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# Supplementary Information for

## Formation of Nano-Magnesite in the Calcareous Spicules Prepared under Ambient Conditions

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## Materials and Methods

**Sample Preparation.** All chemicals were obtained from Acros unless stated otherwise. A lipid thin film was prepared by dissolving 0.31 g of L-α-lecithin (Sigma-Aldrich, product number P-3644) in 3 mL of dichloromethane, followed by rotary evaporation (190 rpm, 40 °C) for 5 min and then drying in vacuo for an hour. To the thin film, a solution of 5 mL of $CaCl_2$ (0.2 M) and $MgCl_2$ (1.0 M) at pH 8.75 was added. The solution mixture was sonicated for 5 min. Then, a solution of 5 mL of $(NH_4)_2CO_3$ (0.2 M, pH 8.75) was added under sonication. The solution was incubated under quiescent conditions at room temperature for different setting time. The final pH was in the range of 7.2 to 7.6. After 10 mL of dichloromethane was added to the solution mixture, the precipitate of calcium carbonate was collected through repeat centrifugation (10000× *g*, 20 min) and resuspension in dichloromethane. The solid sample was flushed with nitrogen gas before measurements.

**Sample Characterization.** Scanning electron microscopy (SEM) images were taken on JEOL JSM-7600F and Hitachi S4800 field emission scanning electron microscopes. The energy dispersive X-ray (EDX) analysis was performed on a LEO 1530 field emission scanning electron microscope equipped with EDX accessories. High-resolution transmission Electron Microscopy (HRTEM) imaging was carried out on a Philips Tecnai F20 field emission gun transmission microscope and a Philips Tecnai T20 $LaB_6$ gun transmission microscope operating at 200 kV.

**Focused Ion Beam Preparation.** Focused ion beam (FIB) milling was performed with a SEIKO SMI 3050 SE system, following the procedure documented in the literature.[1] Briefly, a linear Pt "strap" of thickness 20 to 50 nm was deposited on the sample surface, followed by carbon deposition of ~1 μm in thickness. After excavating trenches on both sides of the strap by a 30 kV $Ga^+$ beam, the foil was thinned to a thickness of 70 to 80 nm at a beam current of ~100 pA.

**Transmission X-ray Microscopy.** TXM images were obtained at TLS BL01B1 beamline of NSRRC. A superconducting wavelength shifter source is used for providing a photon flux of $4 \times 10^{11}$ photons $s^{-1}$ (0.1% bw)$^{-1}$ in the energy range of 5–20 keV in this beamline. A monochromator utilizing a pair of Ge(111) crystals selected monochromatic X-rays with an energy of 8 keV. The image of the specimen was magnified using a Fresnel zone plate with 45-nm outer zone width, which provides about 60-nm spatial resolution using the first-order diffraction mode. Fresnel phase contrast images are performed using a phase ring installed at back focal plane of the zoneplate for imaging of low atomic number materials. After acquiring a series of 2D projections with the sample rotated stepwise, Faproma alignment algorithm was used for correcting vertical and rotational motion errors of each projection for improving 3D reconstruction quality. The aligned projections were reconstructed by applying a maximum likelihood estimation based reconstruction algorithm using 181 sequential projections taken with the azimuth angle rotating from −90° to +90°. The final 3D tomography was visualized using Amira 3D software. Powder specimens were sparsely spread on thin Kapton tape, and no any other specimen preparation process was needed before the observation.

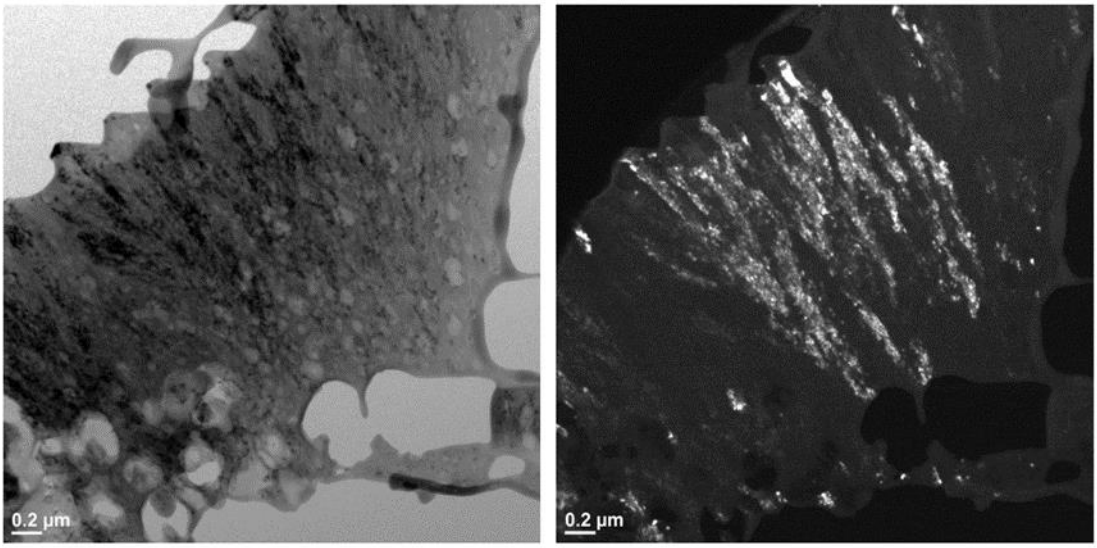



**Fig. S1**. Bright-field (left) and dark-field (right) TEM images of a thin foil of S24h.

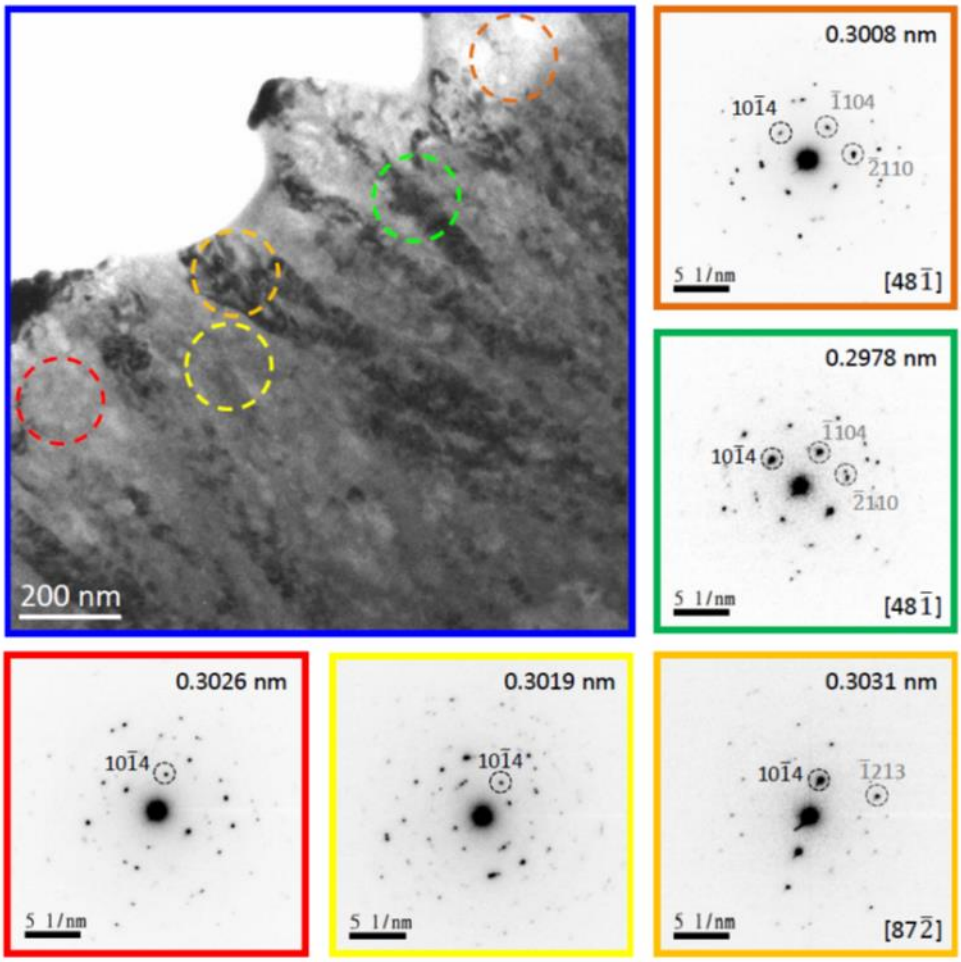



**Fig. S2.** SAED patterns of the S24h sample measured at the regions enclosed by the dashed circle. The frame color of the SAED patterns matched the color of the dashed circles. The diffraction intensities taken in the dark-stripe regions (orange and green) are higher than other regions (red, yellow, and brown). Thus, the dark and light regions did not have identical crystallinity and the appearance of dark stripes was owing to their higher crystallinity. Note that the orientation of the SAED patterns of the brown and green boxes are similar but they had different intensities. Some SAED patterns could be assigned largely to a single calcitic lattice (brown, green, orange), whereas the others were polycrystalline in nature (red and yellow). The d-spacing of (**$10\bar{1}4$**) obtained from the SAED patterns, which ranged from 0.2978 to 0.3031 nm (listed on top right corner of the color boxes), revealed that the substitution level of Mg in the calcite lattice varied considerably in the samples.

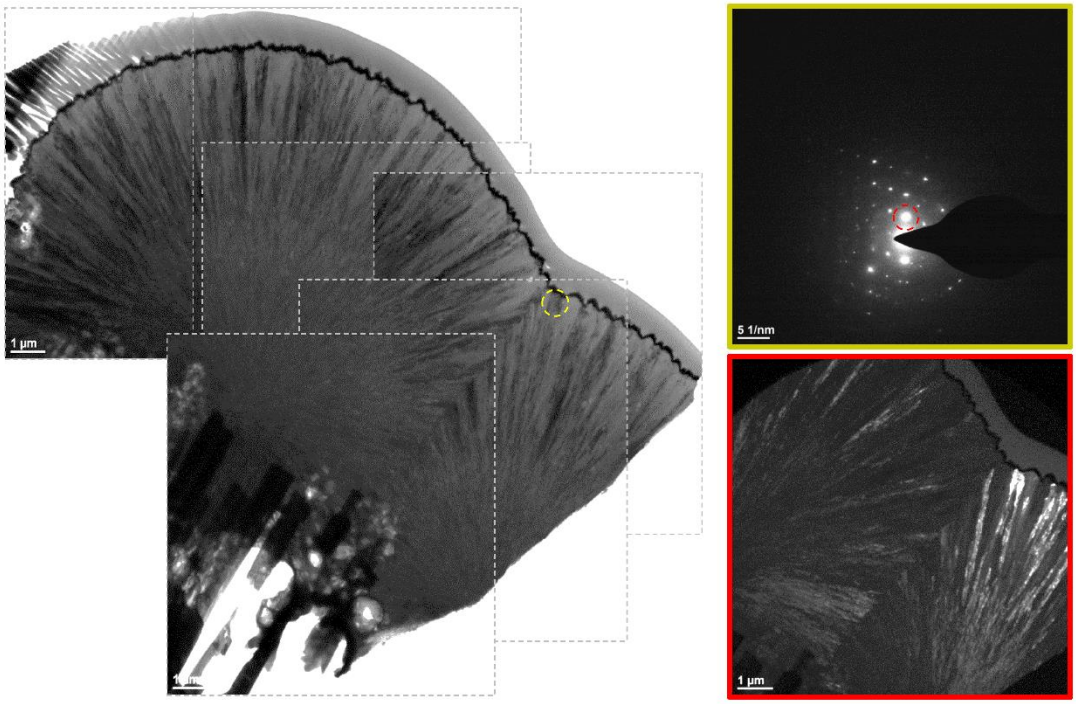



**Fig. S3.** SAED pattern, bright-field and dark-field images of a twin-spherulite acquired for the FIB foil of S48h. The SAED pattern (with dark yellow frame) was taken from the region highlighted in the bright-field image by a dashed circle in dark yellow. The dark-field image (with red frame) was taken with respect to the diffraction spot of the SAED pattern highlighted by a dashed circle in red. A sharp boundary is observed between the two hemispherulites.

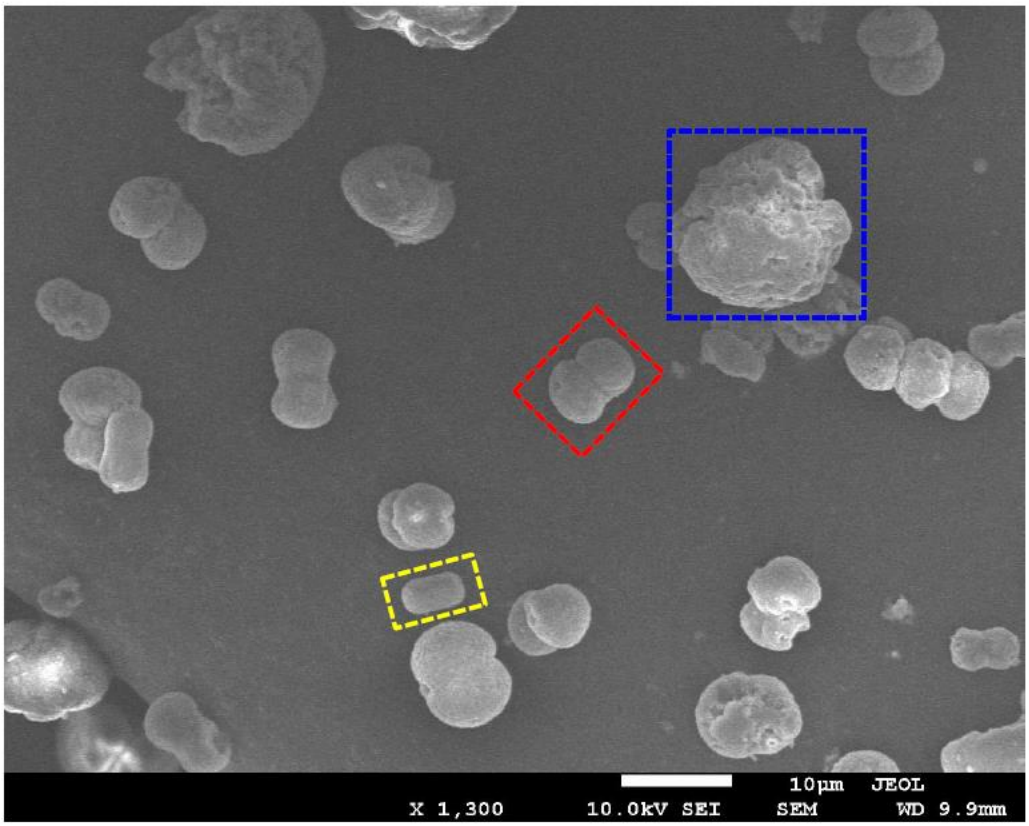



**Fig. S4.** Typical SEM image of S16h, where three distinctive morphologies of different size could be observed: capsules (yellow), spherulites (blue), and twin-spherulites (red). No spicules were found.

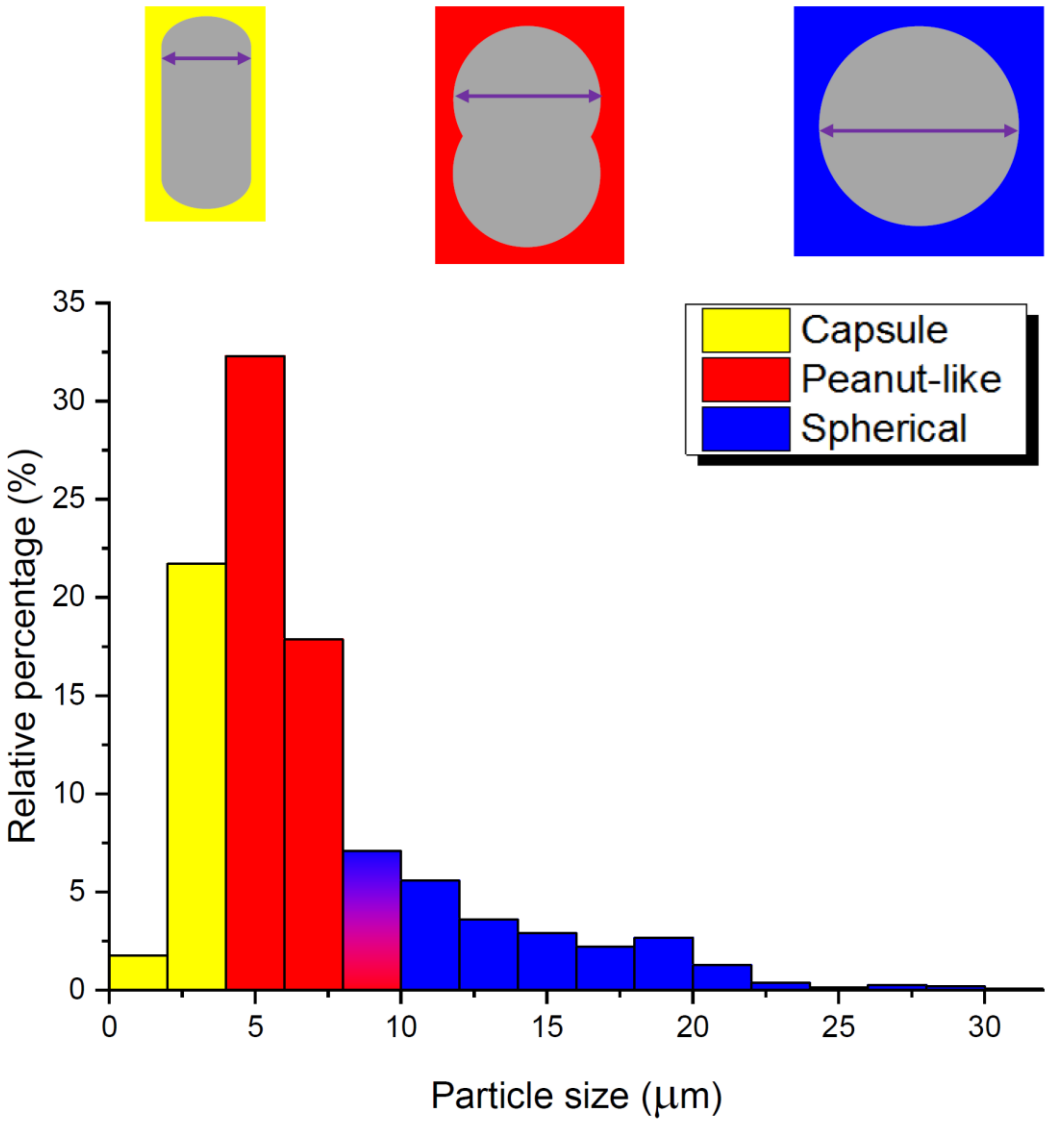
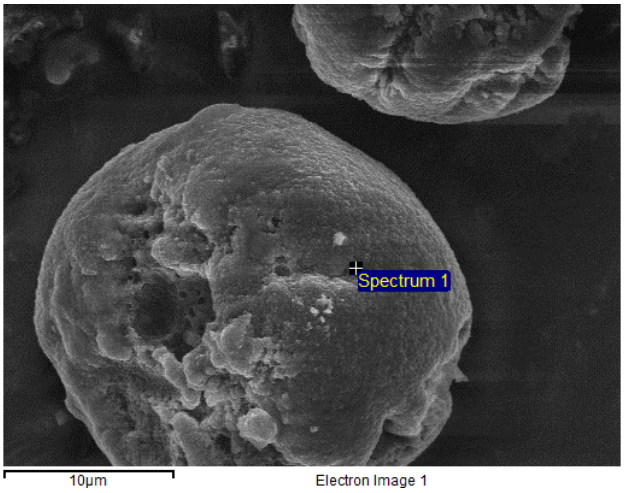
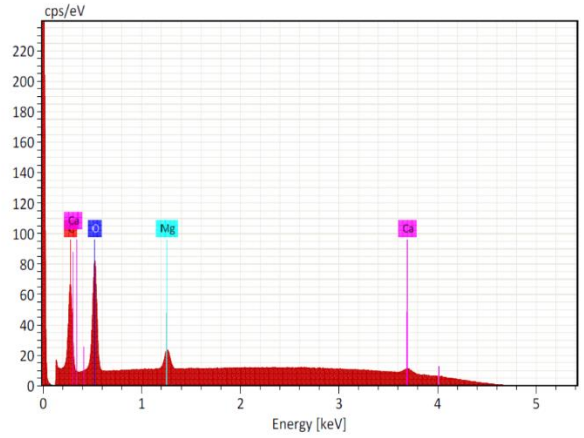



**Fig. S5.** Distribution of the crystallite size of the S16h sample obtained by analyzing the SEM images of 968 particles. The crystallite size was defined as the length of the arrows. The ratios of the counts for the capsules, spherulites, and twin-spherulites were approximately 1:2:2, respectively.




| Element | Weight% | Atomic% |
|---|---|---|
| C K | 16.01 | 24.39 |
| N K | 3.87 | 5.06 |
| O K | 47.50 | 54.33 |
| Mg K | 4.40 | 3.31 |
| Cl K | 0.41 | 0.21 |
| Ca K | 27.81 | 12.70 |
| Totals | 100.00 | |

**Fig. S6**. SEM-EDX data acquired for spherulites in S50h. The Mg content, Mg/(Mg+Ca), was calculated as 21%.




| Element | At. No. | Line s. | Netto | Mass [%] | Mass Norm. [%] | Atom [%] | abs. error [%] (1 sigma) | abs. error [%] (2 sigma) | abs. error [%] r (3 sigma) |
|---|---|---|---|---|---|---|---|---|---|
| Carbon | 6 | K-Serie | 119512 | 0.35 | 6.92 | 12.36 | 0.06 | 0.13 | 0.19 |
| Oxygen | 8 | K-Serie | 251317 | 1.93 | 38.51 | 51.62 | 0.23 | 0.47 | 0.70 |
| Magnesium | 12 | K-Serie | 65101 | 0.98 | 19.63 | 17.32 | 0.08 | 0.15 | 0.23 |
| Calcium | 20 | K-Serie | 30149 | 1.75 | 34.94 | 18.70 | 0.18 | 0.37 | 0.55 |
| | | | **Sum** | **5.01** | **100.00** | **100.00** | | | |

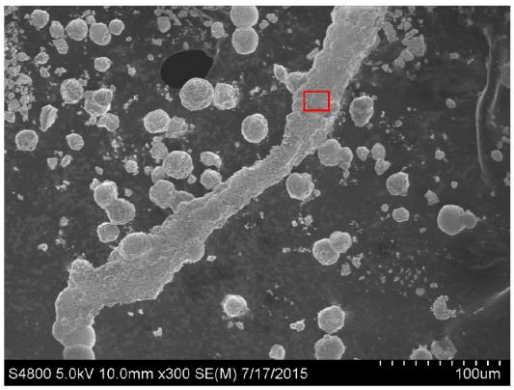



**Fig. S7**. SEM-EDX data acquired for spicules in S28hA4m. The Mg content, i.e., Mg/(Mg + Ca), on the spicule surface was calculated to be 48%. Some spherulites in aged samples did show a large variation in Mg content (14–60%). It indicated that the segregation of Ca- and Mg-rich phases could occur in both the spherulites and spicules during the aging process.


**Fig. S8.** SEM image of S3dA2m.

Movie S1 (separate file). Three dimensional tomograph of a spherulite of S16h.

## References

(1) Heaney, P. J.; Vicenzi, E. P.; Giannuzzi, L. A.; Livi, K. J. T. Focused Ion Beam Milling: A Method of Site-Specific Sample Extraction for Microanalysis of Earth and Planetary Materials. *Am. Mineral.* **2001**, *86* (9), 1094–1099. https://doi.org/10.2138/am-2001-8-917.