

# Amorphous calcium carbonate stabilised by poly(propylene imine) dendrimers

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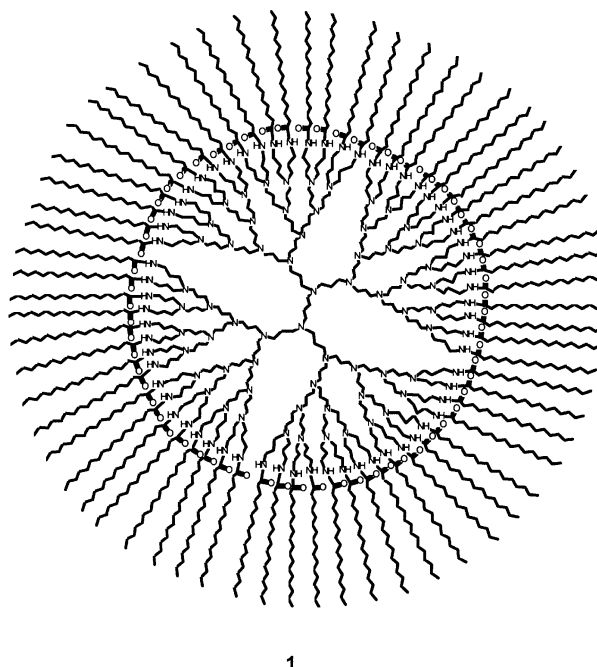
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In the presence of octadecylamine, poly(propylene imine) dendrimers modified with long alkyl chains self-assemble to form well-defined aggregates that stabilise for periods exceeding 14 days the normally unstable amorphous calcium carbonate (ACC) which persists in competition with the thermodynamic product, calcite.

Templates that consist of organised biomacromolecules are known to direct the formation of most biominerals, in many cases leading to materials with unique shapes and properties.<sup>1</sup> In synthetic assays, the use of ordered supramolecular assemblies, such as micelles,<sup>2</sup> monolayers,<sup>3</sup> vesicles,<sup>4</sup> inverted micelles<sup>5</sup> and lyotropic liquid crystalline systems,<sup>6</sup> 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 mollusc shells, for example.<sup>7</sup> In nature, all three crystalline calcium carbonate polymorphs, calcite, aragonite and the metastable vaterite, are observed. In addition, amorphous calcium carbonate, which is normally unstable at room temperature and pressure, has been reported.<sup>8</sup> In synthetic milieu this amorphous phase is unstable and transforms readily into one of the crystalline polymorphs. 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 stabilise spheroids of amorphous calcium carbonate (ACC) for extended periods of time.

Poly(propylene imine) dendrimers modified with long aliphatic chains are a new class of amphiphiles which display a variety of aggregation states due to their conformational flexibility.<sup>9</sup> Globular aggregates are formed when C<sub>16</sub>-modified 5th generation dendrimer **1** is dispersed in aqueous media.<sup>10</sup> In the present work we have modified the outer surface of this dendrimer assembly by addition of **1** to aqueous solutions containing single chain surfactants (octadecylamine, OA, and cetyltrimethylammonium bromide, CTAB) at concentrations below their CMC (Scheme 1). In this way we were able to generate solid self-reinforced aggregates with different shape, size and surface chemistry depending on the choice of surfactant.<sup>†</sup> It was found that aggregates arising from the interaction of **1** and octadecylamine (OA) expressed a persistent polyhedral shape,<sup>11</sup> as well as a narrow size distribution (Fig. 1a,b). The extremely high fluorescence depolarization anisotropy values found for the aggregates at room temperature ( $r = 0.32$ ) indicate that they have a very rigid structure.<sup>‡</sup> 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 (not shown). Fluorescence depolarisation measurements showed that these aggregates are less rigid ( $r = 0.24$ ) with respect to the dendrimer/octadecylamine aggregates.

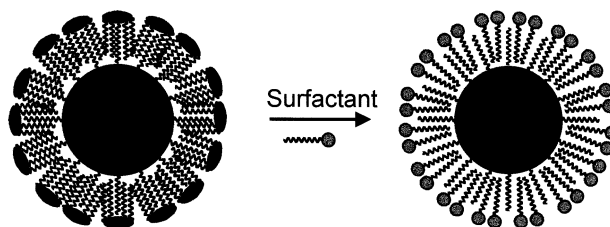
The remarkable rigidity and the well-defined size and shape of the aggregates consisting of **1** and OA prompted us to exploit them as 3D templates in calcium carbonate crystallisation



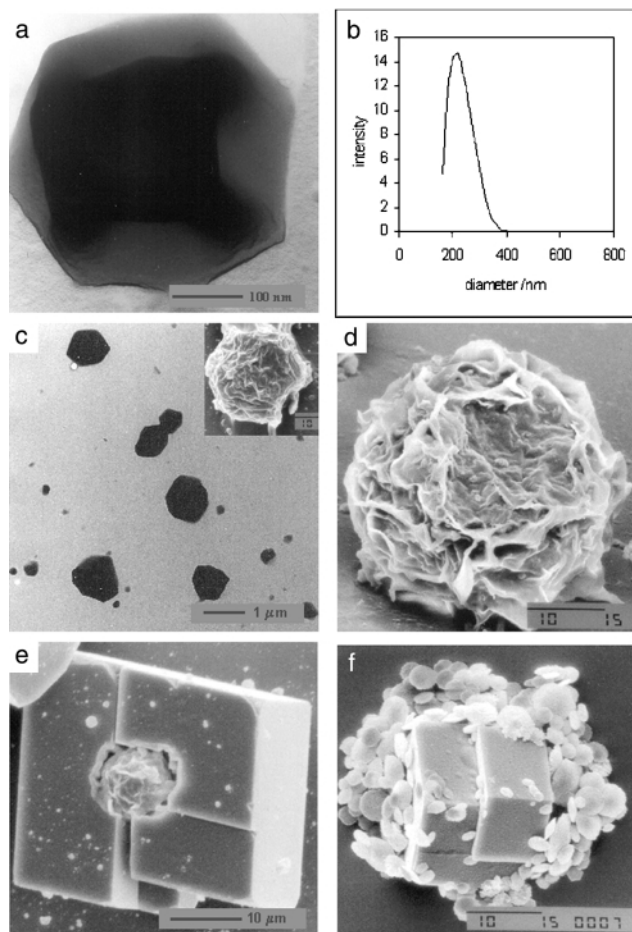
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assays.§ The addition of these novel templates to such an assay catalysed the formation of amorphous calcium carbonate. Indeed, calcified aggregates, which had retained their polyhedral shape, were recovered after only 15 min (Fig. 1c). FTIR showed strong peaks characteristic for hydrated ACC at 3433 and 1070 cm<sup>-1</sup>,<sup>12</sup> whereas only minor peaks characteristic of calcite were observed (1420, 875 and 712 cm<sup>-1</sup>). Selected area electron diffraction analyses confirmed that the vast majority of these particles consisted of amorphous material.

Over the course of 4 days a discrete population of {10.4} rhombohedral calcite crystals was also identified in the bulk solution of **1**/OA doped assays, alongside large isolated ACC particles (diameter ≥ 10 μm; Fig. 1d) a small portion of which still possessed a polyhedral shape (Fig. 1c, inset). It is notable that 80% of the rhombohedra enveloped an ACC sphere (Fig. 1e). The spatial juxtaposition of the two CaCO<sub>3</sub> phases argues



**Scheme 1** Schematic representation of the modification of the outer layer of dendrimer-based aggregates using single chain surfactants.



**Fig. 1** (a) Transmission electron microscopy (TEM) image (platinum shadowing) of an aqueous dispersion of 1/OA. (b) Size distribution of aggregates of 1/OA as determined by dynamic light scattering. (c) TEM image of the crystallisation assay doped with 1/OA after 15 min, inset: scanning electron microscopy (SEM) image of the assay after 1 h. (d–f) SEM images of the assay doped with (d,e) 1/OA and (f) 1/CTAB. Images were recorded (d,f) after 4 days and (e) after 14 days.

strongly for a critical role of the spheres in fostering the nucleation and growth of the crystalline form.

Another notable feature of the 1/OA doped crystallisation assays was the persistent formation of ACC spheres at the air/solution interface. It is our contention that the continuous formation of spheres at this location is linked to the active adsorption of the 1/OA aggregates at the air/solution interface. 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  $\mu\text{m}$ . In contrast, the particle size profile of ACC spheres recovered from bulk solution over the same time period showed a gradual shift in size to larger dimensions (> 10  $\mu\text{m}$ ). This was accompanied by a diminution 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  $\mu\text{m}$ , but comprised only 10% of the population. From the integration of FTIR spectra it was concluded that at this stage 20% of the calcium carbonate was still present in the form of spherical ACC.

Aggregates prepared of 1 alone also templated the formation of ACC spheres. 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: after 24 h 80% of the sample had been converted to calcite compared to 16% in the case of the 1/OA aggregates. The use of aggregates

prepared from 1 and CTAB did not lead to the generation of ACC. Instead, the formation of calcite crystals covered with disc-shaped vaterite crystals was observed (Fig. 1f). Over a period of 5 days these vaterite discs disappeared and only calcite crystals remained.

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.<sup>13</sup> In the present case this process also effects the gradual release of the 1/OA aggregates originally occluded within the calcium carbonate spheres. Thus, the depletion of polymeric complex, which 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 which dissolves. One outcome of this activity will be the iterative formation of an active organic template, capable of inducing the kinetic precipitation of calcium carbonate at the air/solution interface leading to a high local nucleation density of ACC.

Although ACC has been observed as a short-lived intermediate in the presence of various scale prevention agents, to our knowledge only biological systems allow the coexistence of amorphous and crystalline calcium carbonate for extended periods of time.<sup>12</sup> The nucleation of the kinetic product at the expense of the thermodynamically stable form, calcite, and the stabilisation of the amorphous phase against transformation results in the formation of a unique inorganic–organic hybrid material.

## Notes and references

† A solution of 18.5 mg of 1 and 15.5 mg surfactant in 100  $\mu\text{l}$  THF/EtOH (2:1 v:v) was injected into 6 ml Ultrapure water at 60  $^{\circ}\text{C}$  and sonicated for 1 hour. Subsequently, 1.0 ml aggregate suspension was injected into 25 ml Ultrapure water at room temperature. A detailed investigation of the structures of these aggregates will be published elsewhere.

‡ Fluorescence depolarization was carried out using DPH which in a frozen system has an intrinsic anisotropy value of  $r_0 = 0.362$ .<sup>14</sup>

§ The aggregate dispersion (1.0 ml) was injected into 25 ml of a supersaturated solution of calcium carbonate ( $[\text{Ca}^{2+}] = 9 \text{ mM}$ ;  $[\text{I}] = 5.3 \mu\text{M}$ ;  $[\text{surfactant}] = 0.37 \text{ mM}$ ).<sup>15</sup>

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