Control over Calcium Carbonate Phase Formation by Dendrimer/Surfactant Templates

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Abstract: Poly(propylene imine) dendrimers that are modified with long alkyl chains self-assemble to form well-defined aggregates. The geometry and surface chemistry of the dendrimer assemblies can be varied through the addition of surfactants. These dendrimer/surfactant aggregates can be tuned to template the formation of the different phases of calcium carbonate. The use of octadecylamine results in the formation of polyhedral aggregates that become embedded within an amorphous calcium carbonate phase that persists in competition with the thermodynamic product, calcite. In combination with hexadecyltrimethylammonium bromide, small spherical aggregates are formed that induce the formation of vaterite. The use of the negatively charged surfactant SDS results in growth retardation by the Ca^{2+}-induced agglomeration of dendrimer/surfactant aggregates into giant spherical particles. Eventually these particles become overgrown by rhombohedral calcite.

Keywords: amorphous materials · biomimetic synthesis · calcium carbonate · dendrimers · self-assembly

Introduction

Templates that consist of organized biomacromolecules are known to direct the formation of most biominerals, in many cases leading to materials with unique shapes and properties.[1] For synthetic polymers, however, it has been difficult to unambiguously assign structure – function relationships in the context of their activity in crystallization assays, since they mostly occur in random-coil conformations. In contrast, the use of ordered supramolecular assemblies, such as micelles,[2] monolayers,[3] vesicles,[4] inverted micelles,[5] and lyotropic liquid crystalline systems,[6] allows for the controlled nucleation of inorganic materials on molecular templates with well-defined structure and surface chemistry.

One of the most abundant biological minerals is calcium carbonate, the main constituent of mollusk shells, for example.[7] In nature, four crystalline calcium carbonate polymorphs, calcite, aragonite and the metastable forms vaterite and monohydrocalcite, are observed. In addition, amorphous calcium carbonate (ACC), which is normally unstable at room temperature and pressure, has been reported.[8] It has been demonstrated that in vitro experiments with proteins extracted from biogenic ACC result in the formation of stable ACC.[8c] The use of synthetic macromolecules generally results in the formation of habit-modified calcite and occasionally vaterite. Only a few examples are known in which aragonite was formed without the addition of metal ions,[9] whereas the amorphous phase is only observed as a short-lived transient phase.[10]

Here we report on a synthetic system in which aggregates consisting of assemblies of poly(propylene imine) dendrimers modified with long hydrocarbon chains and single-chain surfactants are used to control calcium carbonate formation. The surface chemistry of these dendrimer/surfactant aggregates can easily be varied by the choice of surfactant. In this way, different phases of calcium carbonate, including ACC,[11] as well as composites can be obtained by using a single dendrimer scaffold.

Results and Discussion

Dendrimer/surfactant aggregates: Poly(propylene imine) dendrimers modified with long aliphatic chains (**1**) are a new class of amphiphiles that have a variety of aggregation states due to their conformational flexibility.[12] In organic

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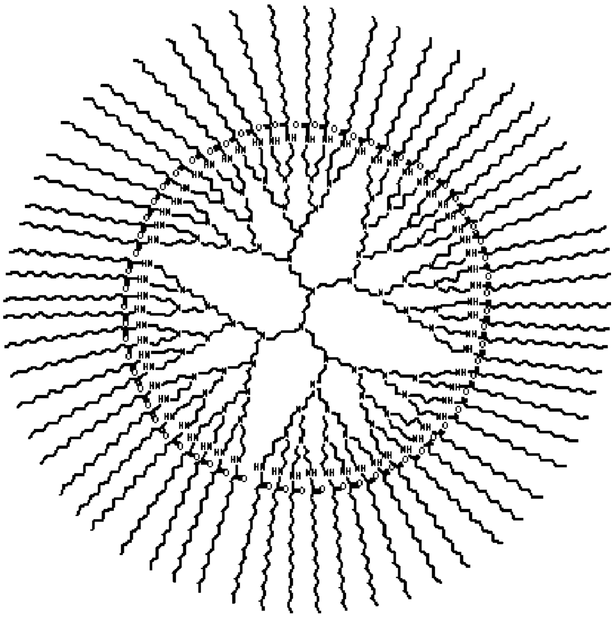

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solvents they are present as inverted unimolecular micelles with a radial structure, whereas they adopt a conformation in which all alkyl chains are oriented away from the aqueous phase when localized at an air/water interface. Globular aggregates are formed when **1** is dispersed in aqueous media (Figure 1a).[13] In the present work, we have modified the outer surface of this dendrimer assembly by the addition of single chain surfactants (Scheme 1) containing an amine functionality (octadecylamine, OA), a quaternary ammonium group (hexadecyltrimethylammonium bromide, CTAB), or a sulfate head-group (sodium dodecylsulfate, SDS).[14] This was achieved by the injection of a solution of **1** and the elected surfactant in THF/EtOH (2:1, *v*/*v*) into hot water, followed by sonication for 1 hour. In the resulting solutions, the surfactants are at concentrations below their critical micelle concentration. In the case of SDS, the aggregates immediately precipitated. This precipitation process was attributed to electrostatic interactions between the negatively charged surfactant head-groups and the nitrogens of the dendritic core, resulting in an aggregate with an apolar periphery. In this case, stable aggregates could be obtained by first dissolving the surfactant in the hot water followed by injection of **1**. Best results (stable, well-defined aggregates) were obtained when the alkyl chain length of the surfactant did not differ by more than two carbon atoms from the length of the palmitoyl chain of the dendrimer.[15] In this way we were able to generate solid self-reinforced aggregates of varying shape, size, and surface chemistry depending on the choice of surfactant.

It was found that aggregates arising from the interaction of **1** and OA expressed a persistent polyhedral shape (Figure 1b)[16] as well as a narrow size distribution around 250 nm (dynamic light scattering, DLS). Electron microscopy on microtomed samples of **1**/OA based aggregates (Figure 1c) showed large regions with no specific morphology and small regions with a morphology similar to the structure found in annealed films of **1** (Figure 1d) wherein the dendrimers are microphase separated. Moreover, powder X-ray diffraction (PXRD) and electron diffraction on the **1**/OA aggregates demonstrated the absence of long-range order. In addition, fluorescence depolarization experiments were carried out in order to study the microviscosity and phase transitions of the

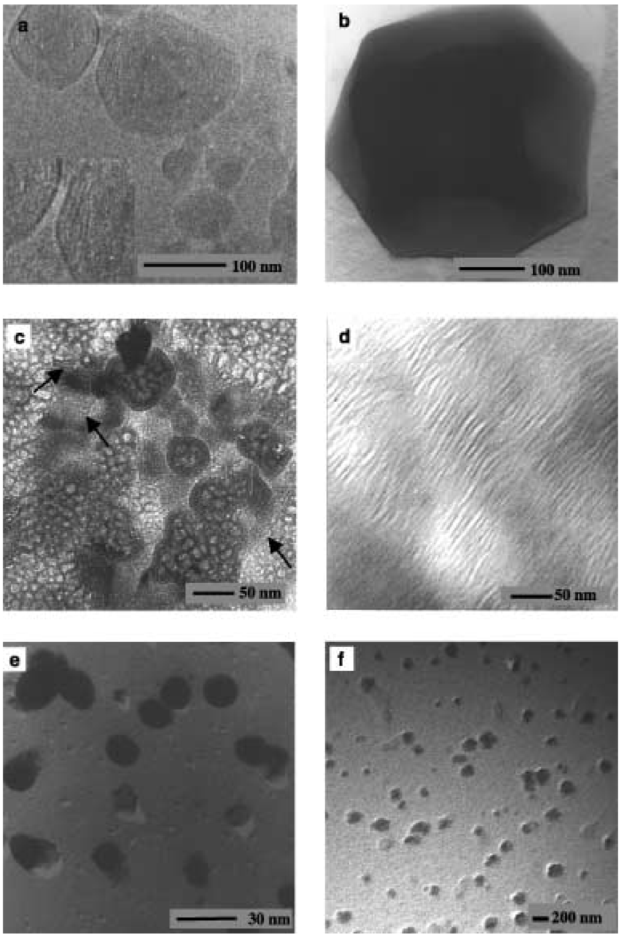
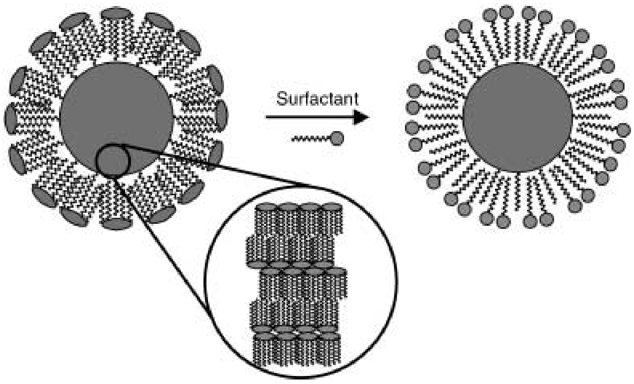


Figure 1. Transmission electron micrographs of a) an aqueous dispersion of **1**, b) an aqueous dispersion of **1**/OA, c) a microtomed sample of aggregates of **1**/OA showing phase separated regions (arrows) in an amorphous matrix, d) an annealed thin film of **1**, e) an aqueous dispersion of **1**/CTAB, and f) an aqueous dispersion of **1**/SDS.



Scheme 1. Effect of the addition of surfactant to the surface of an aggregate.

aggregates (Figure 2a). The extremely high anisotropy value found at room temperature ($r = 0.32$) indicates that they have a very rigid structure.[17] Aggregates prepared from **1** alone showed a single phase transition at 38 °C with this technique, whereas differential scanning calorimetry revealed a T_m of 76 °C for thin films of neat **1**. The observation, by using fluorescence-depolarization spectroscopy, of two phase transitions at 38 and 60 °C is consistent with our proposed model, wherein a core, which consists of small domains of microphase-separated dendrimers in semi-crystalline packing and large amorphous regions, is solubilized by a surrounding layer of surfactant molecules (Figure 2b).

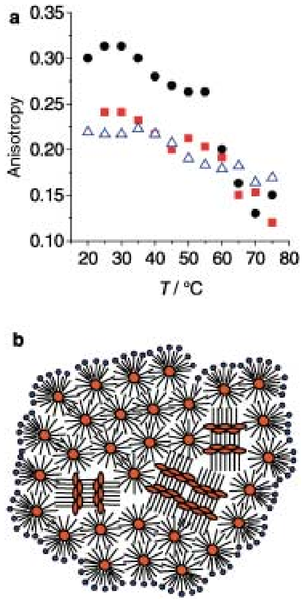


Figure 2. a) Fluorescence depolarization curves for aqueous dispersions of **1**/OA (●), **1**/CTAB (●) and **1**/SDS (△). b) Schematic representation of the proposed structure of the aggregates consisting of **1** and OA.

When the dendrimer was mixed with CTAB, spherical aggregates with a narrow distribution and an average size of 20 nm (determined by DLS) were formed (Figure 1e). The smaller size of these aggregates was attributed to the higher surface charge (+1) relative to the **1**/OA aggregates. A pK_a value of 6.1 was observed in the latter case; this indicates that approximately 50 % of the amino groups are protonated under the conditions used. The higher surface charge for **1**/CTAB favors more curved surfaces and hence smaller aggregates. Fluorescence-depolarization measurements showed that these aggregates are less rigid ($r = 0.24$) with respect to the **1**/OA aggregates (Figure 2a). In addition, two phase transitions are again observed (although they are less pronounced); this suggests an internal structure similar to that of **1**/OA.

Aggregates of **1**/SDS were 140 nm in size on average and displayed a spherical but often imperfect morphology (Figure 1f). This, and the larger size compared with the **1**/CTAB aggregates (which have a similar surface charge), was attributed to the different preparation method. Since **1** is added to a solution already containing the surfactant, the local concentration of surfactant is lower than when both components are simultaneously added. Under these conditions, larger core structures may be formed before the formation of a terminating surfactant layer takes place. Notably, similar size aggregates were observed for **1** alone.[13] Fluorescence-depolarization studies showed that the microviscosity and phase behavior of these systems was similar to those of aggregates of **1**/CTAB (Figure 2a).

Crystallization experiments: The remarkable rigidity and the well-defined size and shape of the aggregates consisting of **1** and OA prompted us to exploit them as 3D templates in calcium carbonate crystallization assays.[18] The addition of these novel templates to such an assay resulted in the formation of amorphous calcium carbonate. Indeed, calcified aggregates that had retained their polyhedral shape were isolated after only 15 minutes (Figure 3a). Selected-area electron-diffraction analyses confirmed that the vast majority of these particles consisted of amorphous material. Spherical particles isolated after 1 day were amorphous according to PXRD. IR microscopy showed peaks characteristic for hydrated ACC at 1455 and 1425 cm^{-1} (double band), 1072 cm^{-1}, and 873 cm^{-1} (Figure 4a).[8c] In addition, absorptions originating from the organic component were present. The peak at 712 cm^{-1}, typical for calcite, was not observed. Raman microscopy of the spheres also showed that the typical calcite peak (at 714 cm^{-1}) was absent. The peak at 1086 cm^{-1} corresponding to ACC could not unequivocally be identified, since it was masked by vibrations originating from the

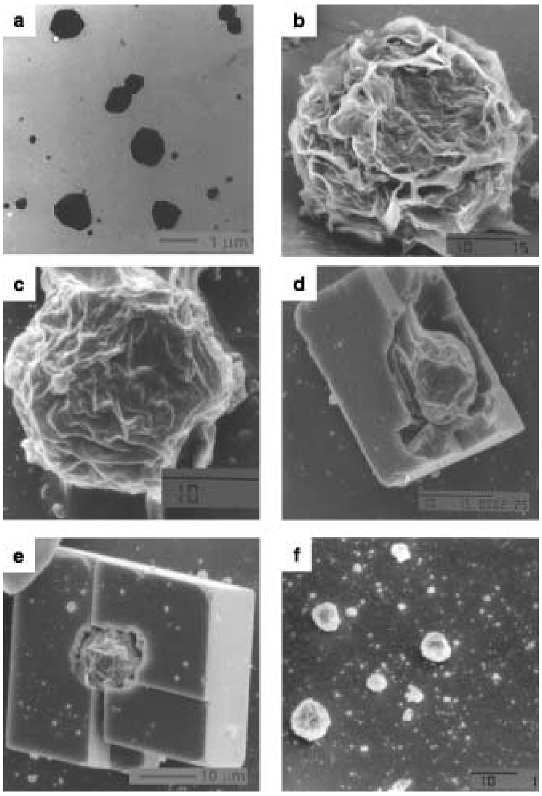


Figure 3. Transmission electron micrograph of the crystallization assay doped with **1**/OA showing the ACC particles after a) 15 min, b) 1 day, c) and d) 4 days, e) 14 days, and f) 1 h (surface particles).

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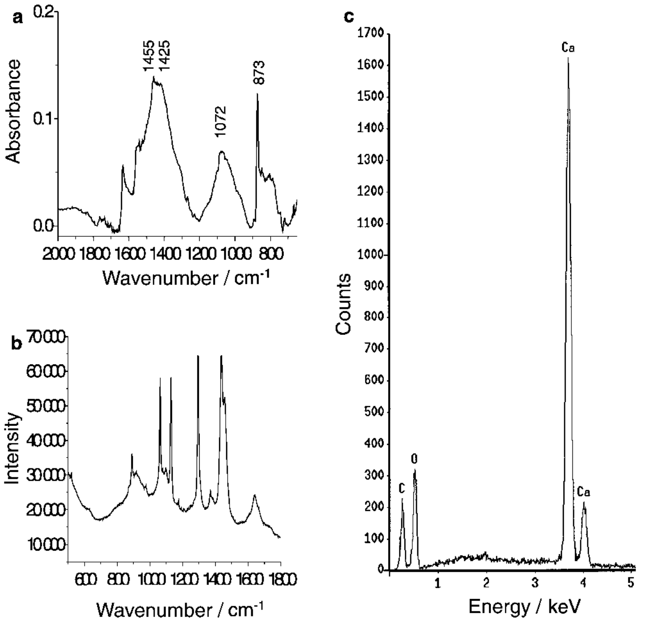


Figure 4. a) IR microscopy spectrum and b) Raman spectrum of a particle isolated after 1 day. c) EDX spectrum of an ACC sphere.

aggregates (Figure 4b). In addition, energy-dispersive X-ray analysis demonstrated the presence of $CaCO_3$ (Figure 4c).

Over the course of four days, a discrete population of {10.4} rhombohedral calcite crystals was also identified in the bulk solution of **1**/OA doped assays (Figure 5a), together with large isolated ACC particles (diameter $>10\,\mu m$; Figure 3b), of which a small portion still possessed a polyhedral shape (Figure 3c). It is notable that 80 % of these rhombohedra were associated intimately with ACC spheres, that is, these spherical particles were found to be enveloped by the crystalline form (Figure 3d, e). The spatial juxtaposition of the two $CaCO_3$ phases argues strongly for a critical role of the hybrid organic/inorganic spheres in fostering the nucleation and growth of the crystalline form. Another notable feature of the **1**/OA-doped crystallization assays was the persistent formation of ACC spheres at the air/solution interface (Figure 3f). It is our contention that the continuous formation of spheres at this location was linked to the surface activity of the surfactant/dendrimer complex. More specifically, that the active adsorption of the **1**/OA aggregates at the air/solution interface catalyzed the formation of ACC. It is significant that particle-size analysis of spheres harvested from this location over a fourteen-day period revealed a population of ACC spheres with diameters consistently in the range of 1–10 μm. In contrast, the particle-size profile of ACC spheres recovered from bulk solution over the same time period (Figure 5b) showed a gradual shift in size to larger dimensions ($>10\,\mu m$). This was accompanied by a decrease in the total number of isolated spheres and an increase in the proportion of spheres, which were associated with calcite. The ACC spheres allied with calcite were very uniform in size, having diameters of 8 μm, but comprised only 10 % of the population.

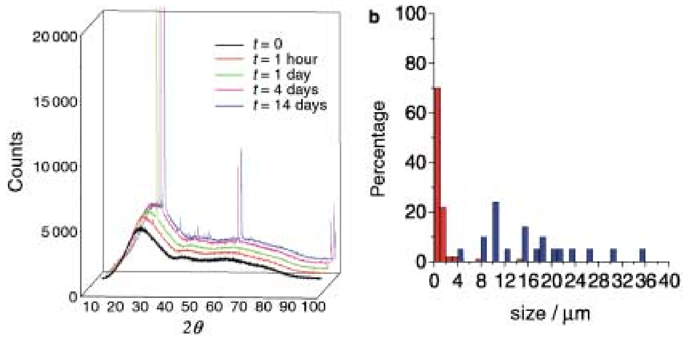


Figure 5. a) Powder diffraction patterns recorded from composites harvested from assays doped with **1**/OA showing the development of calcite and the persistence of ACC over the course of 14 days; b) Histograms indicating the size distributions of ACC spheres in bulk solution after 1 day (red) and 14 days (blue). Over the course of 14 days the population shifts toward larger particles, while the total amount of spheres is reduced to approximately 10 % of the initial population

Aggregates prepared of **1** alone also templated the formation of ACC spheres (Figure 6a). However, in this case the amount of ACC was reduced, and the transformation to calcite was more rapid than observed previously with the OA-modified aggregates: the ACC content of the sample after 24 hours was estimated to be only 10 % (Figure 6b).

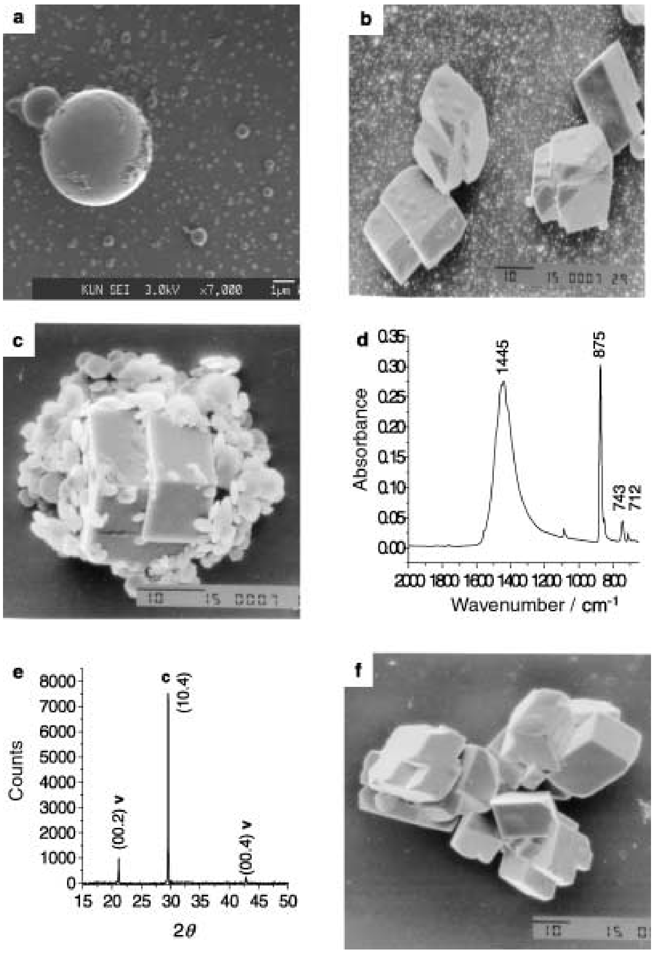


Figure 6. a)–c), f) Scanning electron micrographs of the crystallization assay doped with a) and b) **1**, c) and f) **1**/CTAB. Samples were collected after a) 5 h (surface particles), b) 4 days, c) 1 day, and f) 4 days. d) FTIR spectrum of crystals grown in the presence of **1**/CTAB recorded after 24 h. e) PXRD spectrum of **1**/CTAB recorded after 24 h; calcite reflections are marked "c", vaterite reflections "v".

The use of aggregates prepared from **1** and CTAB did not lead to the generation of ACC. Instead, the formation of rhombohedral calcite covered with disc-shaped crystals (Figure 6c), which according to FTIR (Figure 6d) and PXRD (Figure 6e) consisted of vaterite, was observed. Similar crystals have been observed when monolayers of stearic acid were used as template.[19] Over a period of five days, these vaterite discs were transformed into calcite (Figure 6f).

Addition of aggregates of **1**/SDS to the crystallization assay[20] resulted in the formation of spheres with diameters of 1–20 µm(Figure 7a). In addition, only a few calcite crystals were observed. PXRD and electron diffraction did not provide evidence for any long-range order. FTIR (Figure 7b, lower trace) showed only weak carbonate signals characteristic for calcite. However, strong peaks that could be attributed to the injected aggregates were observed. In addition, energy-dispersive X-ray (EDX) analysis of the spherical particles showed the presence of only minor amounts of calcium (Figure 7c). From this it was concluded that the spherical particles consist mainly of organic material. Indeed, aggregate preparation in a 9 mM $CaCl_2$ solution also resulted in the formation of giant spheres, in contrast to the much smaller aggregates observed in pure water (Figure 7d). Over the course of four days, calcite crystals were observed, into which the organic spheres had been incorporated (Figure 7e). As expected, at this stage, calcite vibrations dominated the FTIR spectrum (Figure 7b, upper trace), and PXRD also showed the development of the nonoriented crystalline phase.

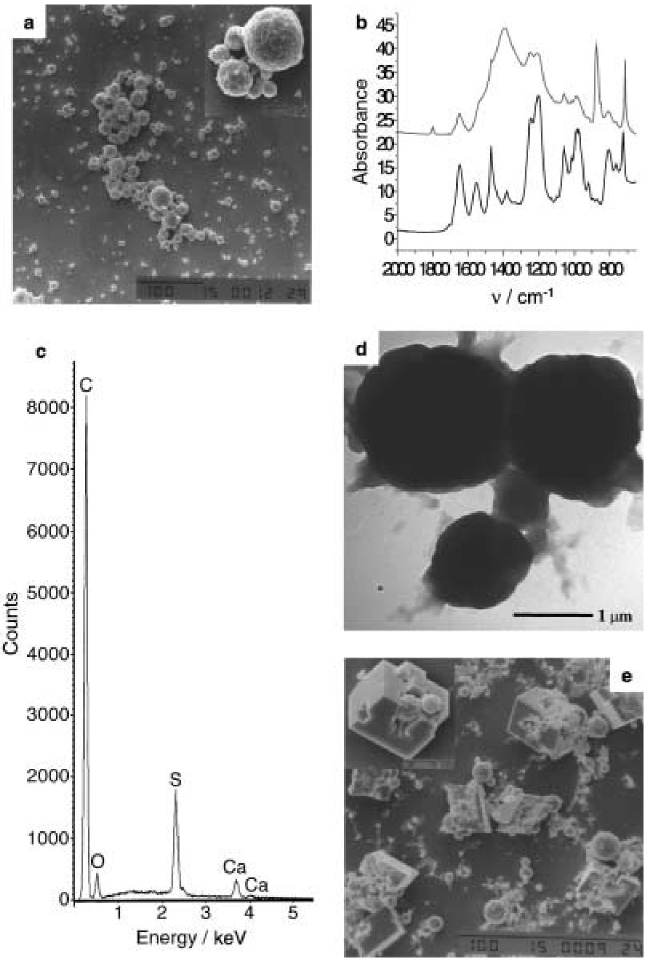


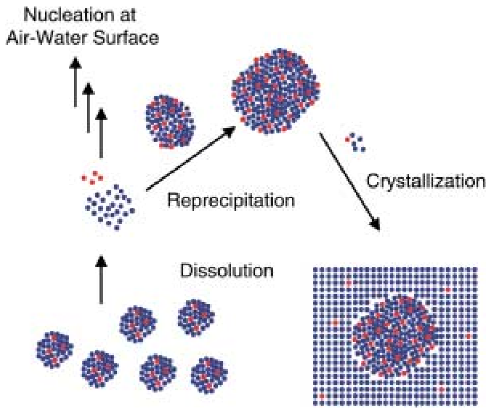
Figure 7. a), e) Scanning electron micrographs of assays doped with **1**/SDS of samples collected after a) 1 day and e) 4 days. b) FTIR spectrum of **1**/SDS assays recorded after 24 h (lower trace) and 4 days (upper trace). c) EDX spectrum of **1**/SDS spheres isolated after 1 day. d) Transmission electron micrograph of **1**/SDS in 9 mM $CaCl_2$.

Mechanistic Considerations: Although amorphous phases are generally kinetic products, it has been proposed that the formation of ACC follows from the inhibition of nucleation of the crystalline forms.[21, 8c, g] Under the crystallization conditions used in the present system only half of the surfactant molecules in the **1**/OA aggregates are protonated; this allows the formation of hydrogen bonds between neutral and protonated OA molecules. This, in combination with the extremely high rigidity of the aggregates, will limit the possibilities of the templates to adapt themselves so as to match a specific crystal face, and could possibly account for the formation of ACC rather than a crystalline phase. In the absence of surfactants, the template surface consists of molecules of **1**, which may be regarded as an assembly of head-group-polymerized surfactants. Consequently, the polar groups on the surface of these aggregates will also, in this case, exhibit a limited translational mobility.

The low charge density of the aggregates obtained from pure **1** and from **1**/OA ($\approx +0.5$ per surface amino group) most probably also relates to the inhibition of nucleation and growth of the crystalline polymorphs.

The stabilization of ACC is probably due to the presence of surface-adsorbed organic material that prevents dissolution of the ACC. However, at this stage it cannot be excluded that a continuous distribution of organic material throughout the ACC phase is needed to prevent crystallization from within the particles. The gradual dissolution of the small ACC particles and maturation of larger ones can be attributed to the process of Ostwald ripening, wherein particles with a high surface-to-volume ratio provide a continuous source of materials for those with lower surface activity.[22] In the present case, this process also effects the gradual release of the **1**/OA aggregates originally associated with the calcium carbonate spheres (Scheme 2). Thus, the depletion of macromolecular complex that accompanies ACC formation at the air/solution interface will be offset by the dynamic adsorption of surfactant/dendrimer aggregates released into bulk solution from the proportion of small ACC spheres that dissolves. One outcome of this activity will be the iterative formation of an active organic additive, capable of inducing the kinetic precipitation of calcium carbonate at the air/solution interface, which leads to a high local nucleation density of ACC. Another effect is the gradual decrease in the bulk of the solution of the additive concentration; this, may account for the increasing significance of the thermodynamically controlled precipitation of calcite, which results in the encapsulation of ACC particles.

The high positive surface charge of the CTAB-based aggregates (+1 per surfactant) may be expected to lead to the deposition of an initial layer of carbonate ions. It has been



Scheme 2. Schematic representation of the proposed aging mechanism in crystallization assays doped with **1**/OA.

proposed that accumulation of carbonate ions at the template surface preferentially stabilizes the formation of negatively charged nuclei and, hence, vaterite formation.[23, 19a] Although, the initially generated phase is again the kinetically favored polymorph rather than the thermodynamic most stable form, it is only a transient phase, and transformation into calcite is completed within four days when the material is in contact with water. This is in agreement with the generally observed lifetime of vaterite (60 hours) in synthetic systems.

The introduction of the **1**/SDS aggregates into a medium containing Ca^{2+} induces the agglomeration of the aggregates into giant spheres. This process depletes the solution of Ca^{2+} ions, therefore lowering the level of supersaturation.[23] This, in turn, leads to retardation in the formation of calcium carbonate. In time, the thermodynamically favored rhombohedral calcite nucleates and incorporates the organic spheres floating in solution, yielding a organic/inorganic composite.

Conclusion

The mixing of amphiphilic dendrimers and single-chain surfactants leads to stable shape-persistent aggregates with tunable surface chemistry. It has been demonstrated that the use of amine-derived surfactants results in the generation and (temporary) stabilization of different metastable phases of calcium carbonate, whereas the use of a surfactant with a sulfate head group leads to retardation of calcium carbonate growth due to the very strong interaction with calcium ions. The use of a single scaffold able to complex a variety of surfactants opens a way to a combinatorial approach toward the synthesis of different calcium carbonate polymorphs and crystal habits by combination of this scaffold with the large variety of surfactants that is commercially available.

The self-reinforcing combination of **1** and OA was able to significantly stabilize ACC particles. Although ACC has been observed as a short-living intermediate in the presence of various scale prevention agents, to our knowledge only templates from biological origin allow the coexistence of amorphous and crystalline calcium carbonate for extended periods of time.[8] The durability of ACC in this system suggests a key role for the organic component in first favoring the formation of the kinetic product at the expense of the thermodynamically stable form, calcite, and secondly in stabilizing the amorphous phase against transformation resulting in the formation of a unique inorganic/organic hybrid. Indeed there are numerous examples where the synchronous stabilization of amorphous and crystalline phases leads to remarkable materials properties. As the blending of amorphous and crystalline phases has been demonstrated to significantly enhance the toughness of materials, the present results open the way for the generation of new composites with improved materials properties.

Experimental Section

Calcium carbonate (precipitated, p.a.) was purchased from Merck, hexadecyltrimethylammonium bromide (>99%) and sodium dodecylsulfate (>99%) were purchased from Sigma and octadecylamine (99%) from Fluka. THF (AR—analytical reagent) and ethanol (AR) were purchased from Biosolve Ltd. All reagents and solvents were used as received. Ultrapure water was generated by using a Barnstead Easypure LF water-purification system. Fluorescence spectra were recorded on a Perkin–Elmer LS50B spectrometer. Transmission electron microscopy was performed on a Jeol 100CX or Philips EM201 microscope at an accelerating voltage of 60 kV. Scanning electron microscopy studies on gold-sputtered samples were performed on a Philips SEM-500 microscope at an accelerating voltage of 15 kV. PXRD measurements were performed on a Philips PW1710 diffractometer equipped with a Cu LF X-ray tube operating at 40 kV and 55 mA. Raman microscopy was performed on a Dilor Labram Raman microscope in reflection mode. IR microscopy was performed on a BIO-RAD UMA500 IR microscope in transmission mode with a ZnSe sample holder.

Aggregate preparation: Typically, a solution of **1** (0.83 mmol) and surfactant (58 mmol) in THF/EtOH (2:1, *v*/*v*, 100 μL) was injected into ultrapure water (7.0 mL) at 60 °C and sonicated for 1 hour. In the case of the negatively charged surfactant SDS, the surfactant was dissolved directly in the ultrapure water before the injection of a solution of **1** in THF/EtOH. For characterization of the aggregates the suspension (1.0 mL) was injected into ultrapure water (25.0 mL) at room temperature.

Crystallization setup: The aggregate dispersion (1.0 mL) was injected into a supersaturated solution of calcium carbonate (25.0 mL) prepared by the Kitano method ($[Ca^{2+}] = 9$ mM; $[\mathbf{1}] = 5.3$ μM; [surfactant] = 0.37 mM).[20] The crystals, which had grown in the bulk of the solution and at the air/water interface, were collected on glass microscope cover slips.[18] Blank experiments performed to investigate the role of air-drying of artifacts only showed the formation of calcite and vaterite.[24]

Fluorescence depolarization experiments: Aggregate dispersions containing all-*trans*-1,6-diphenyl-1,3,5-hexatriene (DPH) as fluorescent probe were prepared as described above. Concentrations were [dendrimer] = 68 μM and [DPH] = 34 μM. Fluorescence-emission spectra were recorded at $\lambda_{ex} = 382$ nm. The anisotropy was measured over a temperature range of 20–75 °C with steps of 5 °C and 15 min equilibration periods.

Electron microscopy: TEM samples were obtained by placing a drop of solution onto a carbon-covered, formvar-coated copper grid followed by immediate drainage. Aggregate samples were platinum shadowed.

Microtomy experiments: Microtomy was performed on a sample of lyophilized **1**/OA aggregates embedded in LR White Resin (London Resin Company) followed by polymerization at 60 °C for 24 hours. Subsequently, thin coupes were cut by using a diamond knife. After staining, the coupes were evaluated by using transmission electron microscopy at an accelerating voltage of 60 kV.

Powder X-ray diffraction: PXRD was performed by mounting the cover slips on a silicon wafer. Experiments were carried out in the range $2\theta = 10$–$100°$ with a step width of 0.02 and a step time of 25 s.

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