overall CaCO₃ concentration. The mechanism of particle growth follows a monomer addition mechanism with the monomers corresponding to either small clusters and/or to the constituent ions.

It has meanwhile been emphasized in several articles that the ACC phase adopts a key role in the process of biomineralization. Due to its high solubility and amorphous nature, ACC may react specifically on the impact of structure directing agents like water-soluble polymers and hence can readily be guided into subcellular compartments and molded into a mineral with hierarchical structure and interesting material properties.

An impressive set of data has been collected on the mode of action of polymeric additives. Significant progress came along with the polymer-induced liquid precursor process (PILP) introduced by Gower et al. They added CO₃²⁻ ions to aqueous 20 mM CaCl₂ with 0.02 μg/L polysapartate and observed the separation of an amorphous liquid phase. The amorphous phase transformed into crystalline phases, retaining the shape of the original amorphous domains. This polymer-assisted phase formation is thus of great relevance for the crystallization in confined compartments during biomineralization. The polymers are incorporated into the ACC with the extent of incorporation depending on the polymer concentration and on the age of the phase. Incorporation of the polymeric additives increases the lifetime of this phase thereby providing enough time to mold the material into morphological textures of living systems. If additives are applied in similar amounts as CaCO₃, neutron scattering experiments with appropriate contrast variation revealed that even calcite crystals include a considerable amount of polymeric additive.

Sodium polyacrylate (NaPA) with its regular array of anionic COO⁻ residues is considered to be a particularly interesting system for investigations of the impact of such polymeric additives on the process of CaCO₃ mineralization. It is widely used as scale inhibitor in technical applications and has model character for structure directing agents in biomineralization. Rieger et al. to begin with, indicated that a copolymer of acrylate and maleate is able to fully stabilize ACC precipitates if the concentration exceeds some 0.05 g/L. The ACC occurred as particles smaller than 50 nm, which were embedded in a network of interconnected NaPA chains. DiMasi et al. later reported on the NaPA assisted formation of an amorphous CaCO₃ film on top of a layer of arachidic acid. X-ray reflectivity experiments indicated that NaPA coils prolonged the lifetime of the ACC film but did not influence the rate of film growth. This effect was attributed to a rough layer of NaPA coils, which acts as a reservoir for Ca²⁺ ions and which confines the ACC film to an interstitial layer bordered by the arachidic film on the opposite side. In close analogy to this NaPA assisted mineral phase formation, Chujo et al. demonstrated an interesting interplay between water-soluble NaPA anions and Ca²⁺ ions, which modifies the mineralization of CaCO₃. If NaPA was added after Ca²⁺ had been mixed with CO₃²⁻ ions, vaterite particles could be generated, which were stable for more than 30 days. If the NaPA was mixed with the Ca²⁺ solution at 2.75 mM ≤ [Ca²⁺] ≤ 5.5 mM prior to combination with CO₃²⁻ ions at a ratio of [COO⁻]/[Ca²⁺] ≥ 0.1, nucleation of crystalline CaCO₃ was inhibited but nanometer-sized ACC particles were formed instead. Successively, the authors had a closer look into the impact of premixed CaPA solutions and developed a controlled addition method to generate stable ACC particles, preferably with a NaPA sample of a molar mass of 5 kD. The size of the stabilized ACC particles could be controlled by the equilibration time of premixed CaCl₂ and PA in solution prior to the addition of ammonium carbonate solution. The resulting CaPA particles got larger with increasing equilibration time and retained their size while being transformed into ACC particles. The mechanism could consistently be explained by a particle—particle agglomeration of CaPA colloids induced by the Ca²⁺ ions. Direct evidence for the formation of CaPA particles via agglomeration could indeed be provided by time-resolved static light scattering experiments on a high molecular weight NaPA sample. As suggested by Chujo et al., the preformed CaPA domains incorporate CO₃²⁻ ions, thereby acting as reactors for the ACC formation. This may be attributed to the large amount of NaPA (corresponding to 2 mM of carboxylate residues) applied in comparison to the CaCO₃ content (5 mM) in solution. An increased stability of the ACC particles with reduced equilibration time of CaPA was attributed to a lower amount of bidentate PA-Ca²⁺ complexes, which gradually replace the less well ordered, intermediate PA-Ca²⁺-OH₂ complexes.

Further information on the impact of NaPA was provided by Colfén et al. who used an ion selective electrode to follow the evolution of the Ca²⁺ ion concentration while titrating a solution of Na₂CO₃ in the absence and presence of NaPA. In the absence of NaPA, the increase of the amount of dissolved Ca²⁺ ions lay below the trend expected from the dosed amount. This discrepancy was interpreted by the formation of CaCO₃ clusters, which eventually formed nuclei. Addition of NaPA chains introduced an offset, which increased with the NaPA concentration and which indicated partial removal of Ca²⁺ ions via sequestration. The gradient of the increasing Ca²⁺ concentration was not affected by the presence of NaPA chains. More important, the appearance of nucleation which caused a breakdown of the Ca²⁺ content in solution was significantly delayed. Apparently, the NaPA coils did not interfere with the cluster formation but protected the CaCO₃ clusters, thereby postponing nucleation toward higher Ca²⁺ concentrations.

Taken together, these results suggest a complex pattern of pathways along which NaPA colloids may influence the formation of ACC particles. It remains to be clarified which condition or additive property favors a certain mode of interaction. To give but an example, Chujo et al. could establish that preformed CaPA aggregates act as templates for the formation of ACC-PA hybrid particles. However, even if we restrict ourselves on this detailed aspect, we still do not know whether this is a general feature of NaPA assisted ACC formation or whether this only holds at fairly high NaPA concentrations.

At this point, we would like to draw the reader’s attention to a work by Wegner et al. who applied a procedure to form ACC, which is of direct relevance to the present work. In this procedure, CaCO₃ formation was initiated by an in situ generation of CO₃²⁻ ions via hydrolysis of the dialkylcarbonates. Hydrolysis requires a significant lag-time to produce a CO₃²⁻ ion concentration, high enough to hit the solubility limit of ACC. Thus, the advantage of this procedure is twofold: (i) it avoids inhomogeneities, which inevitably occur during the initial stages of direct mixing of component solutions, i.e., CaCl₂ and Na₂CO₃; (ii) the gradual approach of a supersaturated state may be closer to application conditions than an instantaneous supersaturation via rapid mixing of component solutions. It is these advantages which
motivated us to subject the ACC formation induced by an ester hydrolysis\textsuperscript{28,29} to a time-resolved static light scattering (TR-SLS) investigation. In using an instrumental development by Becker and Schmidt,\textsuperscript{30} we could indeed successfully demonstrate applicability of TR-SLS onto ACC formation in a preceding work.\textsuperscript{31} ACC formation was carried out via the hydrolysis of the dimethyl ester of carboxylic acid (DMC) at a DMC concentration of 10 mM in the presence of a Ca\textsuperscript{2+} concentration of 10 mM. The process was initiated by the addition of a variable amount of NaOH, which catalyzed the ester hydrolysis. Under such conditions, the lag-time lasted a few hundred seconds and the successive nucleation and growth could be followed over at least 200 s, resulting in the time-resolved evolution of the particle mass and size.\textsuperscript{31} Correlation of these data yielded power laws, which indicated a growth of compact particles according to a monomer addition mechanism.\textsuperscript{31}

The present work extends this investigation of ACC formation induced by DMC hydrolysis to the growth of ACC particles in the presence of NaPA. First, we reproduce our earlier results on the formation process in the absence of any additives whereby we use additional time-resolved SAXS experiments. Compared to light scattering, SAXS extends the regime of momentum transfer giving access to full scattering curves and improves the time resolution to the regime of 100 ms. The results serve as a reference system for the second part of the present work, reporting on ACC formation experiments in the presence of NaPA as additives. For this NaPA directed ACC formation, we will analyze the impact of additive concentration, which covers a regime of 0 \( \leq c_{\text{PA}} \leq 100\) mg/L. Application of two NaPA samples with molar mass values of 2 kDa and 97 kDa provides a first insight into the impact of molar mass of the NaPA additive.

### Experiments and Data Evaluation

**Materials.** Calcium chloride hexahydrate CaCl\textsubscript{2}-6H\textsubscript{2}O (Assay \( \geq 99\%\)) and sodium carbonate Na\textsubscript{2}CO\textsubscript{3} (Assay \( \geq 99\%\)) was purchased from Fluka (Buchs, CH). The dimethyl ester of the carboxylic acid denoted as DMC (Assay \( \geq 99\%\)) stemmed from Acros Organics (New Jersey, USA). Sodium hydroxide (Assay \( \geq 99\%\)) was supplied by Merck (Darmstadt, FRG). Bidistilled water (conductivity below 0.1 \( \mu \text{s/cm} \)) served as solvent. Two different sodium polycrylate samples have been used as polymeric additive. PAkD2 is a commercial product from BASF with a molecular weight close to 2 kDa. An aqueous solution of the raw material was dialyzed against distilled water for two days at pH = 10 and for two days at pH = 8. The surrounding water was exchanged twice per day. Dialysis was performed with THOMAPOR standard hoses (type RCT 88) surrounding water was exchanged twice per day. Dialysis was performed with THOMAPOR standard hoses (type RCT 88) and the dimethylester of the carboxylic acid (DMC) (10 mM) and (ii) an aqueous NaOH solution, were required. Calcium chloride and DMC were dissolved in double distilled water, both at a concentration of 20 mM. Sodium hydroxide was prepared in a separate solution at a concentration of 40 mM.

Five milliliters of each component solution were filtered into the scattering cell through nonsterile Millex-GS filters with a pore width of 0.22 \( \mu \text{m} \) (MF-Millipore Membrane, mixed cellulose esters) from Millipore Corp. (Bedford, MA USA). Combination of the CaCl\textsubscript{2}/DMC solution with the NaOH solution in the scattering cells fixed time zero of the respective particle growth experiments. In experiments where a polymeric additive was used in addition, it was included in the aqueous NaOH solution. The additive concentrations of sample PAkD2 covered a range of 26–100 mg/L. With sample Paa85k, this range had to be confined to 1.5–26 mg/L.

**Time-Resolved Static Light Scattering (TR-SLS).** Cylindrical quartz glass cuvettes with a diameter of 20 mm from Hellma (Müllheim, FRG) were used as scattering cells. The scattering instrument is a home-built goniometer with a He–Ne laser as light source, operating at a wavelength of 633 nm. It enables us to simultaneously record time-resolved static scattering at \( 2 \times 19 \) fixed angles\textsuperscript{30} arranged in pairs on both sides of the beam symmetrically and covering a regime of 25.84° < \( \theta < 143.13\)°. The final concentration of the Ca\textsuperscript{2+} and DMC was 10 mM and the final NaOH concentration was 20 mM. Filtration of the NaOH solution as the second component into the scattering cell defines time zero of each time-resolved particle growth experiment. Recording of an angular dependent curve takes 2 ms. Each scattering curve was based on 100 recordings requiring 0.2 s as actual measurement time. Time interval between two succeeding curves was 2 s. Measuring temperature was 25 °C.

**Processing of the Scattering Curves.** Scattering curves were recorded as the Rayleigh ratio of the aggregates \( \Delta R_0 \) which was calculated from the difference between the Rayleigh ratio of the particle solution and of the solvent at variable scattering angle \( \theta \). In the course of a growth process, the recorded scattering intensity may increase considerably. This increase occasionally causes an overflow, preferentially at one of the detectors at low scattering angles. Once such an overflow occurred, the signal of the respective detector had to be discarded from the time of its first appearance onward.

In the regime of low momentum transfer \( q \) of scattered light, scattering curves can be approximated according to the following series expansion

\[
\frac{Kc}{\Delta R_0} = \frac{1}{M_w} \left[ 1 + \frac{R_g^2}{3} \left( \frac{q}{q_s} \right)^2 \right] + A_2 c
\]  

(1)

with \( K \) the contrast factor of ACC in solution, \( \Delta R_0 \) the Rayleigh scattering of the ACC particles at the scattering angle \( \theta \), \( c \) the concentration of CaCO\textsubscript{3} in g/L, \( M_w \) the weight averaged molar mass, and \( R_g^2 \) the z-averaged squared radius of gyration of the growing particles and \( A_2 \) the second osmotic virial coefficient. The contrast factor

\[
K = \frac{4\pi^2 n^2}{N_A \lambda^4} \left( \frac{dn}{dc} \right)^2
\]  

(2)

and the momentum transfer

\[
q = \frac{4\pi n}{\lambda} \sin(\theta/2)
\]  

(3)

was calculated with \( N_A \) Avogadro’s number, with \( \lambda = 633 \) nm the laser wavelength, with \( n = 1.333 \) the refractive index of H\textsubscript{2}O as the solvent, and with \( dn/dc \) the refractive index increment of ACC in water. A value of \( dn/dc = 0.1 \text{ mL/g} \) was estimated for

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\textsuperscript{51} In Situ Formation of Carbonate by Ester Hydrolysis.\textsuperscript{28,29} Two solutions, (i) an aqueous solution including Ca\textsuperscript{2+} ions and the dimethylester of the carboxylic acid (DMC) and (ii) an aqueous NaOH solution, were required. Calcium chloride and DMC were dissolved in double distilled water, both at a concentration of 20 mM. Sodium hydroxide was prepared in a separate solution at a concentration of 40 mM.

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amorphous calcium carbonate by means of a time-resolved dn/dc experiment (Jianke Instrument Ltd., China) in a concentration regime of 0.3 g/L < c < 1.0 g/L induced by mixing appropriate Na₂CO₃ solutions with CaCl₂ solutions at 10 °C at 8 different concentrations against the respective NaCl solution as background solvent. Stable dn/dc values were observed 30 s after mixing the component solutions. Appropriate correction of the ACC concentration for soluble CaCO₃ was done by using a solubility product of 4 × 10⁻⁷ (mol/L)² for ACC⁹ and a dn/dc value of 0.259 g/mL for soluble CaCO₃ salt. The CaCO₃ concentration and the concentration of ACC particles depend in a complex way on the growth time and an appropriate extrapolation of eq 1 to c = 0 is impossible. However, neglect of the concentration dependent term in eq 1 seems to be justified in light of the very low concentrations applied in the present work.

In almost all cases, the particles were denser than linear polymer coils and their scattering curves showed an upturn in the Zimm representation.³³ Therefore, an alternative data evaluation by means of a Guinier plot

\[
\log \left( \frac{\Delta R_g^2}{Kc} \right) \cong \log \left( \frac{\Delta R_\theta=0^2}{Kc} \right) - \frac{R_g^2}{3} q^2
\]  

turned out to be more appropriate and was applied out through the present work. The q-regime used for the Guinier analysis covered a range of 3.5 × 10⁻⁵ nm⁻² < q² < 2.45 × 10⁻⁴ nm⁻², which kept the upper limit of qRg below 2.5 even for the largest-sized values reached. This ensures that the established Rg values can still be considered to be reliable estimates.²⁵

**Time-Resolved SAXS Experiments.** Formation of ACC particles was analyzed by time-resolved SAXS at the ID02, in combination with a stopped-flow device.³⁵ The stopped-flow apparatus (SFM-3, from BioLogic, Pont de Claix, France) was equipped with 3 motorized syringes, which could be independently controlled by computer. Separate NaOH solutions were prepared at a concentration of 50 mM, 40 mM, 32 mM, and 25 mM, respectively. Each of these solutions was mixed with a solution, which includes CaCl₂ and the dimethyl ester of carboxylic acid (DMC). Equal amounts of both solutions were mixed at a flow rate of 2 mL/s. The X-ray wavelength was 0.995 Å and the sample to detector distance 8 m. Acquisition time for a single frame was 20 ms.

**Processing of the Scattering Curves from Time-Resolved SAXS.** SAXS intensities were calibrated by means of the known standard scattering of water at 25 °C corresponding to a differential scattering cross section of dΣ/dΩ = 1.6 × 10⁻³ mm⁻¹.³⁵ Accordingly, all original scattering curves were expressed as differential scattering cross sections I(q) = dΣ/dΩ(q) in units of reciprocal mm versus momentum transfer q with q defined by eq 3. Intercept and initial slope of each scattering curve is proportional to the apparent weight averaged molar mass and the z-averaged squared radius of gyration, respectively, and is established by means of a Guinier fit (following eq 4) to the scattering curves in the regime of 0.0011 nm⁻¹ < q < 3/Rg. The upper q-limit was thereby adjusted in order to ensure that qRg kept below 3. Accordingly, this upper q-limit decreased and with it the number of data points used for a fit as the particle size increased.

**Correlation between Particle Mass and Particle Size.** TR-SLS and TR-SAXS enable the simultaneous recording of the mass and size of growing particles. A correlation between these two parameters bears structural and kinetic information. A simple topological consideration results in a scaling law of the following type

\[ R_g = KM_w^{\alpha} \]

with a shape-sensitive exponent α. For self-similar structures like spheres, Gaussian coils and infinitely thin rods, this exponent is α = 1/3, α = 1/2, and α = 1, respectively. In a kinetic experiment, based on time-resolved scattering experiments the situation usually is more complex due to a bias in the concentration c of the scattering particles used in eqs 1 and 4 and—in case of a variable polydispersity—due to the different ways polydispersity affects the z-averaged R_g² and the weight averaged M_w. The mass from SLS stems from the intercept ΔRₜₐₕ which corresponds to the product M_w.c. Both factors in this product in general refer to all solute species including constituting ions, small multitudes of CaCO₃ units, and clusters, which may all act as monomers. In the discussion to follow, we thus use the term “monomer” for any of these low mass species. The concentration c is a constant value known a priori from the weight of the solute and therefore is not capable of discriminating between varying fractions of monomers and growing particles during the growth process. The implications thereof shall be illustrated with two limiting situations:

1. In simple monomer-addition growth, monomers with the same scattering contrast as the growing polymeric particles are assumed to be gradually incorporated into the growing particles. The monomer fraction decreases while the particle fraction increases, forming a bimodal system with changing composition. The solid concentration c includes both fractions indiscriminately. Beyond 20% of monomer consumption, a plot of the square root of the z-averaged size versus the weight averaged mass approaches a power law with an exponent that is just half of the value corresponding to the respective pure self-similar particles.³¹ Along this argumentation, infinitely thin rods decrease their exponent from α = 1.0 to 0.5, ideally flexible polymer coils from α = 0.5 to 0.25, and regular spheres from α = 0.33 to 0.165.

2. In a second model, growth occurs via coalescence of particles, which obey Brownian dynamics. The time-dependent evolution of particle size and its distribution are provided for spherical particles by the approximate solution of the respective differential equations by v. Smoluchowski.³⁶ Application of the v. Smoluchowski model leads to a fast increase of the polydispersity index from 1 toward a plateau value at 2.³¹ Once the degree of polydispersity is constant, the exponent α in the size versus mass correlation approaches the value of 1/3 for growing compact spheres or cubes. In this growth model, distinction between monomers and particles becomes obsolete and the value of c of total solid determined by weight refers to the concentration of the coalescing particles. The system can thus be considered to include particles with a more or less broad distribution in size. As a consequence, there is no bias in M_w,c in this case, since M_w,c is the weight averaged mass of the particle ensemble.

The bias with respect to c in the case of a bidisperse system as occurs in a monomer addition model can be resolved with the so-called Porod invariant, if the following conditions are fulfilled.
such a case, time-resolved evolution of the OH⁻ ions and of the methyl ester residues. At equimolar amounts, a lag-time of 360 ± 20 s has been established. After this time, the accumulated CO₃²⁻ concentration had generated a level of supersaturation which has been sufficient for the formation of ACC. Further kinetic analysis revealed that this time is within experimental uncertainty indistinguishable from the time required to reach the solubility product. In a preceding work applying TR-SLS, a first indication could be provided for a monomer addition process as the growth mechanism for ACC formation. The shape of the growing particles was sphere-like with a narrow distribution of the particle size. This growth is thus similar to the process observed after a fast mixing of a CaCl₂ solution with a Na₂CO₃ solution. In the first part of the present work, TR-SLS is combined with small-angle X-ray scattering. This extension is aiming at a more detailed description of the ACC particle formation induced by the hydrolysis of DMC in the absence of additives. As in the preceding work, TR-SLS experiments are initiated by hand mixing also in the present study, requiring a few seconds. Contrary to the TR-SLS, TR-SAXS are performed with a stopped-flow device, which better defines the starting conditions. The reason for an additional time-resolved SAXS in combination with a stopped-flow device is twofold: (i) Since a lag-phase along the ester-hydrolysis precedes the ACC formation, it seems very likely that the process triggered via mixing the component solutions by means of a stopped-flow device does not differ from the process initiated by mixing the component solutions sequentially by hand. Consequently, SAXS applied with a stopped-flow device is expected to demonstrate that the mode of mixing does not influence particle formation which follows in situ ester hydrolysis, thereby confirming our earlier findings established by TR-SLS. (ii) We intended to extend these earlier findings now with time-resolved SAXS as an alternative method. SAXS enables a full analysis of form factors and makes accessible the invariant QΔp, which helps to further confirm the established mechanism of ACC formation. Finally, combined TR-SAXS and TR-SLS provide an excellent reference system, which is a crucial prerequisite for a successful investigation of the impact of NaPA on the very process.

We applied four different NaOH concentrations to initiate hydrolysis of DMC, i.e., 25 mM, 20 mM, 16 mM, and 12.5 mM [NaOH], and analyzed the resulting process with TR-SAXS measurements. In the case of 20 mM [NaOH], we redid in addition TR-SLS experiments, as [NaOH] = 20 mM is the stoichiometric amount necessary to hydrolyze all ester residues and as all TR-SLS experiments performed in the presence of NaPA will also be applied at [NaOH] = 20 mM. Figure 1

Figure 1. Time-resolved SAXS experiments on ACC formation induced by DMC hydrolysis at T = 25 °C at variable NaOH concentration. The CaCO₃ content in all cases is 10 mM. The symbols indicate NaOH concentrations as follows: ( △ ) 25 mM; ( ○ ) 20 mM; ( ◊ ) 16 mM; ( □ ) 12.5 mM. An additional TR-SLS experiment at 20 mM NaOH ( ● ) compares light scattering data directly with SAXS results. Part A represents the evolution of the apparent molar mass as \( \bar{M}_p \) versus t; Part B the size as \( R_g \) versus t and Part C the mass fraction of ACC as \( Q_p \) versus t.

The Porod invariant \( Q \) in general is calculated as the integral

\[
Q = \int q^4 I(q) dq
\]

and stems from the intensity of all scattering species. The actually recorded scattering curves inevitably miss an initial \( q \)-regime between \( q = 0 \) and \( q_1 \) with \( q_1 \) the first \( q \)-value accessible to the SAXS detector. As long as the corresponding section of the integral in eq 6 stays far enough to the left of the maximum value of \( I(q)q^4 \), it can be satisfactorily estimated by the triangle \( q_1 [I(q_1) - I_m] \)/2. An example is outlined in the Supporting Information. As the monomers (due to their small size) contribute as \( q \)-independent scattering in the \( q \)-regime used for the integral in eq 6, appropriate subtraction of this contribution denoted as \( I_m \) has to be performed in addition. The resulting experimental estimation of the invariant reads

\[
Q_p = q_1 [I(q_1) - I_m]/2 + \int_{q_1}^{q_2} [I(q) - I_m]4\pi q^2 dq
\]

with \( q_1 \) and \( q_2 \) the lower and upper limit, respectively, of the SAXS detector. This \( Q_p \) can be attributed to the mass fraction of ACC particles formed on the expense of the monomers. In such a case, time-resolved evolution of \( Q_p \) provides an adequate measure of the increase of the particle mass fraction in situ for a monomer addition process.

RESULTS: THE PARTICLE FORMATION FROM HOMOGENEOUS CARBONATE RELEASE

Particle formation induced by the in situ generation of the carbonate ions is based on two component solutions. A NaOH solution is added to a solution, which includes the calcium ions and the DMC. The OH⁻ ions initiate the ester hydrolysis, thereby triggering particle formation. In all experiments, we applied a concentration of 10 mM both for CaCl₂ and DMC. Hydrolysis was induced at various NaOH concentrations including a concentration of 20 mM. The latter corresponds to equal molar amounts of the OH⁻ ions and of the methyl ester residues. At equimolar amounts, a lag-time of 360 ± 20 s has been established. After this time, the accumulated CO₃²⁻ concentration had generated a level of supersaturation which had become sufficient for the formation of ACC. Further kinetic analysis revealed that this time is within experimental uncertainty indistinguishable from the time required to reach the solubility product. In a preceding work applying TR-SLS, a first indication could be provided for a monomer addition process as the growth mechanism for ACC formation. The shape of the growing particles was sphere-like with a narrow distribution of the particle size. This growth is thus similar to the process observed after a fast mixing of a CaCl₂ solution with a Na₂CO₃ solution. In the first part of the present work, TR-SLS is combined with small-angle X-ray scattering. This extension is aiming at a more detailed description of the ACC particle formation induced by the hydrolysis of DMC in the absence of additives. As in the preceding work, TR-SLS experiments are initiated by hand mixing also in the present study, requiring a few seconds. Contrary to the TR-SLS, TR-SAXS are performed with a stopped-flow device, which better defines the starting conditions. The reason for an additional time-resolved SAXS in combination with a stopped-flow device is twofold: (i) Since a lag-phase along the ester-hydrolysis precedes the ACC formation, it seems very likely that the process triggered via mixing the component solutions by means of a stopped-flow device does not differ from the process initiated by mixing the component solutions sequentially by hand. Consequently, SAXS applied with a stopped-flow device is expected to demonstrate that the mode of mixing does not influence particle formation which follows in situ ester hydrolysis, thereby confirming our earlier findings established by TR-SLS. (ii) We intended to extend these earlier findings now with time-resolved SAXS as an alternative method. SAXS enables a full analysis of form factors and makes accessible the invariant \( Q \), which helps to further confirm the established mechanism of ACC formation. Finally, combined TR-SAXS and TR-SLS provide an excellent reference system, which is a crucial prerequisite for a successful investigation of the impact of NaPA on the very process.

We applied four different NaOH concentrations to initiate hydrolysis of DMC, i.e., 25 mM, 20 mM, 16 mM, and 12.5 mM [NaOH], and analyzed the resulting process with TR-SAXS measurements. In the case of 20 mM [NaOH], we redid in addition TR-SLS experiments, as [NaOH] = 20 mM is the stoichiometric amount necessary to hydrolyze all ester residues and as all TR-SLS experiments performed in the presence of NaPA will also be applied at [NaOH] = 20 mM. Figure 1

Figure 1. Time-resolved SAXS experiments on ACC formation induced by DMC hydrolysis at T = 25 °C at variable NaOH concentration. The CaCO₃ content in all cases is 10 mM. The symbols indicate NaOH concentrations as follows: ( △ ) 25 mM; ( ○ ) 20 mM; ( ◊ ) 16 mM; ( □ ) 12.5 mM. An additional TR-SLS experiment at 20 mM NaOH ( ● ) compares light scattering data directly with SAXS results. Part A represents the evolution of the apparent molar mass as \( \bar{M}_p \) versus t; Part B the size as \( R_g \) versus t and Part C the mass fraction of ACC as \( Q_p \) versus t.
SAXS experiment at [NaOH] = 20 mM also with the respective TR-SLS experiment. The apparent molar mass is expressed as the intercept $I_0$ of the scattering curves and the particle size $R_g$ is derived from the initial slope of the scattering curves. The invariant $Q_0$ is established according to eq 7 and can be attributed to the mass fraction of ACC particles formed at the expense of monomers.$^{16}$ The latter parameter is only accessible from SAXS. The increase of the NaOH concentration decreases the lag-time, in agreement with the light scattering results established earlier.$^{31}$ A graphical comparison of lag-times is shown in the Supporting Information. In order to enable direct establishment of the relation between the number density, which can also be established by means of $Q_0$, with $Q_0/Q_p$, the scattering data had to be aligned with the corresponding SAXS values by multiplication with a constant shift factor. As is demonstrated in Figure 1, full agreement of the results from both techniques is recovered.

Further insight into the mechanism of particle formation is provided by correlating the radii of gyration with the mass values according to eq 5. The resulting exponent $\alpha$ of such a correlation depends on the mechanism of particle growth and on the procedure with which the values for the mass $M$ had been established.$^{16,31}$ Two alternative routes are available for the mass values: (i) the intercepts $I_0$ in Figure 1 are used as received and correspond to a weight average, which comprises all CaCO$_3$ species including ACC particles, ions, and clusters, or (ii) the intercepts are divided by the corresponding invariant $I_0/Q_0$, which correspond to the weight average of the ACC particle mass only. If sphere-like or cube-like particles grow according to a monomer addition mechanism, two exponents are revealed, i.e., an exponent of 1/3 for a correlation of $R_g$ with $I_0/Q_0$ and 1/6 for the respective correlation of $R_g$ with $I_0$.$^{16,31}$

As is shown in Figure 2, a double logarithmic plot of $R_g$ versus $I_0$ indeed reveals a slope close to 1/6, whereas a plot of $R_g$ versus $I_0/Q_0$ leads to 1/3. Hence, particles grow in very much the same way as already observed for the abrupt initiation of the process by mixing the Ca$^{2+}$ and CO$_3^{2-}$ solutions directly with a stopped-flow device,$^{16}$ i.e., particle growth also follows a monomer addition mechanism if the CO$_3^{2-}$ concentration evolves in situ via hydrolysis of DMC. It has to be stressed again that data from the TR-SLS experiment agree with the respective results from the TR-SAXS experiment once plotted versus the intercept $I_0$ as this plot recovers $\alpha = 1/6$. However, SLS data do not give access to the invariant $Q_0$ because the $q$-regime in SLS is too small. With only the SLS data available, no plot of $R_g$ versus $I_0/Q_0$ would have been accessible emphasizing the significance of TR-SAXS data. However, agreement of SLS and SAXS results at [NaOH] = 20 mM clearly indicates that the technique of mixing the components has no impact on the mechanism of particle formation if the process is induced via hydrolysis of DMC. Thus, the reliability of the TR-SLS data presented in an earlier paper$^{17}$ is confirmed by the present work. The monomer addition mechanism for the ACC formation is further supported by the evolution of the particle number density, which can also be established by means of $Q_0$, via $Q_0^2/I_0$. Despite the large uncertainty of this composite number, an increase of $Q_0^2/I_0$ can be discerned with time in all cases (Figure SI-3 in the Supporting Information). This excludes growth via coalescence since the latter would inevitably cause a decrease in the particle number density and with it in $Q_0^2/I_0$.

In addition, model fits were performed to the SAXS curves recorded at 20 mM NaOH in order to confirm the model independent data interpretation outlined with eqs 4–7 and in order to extract additional information on the degree of polydispersity of the growing ACC particles. To this end, a Schulz-Zimm distribution$^{35}$ has been applied for the particle volume. Based on this distribution, the scattering curve $I(q)$ was calculated according to

$$I(q) = K_x \int_{V} v(V) V^2 P_V(q) \, dV \cdot S(q)$$

with $K_x$ the contrast factor for X-rays, with $P_V(q)$ the form factor of a homogeneous sphere$^{38}$ of outer sphere radius $R$ and $V = 4\pi R^3/3$, and with $S(q)$ the structure factor adopted by the spheres. The structure factor is either neglected by setting $S(q) = 1$ or explicitly taken into account as suggested by Vrij.$^{39}$ Details of the fit are presented in the Supporting Information.

As is outlined in Figure 3, both types of fits result in a satisfactory description of the scattering curves, thereby confirming the spherical morphology of the growing ACC particles. The resulting particle size and mass values were slightly larger than the results from the Guinier fits if $S(q) = 1$ and slightly smaller if a structure factor was included in the fit. However, the trends of the correlation of $R_g$ versus $M_w$ were parallel in all three cases having exponents close to 1/6 which confirms the monomer addition mechanism, established with the model independent data analysis based on eq 4 and eq 7. The polydispersities obtained from the model fits decreased with increasing reaction time and leveled off at a value for the ratio of the weight averaged volume and the number averaged volume close to $V_w/V_n \approx 1.5$. This is significantly broader than the polydispersities observed in the preceding SAXS experiments$^{12,16}$ at a CaCO$_3$ concentration of [CaCO$_3$] $\leq$ 5 mM and indicates an interesting detail, which shall be addressed once more in the following paragraph.

Two distinct features revealed by the ester-hydrolysis induced ACC formation deserve particular attention and shall be compared with the respective results from preceding TR-SAXS studies$^{12,13,16}$ where the Ca$^{2+}$ and CO$_3^{2-}$ component solutions had been directly injected into the capillary. The first feature refers to particle size, which gets significantly larger.
Upon ester-hydrolysis and which does not provide any evidence for the appearance of a final plateau value. As is shown in Figure 1B and Figure 2, the particle size recorded toward the end reaches values four times larger than observed upon direct mixing of a Ca\(^{2+}\) and a CO\(_3\)\(^{2-}\) solution with a stopped-flow device. At the same time, the extent of reaction expressed by \(Q_p\) in Figure 1C (\(\sim 0.004 \text{ nm}^{-3} \text{ mm}^{-1}\)) is smaller by a factor 1/2 than the limiting values established in ref 16. Taken together, this suggests a number density of particles smaller by 2 orders of magnitude (\(\sim 1/2 \cdot 1/4 = 1/8\)). This is indeed recovered by the number density observed at the end of our recordings (Figure S1-3 in the Supporting Information) for the experimental runs performed at NaOH = 20 mM (2.5 \(\times 10^{-9} \text{ nm}^{-3} \text{ mm}^{-1}\) compared to (2−5) \(\times 10^{-6} \text{ nm}^{-3} \text{ mm}^{-1}\) reported in ref 16). Therefore, a much larger amount of monomeric material is available per growing nuclei than in ref 16, which leads to larger particle size values in the present work. The second feature refers to the rate of particle formation and to the resulting distribution of particle size. Albeit the total amount of CaCO\(_3\) used in the present work is two times larger than in the case of the direct mixing shown in ref 16, the rate of ACC formation induced by the hydrolysis of DMC is more gradual with the nucleation period lasting more than 20 s. During this period of time, the number density represented by \(Q_p^2/I_0\) increases gradually. Contrary to the DMC hydrolysis induced process, the number density reaches a final value after less than a second and stays constant thereafter if Ca\(^{2+}\) cations were directly mixed with CO\(_3\)\(^{2-}\) ions. The extended period of nucleation inevitably causes a broader size distribution in the present case, which has already been suggested by the model fits to the scattering curves presented above. Clearly, the continuing generation of new CO\(_3\)\(^{2-}\) ions keeps the extent of supersaturation at a low but persistent level. An interesting comparison also becomes possible with an earlier investigation where the components had been directly mixed at the same concentration of the components of 10 mM as in the present case. In the process following ester-hydrolysis, a gradual growth of ACC particles could be followed over some 50 s. Contrary to this rate, Bolze et al. observed an almost instantaneous formation of primary particles with a size of 19 nm within the first second. Thereafter, agglomeration seemed to have taken place, which could not be further analyzed appropriately by the selected instrumental settings. Thus, in situ hydrolysis enables a better control of the process and produces more uniform particles compared to the process triggered by direct mixing of the components once the CaCO\(_3\) content is as high as 10 mM.

**RESULTS: IMPACT OF SODIUM POLYACRYLATE ON THE ACC FORMATION**

Two samples of sodium polyacrylate anions differing in their molar mass were selected in order to establish characteristic features of the mode of action of sodium polyacrylate additives on the formation of ACC particles. All experiments were carried out at \(T = 25 \, ^\circ\text{C}\). The Ca\(^{2+}\) and DMC concentrations are 10 mM and particle formation was initiated with the addition of a NaOH solution leading to a final concentration of [OH\(^−\)] = 20 mM corresponding to the stoichiometric amount necessary for complete hydrolysis of DMC. The polymeric additive is added with the NaOH solution.

One series of experiments addresses the influence of a model sodium polyacrylate sample with a molar mass of 97 kDa denoted as Paa8SkD on the mechanism of ACC particle formation induced by DMC hydrolysis. Four different Paa8SkD concentrations at 1.5 mg/L, 2.5 mg/L, 5 mg/L, and 7.5 mg/L have been applied. The concentrations are fairly low as they correspond to molar concentrations of COO\(^−\) residues, which are more than 2 orders of magnitude below the concentration of Ca\(^{2+}\) ions.

Figure 4 compares the increase of the particle mass at all additive concentrations. Use of the polyacrylate chains as an additive slightly decreases the induction time from 360 s down to 320 s. Variation of the polyacrylate concentration does not significantly affect this induction time. This feature contradicts with the findings of Faatz and Wegner. They observed an increase of the induction time from 2200 to 2700 s if an additive was added to the reaction mixture. Although they applied the same Ca\(^{2+}\) and DMC concentrations as used in the present work, their system differs considerably from the present work in terms of the following aspects. The OH\(^−\) concentration applied by Faatz and Wegner was only 8 mM corresponding to an [ester residue]/[OH\(^−\)] ratio of 0.4 and the reaction temperature was fixed at 10 °C instead of 25 °C used in the present work. However, three other differences of the process applied by Wegner et al. were more important. Instead of pure polyacrylate anions considered here, they have used a double hydrophilic block-copolymer with 68 units of ethylene oxide and 8 units of anionic methylymethacrylate monomers. The concentration of the double hydrophilic block copolymer was 1 g/L and thus 2 orders of magnitude larger than the Paa8SkD concentration applied for the present series. As will be shown below, a pronounced increase of the lag-time was in fact observed once a significantly larger amount of NaPA was used, yet with a much lower molar mass value. This points to the third difference which refers to the high molar mass sample Paa8SkD compared to the additives used by Wegner et al.

In order to better understand its implication, we have to first focus on the induction period. During the induction period, an increase of the particle mass and particle size is observed from time zero on if Paa8SkD is added. No such increase had been observed in the absence of NaPA. The growth is accelerated with increasing NaPA concentration but levels off at a value close to \(R_g = 140 \, \text{nm}\).
particle mass values are based on a concentration of 1 g/mL before the lag-period had come to an end in all cases. The end of the lag-period is characterized by a further steep increase of the scattering signal by 2–3 orders of magnitude. During this second period of growth, which corresponds to the onset of ACC formation, no further increase of the particle size is observed. In an additional reference experiment (outlined in the Supporting Information), in the absence of DMC but at otherwise identical conditions and composition as in the experiment with an NaPA content of 7.5 mg/L (represented as black squares in Figure 4) the same aggregation pattern was observed, as represented by the respective trend in Figure 4. Hence, preformed ACC clusters can be discarded, as the possible origin of the aggregation during the lag-period and growth during this first period inevitably corresponds to the formation of CaPA aggregates.25 In the following, a more quantitative discussion of the evolution of apparent mass values is given, which better discriminates both stages.

Despite the occurrence of a first stage where CaPA aggregates are formed and a second stage where ACC formation sets in, the constants used to establish those light scattering results indicated with symbols in Figure 4A and Figure 5 refer to the CaCO₃ component only. The respective particle mass values are based on a concentration of 1 g/mL corresponding to the total content of CaCO₃ in solution and on a dn/dc value of 0.1 mL/g which is close to the value of ACC in water. Though CaCO₃ is the dominating component, it only comes into play in the second stage. As outlined in the Appendix, more sensible mass data can be established for the CaPA aggregates during the lag-period, if the proper NaPA concentrations are used together with a dn/dc = 0.17 mL/g, which corresponds to NaPA in salt-free water and which is supposed to be much closer to the true values of the aggregates than the value of 0.1 mL/g is.40 With these corrections performed, the resulting mass values of the aggregates, denoted as M_{CaPA}, are represented as colored lines in Figure 4A. Those lines lead to final values, which are in the same order of magnitude as the finally formed ACC particles at the end of each experiment respectively. These results suggest that the sharp increase of the signal after the lag-period is predominantly caused by an increase of the amount of scattering species, which is in the same order of magnitude in all four cases and not by gradual particle growth. Two alternative pathways can be discussed as possible mechanisms for the second stage of the process, which corresponds to the ACC formation.

The first pathway assumes that the sharp increase of the scattering signal at the end of the lag-period is caused by an increase of the density of scattering matter within the particles without changing their size. This is possible, as the CaPA aggregates exhibit a partial density of only 0.06 g/mL in the aggregates and thus give way to a large capacity for CaCO₃ incorporation enabling an increase of the resulting partial density by more than a factor of 10. The second pathway attributes the drastic increase of the scattering signal after the lag-period to a nucleation of ACC particles outside the CaPA domains. The successive growth of ACC particles is then modulated by adsorbing PA chains and aggregates. The particles formed during the second stage exhibit roughly the same (apparent) partial density and particle size as the already existing CaPA aggregates. However, comparable values for the true particle densities are only realistic if the particle mass...
values in both stages are true values, which would require a 100% conversion of the scattering matter into particles in the case of the aggregates and of the ACC particles.

As is outlined in the Appendix, both pathways can be reconciled with our experimental results. There are only indirect hints, which are in favor of the first pathway. One hint points to the fact that newly generated ACC particles would have to be synchronized with a partial dissolution of CaPA aggregates without any effect on the average particle size. Simple formation of further particles with the same density as a limiting situation for the second pathway can be totally ruled out, as this would imply an extremely low partial density of only ~0.05 g/mL for these formed ACC particles. Though this is compatible with loosely packed CaPA aggregates, it seems far too low for the more compact ACC particles. Another hint refers to the counterintuitive decrease of the lag-period addressed above. As already mentioned, it may in part be attributed to the high molecular weight of Paa85kD. High molecular weight NaPA samples have a much stronger tendency to form CaPA aggregates than low molecular weight NaPA samples (Figure SI-4 in the Supporting Information). The larger size and/or increased number of CaPA aggregates may slightly accelerate ACC formation, in particular, if the CaPA species acts as a host ore template for the forming ACC. It is this composite CaPA-ACC material which may correspond to the PILP phase reported by Gower et al.18,19 Finally, support for the first pathway is provided by the findings by Chijo et al.24, who clearly identified CaPA aggregates to act as “reaction containers” for ACC upon addition of CO$_3$$^2$ ions.

A plot of the radius of gyration versus the molar mass in Figure 5 reveals parallel scaling regimes, which are all formed during the lag-period and which are shifted toward larger apparent mass values as the polymer concentration increases. As has been just outlined, the shift of the scaling curves is an artifact and can easily be attributed to the increase of the NaPA concentration, which was not taken into account by the evaluation of data in Figure 5 where all $M_w$ data are solely based on the concentration $c$ in g/L corresponding to 10 mM CaCO$_3$ and a $dn/dc$ value of 0.1 mL/g for ACC. However, the relative increase of this apparent particle mass during a single aggregation experiment is still correct and enables quantitative discussion of the respective exponent attributed to the scaling law according to eq 6. The resulting exponents cover a narrow regime in between 0.29 < $\alpha$ < 0.34, being considerably larger than the exponent observed for the ACC formation in the absence of polymeric additives.31

Additional information on the CaPA aggregates and ACC particles can be extracted from the particle scattering curves represented in the Supporting Information. The curves fall in between the decay of a linear coil-like structure and a compact sphere. During the lag-period, the curves overlay nicely, whereas after the lag-period, as the ACC is forming the scattering behavior gets closer to the decay of sphere-like particles. In line with the exponents discussed above, NaPA aggregates during the lag-period and forms ever larger but self-similar structures in qualitative agreement with an earlier investigation with CaPA coils brought to the coil collapse and to the aggregation threshold.25

In order to get further insight into the impact of NaPA chains on the ACC formation, we extended experimental conditions following two strategies. First, we tried to increase the Paa85kD concentration as much as possible. Second, NaPA samples were used with a much lower molecular weight than Paa85kD, the latter case also being closer to commercially applied polyacrylates. Those extensions of experimental conditions also brought us closer to systems analyzed by other groups.14,28,29

In pursuing the first strategy, it turned out that the additive concentration of Paa85kD could not be increased beyond 50 mg/L because solutions got turbid at such high concentrations. Turbidity stemmed from a precipitation of the calcium salt of Paa85kD. Hence, we present one experiment at a Paa85kD concentration of 26 mg/L. No initial increase of the particle size is detectable anymore, i.e., CaPA aggregates reach their final size of 150 nm almost instantaneously. The same is true for the aggregate mass (Figure 6). Beyond the induction time, a second strong growth of the apparent particle mass sets in without any further observable increase of the particle size. Thus, a size versus mass correlation can neither be extracted for CaPA aggregates nor for ACC particles in this case.

The second strategy focused on significantly lower molar mass values of the PA additive. To this end, a commercial BASF sample denoted as PAKD2 had been applied. This sample has a molecular weight of 2000 g/mol. A first experiment at 26 mg/L corresponding to the largest concentration applied with the sample Paa85kD already shows a significant difference. As outlined in Figure 6, no growth of CaPA aggregates is detectable with PAKD2 during the lag-phase. This is in line with the fact that the tendency to form aggregates decreases drastically once NaPA chains get considerably smaller than 100 kDa (see Supporting Information for an illustrative example). At an additive concentration of 26 mg/L, sample Paa85k preforms microgel-like CaPA networks during the lag-period,
whereas the low molecular weight sample PAkD2 does not. Just as important, the induction time is increased by more than 50 s, if PAkD2 was used instead of Paa85kD.

In order to verify this effect, a series of three additional concentrations of PAkD2 was investigated with further ACC formation experiments, and the results thereof were again compared with the particle formation where no additive has been used. Altogether, the four experiments with PAkD2 covered an additive concentration regime of 26 mg/L \( \leq c \leq 100 \) mg/L. For all four experiments, the NaOH concentration and the CaCO\(_3\) concentration were 20 mM and 10 mM, respectively.

The solutions with PAkD2 concentrations of 50 mg/L and larger exhibit a similar two-stage process as observed already in the Paa85kD assisted particle formation. A gradual aggregation of the anionic polycrylate coils induced by Ca\(^{2+}\) ions precedes the actual ACC formation. As mentioned above, this tendency to aggregate is less pronounced than observed for the higher molar mass additives and thus only appears for PAkD2 contents significantly larger than 26 mg/L. The induction time again is indicated by a second steep increase of the particle mass, which is attributed to the onset of ACC formation. Toward the end of the ACC formation step, the increase of the apparent molar mass is connected to a further increase in particle size from \( R_g = 120 \) nm to \( R_g = 150 \) nm. This behavior differs from the trends observed with Paa85kD where the ACC formation step beyond the lag-time is characterized by an increase in particle mass at a constant particle size. Details are represented in Figure 7. A closer look on the particle scattering curves recorded at different times (presented in the Supporting Information) reveals a similar trend as already observed with the experiments assisted by Paa85kD. Scattering curves of the ACC particles fall in between those of compact particles and coil-like aggregates, indicating that the ACC particles incorporate NaPA coils under present conditions.

More important, an increase of the lag-time is observed with increasing content of short chain PAkD2 in Figure 7. Most remarkable, however, is that the ACC particles turned out to be even (meta-)stable for at least 15 min at concentrations of short chain NaPA above 50 mg/L. This could be established by a visual inspection of the scattering cells immediately following the TR-SLS experiments for at least another 15 min. During this inspection, the samples with NaPA contents above 50 mg/L remained clear. Neither the solutions without a polymeric additive nor the solutions with sample Paa85kD where the maximum amount of additive to be used was much less than in the case of PAkD2 showed a similar intermediate stability. Like in the case of Paa85kD assisted ACC formation, the amount of ACC being formed is significantly smaller than in the case of additive free ACC formation. Since the particle size achieved at the end of the ACC formation in the presence of large amounts of PAkD2 is similar to the additive free case, it has to be the number of ACC particles formed, which is significantly lower in the presence of PAkD2. This might be induced by a blocking of small CaCO\(_3\) clusters, thereby preventing them from acting as monomers or as nuclei.

The same two pathways may be discussed for the formation of ACC corresponding to the second step as already considered for the growth assisted by the long chain Paa85k sample: (i) Nucleation and growth of ACC clusters and nanoparticles occur in the preformed CaPA aggregates, which act as sinks of Ca\(^{2+}\) ions and thus generate domains with a locally confined but fairly high Ca\(^{2+}\) content. (ii) Clusters and ACC nanoparticles form, which are interconnected by adsorbing NaPA chains. The chains are withdrawn from solution and replenished from the CaPA aggregates, with the latter serving as reservoirs for NaPA chains.

In order to support the comparative discussion of the two alternative pathways presented above, now for the PAkD2 assisted ACC formation, the same corrections have to be performed as have been done with the apparent mass data of the growth experiments modulated with low amounts of Paa85k. Again, it turns out to be impossible to exclude the scenario of an accumulation of CaCO\(_3\) within preformed CaPA domains.

The final CaPA aggregates based on PAkD2 exhibit much lower apparent mass data than the final ACC particles formed successively after the onset of ACC formation. Noteworthy, the mass data of the CaPA aggregates are also significantly smaller than those of the aggregates formed with Paa85k, while the NaPA concentration is larger by 1–2 orders of magnitude in the case of PAkD2.

Notwithstanding the impossibility to exclude the alternative where CaPA aggregates may transform into ACC particles, there are a few arguments which are in favor of the alternative where CaPA aggregates are off-pathway. First of all, much fewer CaPA aggregates do exist at the onset of ACC formation in the case of the preformed PAkD2 aggregates. Also, the extent of solubility as single chains is much larger than in the case of Paa85k. This may promote formation of mixed CaPA-ACC...
hybrid particles outside from the existing CaPA aggregates. In line with this, a small but sharp increase of $R_g$ is observed close to the onset of ACC formation, which may serve as another hint for the fact that ACC particles may not arise directly from CaPA aggregates.

**SUMMARY**

The formation of amorphous calcium carbonate particles via the hydrolysis of DMC in the presence of $\text{Ca}^{2+}$ cations follows a monomer addition mechanism. Nuclei are formed, once a certain level of supersaturation is reached. During particle growth, constituting ions, units of $\text{CaCO}_3$, or small clusters thereof are considered to act as monomers. In a recent paper by Hedin et al.,\textsuperscript{41} nanostructural features of approximately 2 nm were identified within monodisperse nanoparticles of ACC, quenched from metastable $\text{CaCO}_3$ solutions by destabilization in excess ethanol. These features may correspond to clusters and thus provide an interesting hint for the fact that it is the small clusters which act as monomers. Earlier TR-SLS experiments\textsuperscript{41} indicated a lag-period of some 350 s if a concentration of 10 mM DMC is used in the presence of 20 mM NaOH. These results could be confirmed by the present TR-SAXS experiments. Beyond this, the present SAXS data indicate a nucleation period which extends to 20 s and generates only a low amount of particles compared to the process which is initiated through mixing of a $\text{Ca}^{2+}$ solution with a $\text{CO}_3^{2−}$ solution. For the latter process, a short burst of nucleation creates a number of particles within a second, which is 2 orders of magnitude larger\textsuperscript{16} than the number of particles reached in the present process (induced via hydrolysis of DMC) where the $\text{CaCO}_3$ content is twice as large as the one applied in the direct mixing experiment. Thereby, the NaPA free ACC formation experiments provide excellent reference data for the investigation of the impact of anionic polyacrylate chains on the formation of ACC nanoparticles. Two different NaPA samples have been selected in order to illustrate the influence of NaPA on the ACC formation. One sample ($\text{Paa85k}$) had a molar mass of 97 kD and is considered to represent long chain behavior. A second sample ($\text{PaKd2}$) with a molar mass of only 2 kD had been selected in order to represent short chain behavior.

The high molar mass sample could only be applied in a regime of concentrations up to 50 mg/L, since beyond this regime, aggregation of CaPA generates turbid solutions long before ACC formation sets in. In the concentration regime of $\text{Paa85k}$ accessible to our TR-SLS experiments, CaPA aggregates were formed during the lag-period. The size of those CaPA aggregates was close to the size of the ACC particles formed after the lag-period. Despite this similarity in size, we could not unambiguously discriminate between the following mechanistic alternatives: (i) aggregates act as nucleation centers and as templates or (ii) aggregates are off-pathway and act only as reservoirs for PA chains being incorporated into the ACC particles. However, neither the lag-period nor the amount of ACC formed could be considerably influenced by the long chain NaPA additive.

In the case of the short chain NaPA sample, the tendency to form CaPA aggregates was much lower, which enabled us to extend the amount of NaPA by at least a factor of 4. This extension of the NaPA concentration led to a noticeable increase of the lag-period by 50–100 s. At the same time, solutions with the low molecular weight PA sample exhibit (meta-)stable dispersions of ACC particles. The amount of ACC being formed with these (meta-)stable particles is considerably lower than the latest values being observed in the case where no NaPA had been used. As the particle size values were comparable in both cases, the decreased turnover in the presence of the 2 kD sample is attributed to a blocking of small $\text{CaCO}_3$ clusters which were otherwise ready to act as nuclei or monomers. The same two alternative mechanistic paths can be discussed for the ACC formation modulated by the 97 kD sample as well as modulated by the 2 kD sample, again without being able to make a definite choice.

However, whereas in the case of the 97 kD sample the template path seemed to be more likely to occur (path i), it is the CaPA aggregate reservoirs which may dominate in the case of the 2 kD sample (path ii). A schematic given in Figure 8

![Figure 8](image)

**Figure 8.** Schematic representation of the ACC formation process, observed in the presence of NaPA chains, which act as modulator. During the first step, CaPA aggregates form while the supersaturation level of $\text{CaCO}_3$ is approached via gradual generation of $\text{CO}_3^{2−}$ ions from ester hydrolysis. For the second step, two alternative mechanistic paths are considered: (i) the CaPA act as template/nucleation centers; (ii) the CaPA aggregates act as reservoirs for PA modulator chains which are removed from solution during ACC formation.

In conclusion, short chain NaPA with molar mass values smaller than 10 kD turned out to be much more efficient in modulating the formation of ACC than NaPA chains with mass values of 100 kD are. This holds if the supersaturation level is gradually approached in the presence of the additives. No information is yet available for the case where ACC formation is induced by instantaneously mixing a carbonate solution with a $\text{Ca}^{2+}$ solution.

**APPENDIX 1**

As has been introduced with eq 1, the intensity of scattered light at $\theta = 0$ is determined by $\Delta R_{\theta \phi} = KcM_\gamma$ with $K$ the scattering contrast proportional to $(dn/dc)^2$, $c$ the concentration of the scattering species, and $M_\gamma$ its weight averaged molecular weight. Two issues have to be considered with great care when applying this relationship to the system under present consideration. The scattering species in a two step process may change, and even if a single species dominates the scattering intensity, $c$ may not refer to the strongly scattering species only but include both monomers and particles.

Under the assumption that the scattering signal recorded at times shorter than a certain induction time $t_{\text{ind}} \sim 300$ s stems exclusively from CaPA aggregates, apparent mass values can be recalculated for these intermediates as follows. The concen-
tration value of 1 g/L representing the total CaCO₃ content, which was used to establish all \( M_w \) data in Figure 4A, is replaced by the actual NaPA concentration denoted as \( c_{\text{PA}} \) respectively. Aside from this, 0.17 mL/g established as proper \( dn/dc \) value for NaPA in salt free water is considered to be a better estimate for the \( dn/dc \) value of CaPA aggregates than the value of 0.1 mL/g which refers to the ACC and which was used to calculate values indicated by means of symbols in Figure 4A. Replacements of both parameters results in a correction factor

\[
f_{\text{cor}} = 1 \text{ g/L}(0.1 \text{ mL/g})^2/[c_{\text{PA}}(0.17 \text{ mL/g})^2] \quad (A-1)
\]

for the apparent mass data of the species formed during the lag period. Application of \( f_{\text{cor}} \) transforms the respective apparent mass values \( M_w \) into values denoted as \( M_{\text{CAPA}} \)

\[
f_{\text{cor}} M_w = M_{\text{CAPA}} t < t_{\text{lag}} \quad (A-2)
\]

which are expected to represent the CaPA aggregates much better than the original \( M_w \) values do at \( t < t_{\text{lag}} \) in Figure 4A. As is outlined by the colored lines in Figure 4A, the aggregate mass values \( M_{\text{CAPA}} \) approach the same order of magnitude as the values finally adopted during the second stage by the growing ACC particles. However, we have to stress at this point that the \( dn/dc \) values are crude estimates for both the CaPA aggregates and the ACC particles, respectively.

The mass and size values established in this way and summarized in Table A1 give access to an estimation of the density of the scattering species within the particles. Estimation is performed under the assumption that all mass of the respective species is transformed into particles. In the case of CaPA aggregates this means that \( c_{\text{PA}} \) was completely used up to form CaPA aggregates at all four NaPA concentrations. If this assumption holds, mass and size of the aggregates should correspond to the true values in all four cases. Although size values of the aggregates at all four \( c_{\text{PA}} \) agree, \( M_{\text{CAPA}} \) values get smaller with decreasing \( c_{\text{PA}} \) which may indicate a decreasing extent of aggregation as the polymer concentration decreases.

Hence, the assumption of total conversion of a species into aggregates is valuable for the largest polymer concentration of \( c_{\text{PA}} = 0.0075 \text{ g/L} \) at best. Therefore, we use the largest NaPA concentration to estimate an aggregate density. The density resulting for the final aggregates at \( c_{\text{PA}} = 0.0075 \text{ g/L} \) is therefore

\[
\rho = 3(4.82 \times 10^8 \text{ g/mol})^3/(4\pi 1.49^9 10^{-15} \text{ cm}^3/6.023 \times 10^{23} \text{ mol}^{-1}) = 0.06 \text{ g/cm}^3.
\]

Such a value is indeed typical for polymeric coils penetrated by solvent.

The succeeding second stage of particle formation increases the net scattering signal by a factor of 40. This is indicated by a direct comparison of the signal from the final state of the CaPA aggregate at \( t = t_{\text{lag}} \) (\( M_w \) value in the fourth column of Table A1) with the signal from the last intermediate characterized during the ACC formation in the second phase of the process (\( M_w \) value in the 7th column of Table A1). If the difference in scattering contrast of 2.89 is taken into account, a factor of 14 still remains to be interpreted. As the size of the aggregates and ACC particles is the same, two possible limiting pathways may serve to explain this increase: (i) the factor 14 is caused by incorporation of CaCO₃ units into the existing aggregates, causing an accumulation of the scattering material in the particles by a factor of 14 without changing the size and number of particles; (ii) an increase of the number of particles by a factor of 14 with almost identical partial density of the scattering species as the CaPA aggregates is responsible for the increase of the scattering power by a factor of 40. As is demonstrated below, both alternatives are possible.

At this point, it is helpful to recall that the apparent partial density in the final ACC particles formed in the presence of 0.0075 g/L NaPA is also 0.06 g/mL, as are the densities of the three other ACC samples formed in the presence of the lower NaPA concentrations, since the size and mass values of the final ACC particles are comparable to those of the corresponding CaPA aggregates and comparable to each other. If, in fact, the true partial density of CaCO₃ in ACC would only be 0.05–0.06 g/mL, the limiting density would already be reached in the aggregates and a further accumulation now of ACC is prohibited. Hence, alternative (ii) would be the pathway at hand. Unfortunately, we do not know to which extent CaCO₃ had been transformed into particulate matter. If this extent is considerably smaller than a 100%, the true density of CaCO₃ in the ACC particles would be higher than the one estimated with a total consumption of 1 g/L and may approach values of pure ACC formed in the absence of any polymeric additive. Under the latter condition, mass densities of 1.5 g/mL ≤ ρ ≤ 2 g/mL have been determined for ACC particles with a considerable amount of water molecules still incorporated in the ACC particles. This is compatible with an excess contribution of at least 0.5 g/mL due to incorporation of CaCO₃ without any change in particle size, simply by replacing the penetrated water molecules with CaCO₃ units. If we take together the uncertainty of the extent of CaCO₃ consumption with an inaccuracy of the ratio of the two scattering contrasts applied in Table A1, pathway (i) cannot be ruled out. Indeed, do the CaPA aggregates have enough capacity to incorporate CaCO₃ units since the excess density of polymers is as low as 0.06 g/mL. This makes alternative (i) equally likely to occur. An increase of 0.06 g/mL by a factor of 14 leaves us with 0.84 g/mL, which is still a physically meaningful quantity.

### ASSOCIATED CONTENT

#### Supporting Information

Evaluation of the invariant \( Q_p \) from modified Kratky-plots of the SAXS scattering curves, comparison of induction times for the onset of ACC-formation extracted from SAXS and from SLS, evolution of the particle number density of growing ACC particles at an NaOH concentration of 20 mM established from SAXS curves, description of the model fits based on polydisperse hard spheres to SAXS data, comparison of the
aggregation behavior of two NaPA samples with two different molar mass values in the presence of Ca$^{2+}$ ions in dilute aqueous solution, and evaluation of the impact of DMC with NaOH on the aggregation of high molecular weight NaPA in the presence of Ca$^{2+}$ ions in dilute aqueous solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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