

Fullerenol Entrapment in Calcite Microspheres

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SUPPORTING INFORMATION

1 Experimental section

1.1 Preparation of hybrid microspheres

Calcium chloride dihydrate and anhydrous calcium chloride granular (for drying) manufactured by Merk Co. were used. Ammonium carbonate was supplied by Sigma Co. Fullerenol was supplied by BuckyUSA. Water Millipore grade (18.2 M Ω) was used to prepare solutions.

Calcium carbonate hybrids microsphere precipitation was carried out using microplates, 24 wells (dia. 16 mm) with lid, tissue culture treated polystyrene supplied by IWAK brand and glass cover round slides (12 mm). The 24 wells microplate was prepared introducing an ethanol-washed and post-dried glass cover slide in each well. 750 μ L of a solution of 0.1, 1.0 or 4.0 mg/mL of fullerenol in 10.0 mM calcium chloride solution was introduced to each well. The wells were covered with aluminum foil and punctured by a needle. Then the microplate was placed inside a sealed Plexiglass box containing ammonium carbonate and anhydrous calcium chloride.

The crystallization process started with the vapor diffusion of CO₂ and NH₃ from ammonium carbonate in the calcium chloride solutions at room temperature. After 4 days the crystallization process was stopped by opening the Plexiglass box. First obtained precipitates were washed with deionized water (750 μ L for well) at least twice and then the microplates were air-dried for

two days at room temperature. In each crystallization experiment control wells containing only a pure calcium chloride solution were present. Crystals were initially examined directly in wells by an optical microscope (Leika optical microscope connected to a CCD digital camera).

1.2 SEM

The dried glass coverslips covered with crystals were glued to SEM aluminum stubs. After gold coating, the crystals were observed in the SEM (Philips 515) using a tension of 15 kV.

The presence of fulleranol influenced the crystallization process as a function of its concentration in solution. The morphology of calcite can be modified by the addition of ions or molecules. These additives can interact with unstable crystalline faces during the crystal growth process. As a consequence of this interaction the stabilized faces contribute to the final habitus of the crystal. The stabilization process can be specific on a peculiar ions arrangement of a crystalline face or involve several crystalline faces. This brings to different final morphologies. When the interaction is not specific, as occurs for fulleranol, the stabilization of several faces generates a rounded morphology.

1.3 XRD

X-ray powder diffraction (XRD) patterns were recorded using a Philips X'Celerator diffractometer with Cu K α (1.5814 Å) radiation. The samples were scanned for 2 θ angles between 20° and 60°, with a resolution of 0.02°.

1.4 FTIR

In the FTIR analysis each powdered sample (approximately 0.1 mg) was mixed with about 10 mg of anhydrous KBr. The mixtures were pressed (670.2 MPa) into 7 mm diameter discs. Pure KBr discs were used as background. The analysis was performed at 4 cm⁻¹ resolution using a Nicolet 380 FT-IR spectrometer.

1.5 Photophysical measurements

An ultra pure water obtained using a Milli-Q system by Millipore was used for the photophysical studies.

UV-Vis absorption spectrum was performed by means of a Perkin-Elmer Lambda 45 spectrophotometer. Steady-state emission spectra were recorded on a HORIBA Jobin Yvon Fluorolog 3 spectrometer equipped with a 450 W xenon arc lamp, double-grating excitation and emission monochromators and a TBX-4-X single-photon-counting detector. Emission spectra were corrected for source intensity (lamp and grating) and emission spectral response (detector and grating) by standard correction curves. Emission spectra were measured in optically dilute solutions (O.D. < 0.1 at excitation wavelength).

The experimental error on molar absorption coefficients and emission intensities is estimated to be $\pm 5\%$. Quartz cuvettes with optical path length of 1 cm were used for liquid and solid state (fullerenol-CaCO₃ crystals) measurements in a right angle geometry.

1.6 Fluorescence confocal microscopy measurements

Confocal laser microscopy images were collected using a Nikon Eclipse C1 system equipped with a Nikon Eclipse TE2000 inverted fluorescence microscope using a 488 nm laser source and observing emission at 520 nm

2 Photophysical characterization of fullereneol in water solution

The absorption spectrum of fullereneol $C_{60}(OH)_x$ in water solution ($C = 2 \mu\text{M}$) at room temperature (Figure SI 1) shows a broad band in the UV-Vis region (starting from 600 nm up to 200 nm) that is typical of the fullereneol molecule.

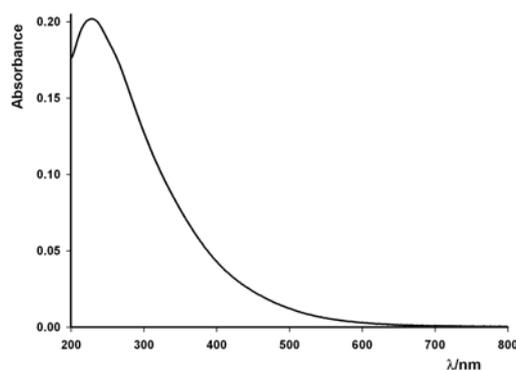


Figure SI 1. UV/Vis absorption spectra of Fullereneol in water solution.

The luminescence spectra of the fullereneol (Figure SI 2) were performed using both excitation and emission slits set at 5 nm band widths and the spectra have an emission band between 400 and 700 nm when excited at 300 nm. The fluorescence quantum yield of the fullereneol in water solution is $\Phi = 5 \times 10^{-3}$ (the quantum yield was determined using perylene in deoxygenated ethanol solution as a reference ($\Phi = 0.97$), whereas its excited-state decay could be fitted with a multi-exponential model (average lifetime = 5 ns).

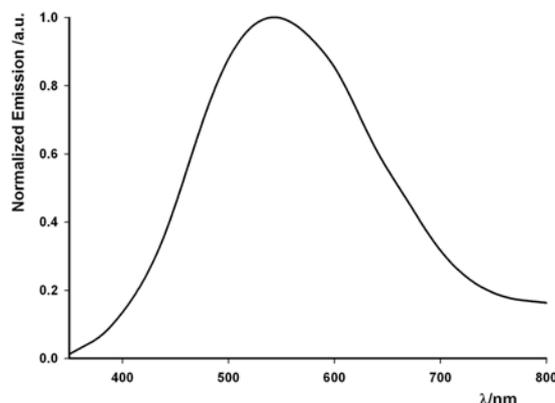


Figure SI 2. Room-temperature emission spectra in water solution of fullereneol (λ_{exc} 300 nm; 5x5 slits)

Thanks to excitation-emission matrix scans, we also observed that the emission spectrum of fulleranol changes with excitation wavelength: a small red-shift occurs going from excitation at 300 to 560 nm.^a

3 Determination of weight and molar percentage of fullereneol in the hybrid microspheres and its release

We dissolved the different crystals in HCl 0.1 M and carried out spectroscopic measurements to determine the content in fullereneol (UV-vis spectroscopy to calculate fullereneol absorbance at 270nm) and CaCO₃ (Flame Atomic Absorption Spectroscopy to calculate the Ca²⁺ ion concentration). The release measurements are carried out in HEPES 0.1 M pH 6.5.

Table SI 1. Summary of the spectroscopic analysis on the dissolved microparticles B, C and D.

Doping degree of fullereneol	Fullereneol Absorbance Measurements ^a		Elemental Analysis Ca ²⁺	Elemental Analysis CaCO ₃ ^b	Weight %	Mole %
0.1 mg/mL	4.04 e-6 M	= 4.3 mg/L	80 mg/L	200 mg/L	2.10 %	0.20 %
1.0 mg/mL	3.37 e-5 M	= 35.72 mg/L	47 mg/L	117.5 mg/L	23.31 %	2.81 %
4.0 mg/mL	3.80 e-5 M	= 40.28 mg/L	26 mg/L	65 mg/L	38.26 %	5.52 %

^a $\epsilon(270 \text{ nm}) = 11569.01 \text{ g/mol}$

^b $40 \text{ g/mol (Ca}^{2+}) = 100 \text{ g/mol (CaCO}_3)$

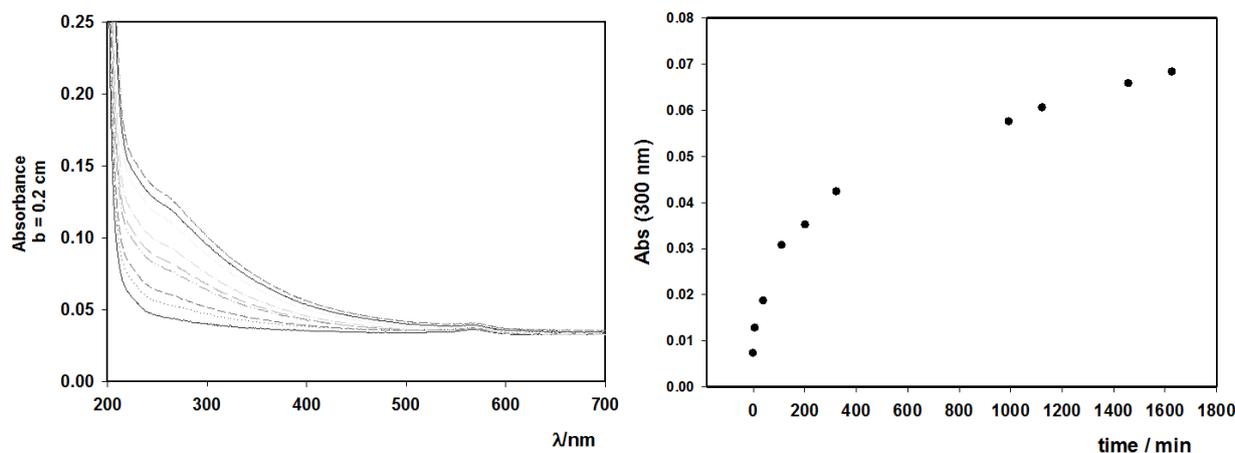


Figure SI 3. Kinetic of the release of fullereneol from sample D at pH 6.5 (environmental temperature)

4 Confocal fluorescence Microscopy Measurements of B and C.

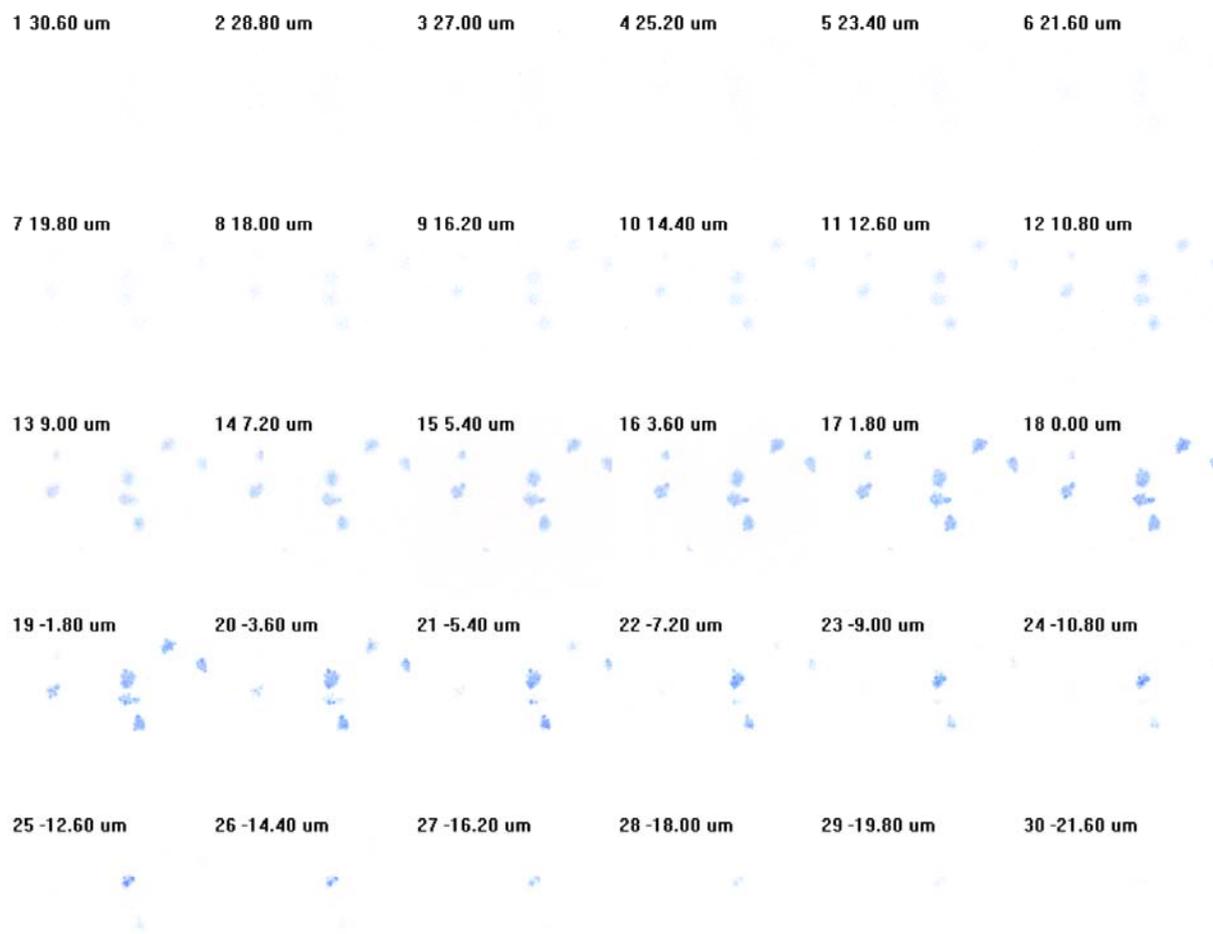


Figure SI 4. Fluorescence images obtained scanning thirty Xy sections of a sample of crystals B with a confocal microscope for different value of z (ranging from 30.60 to -20.60 μm with an interval of 1.80 μm). Inverted colors.

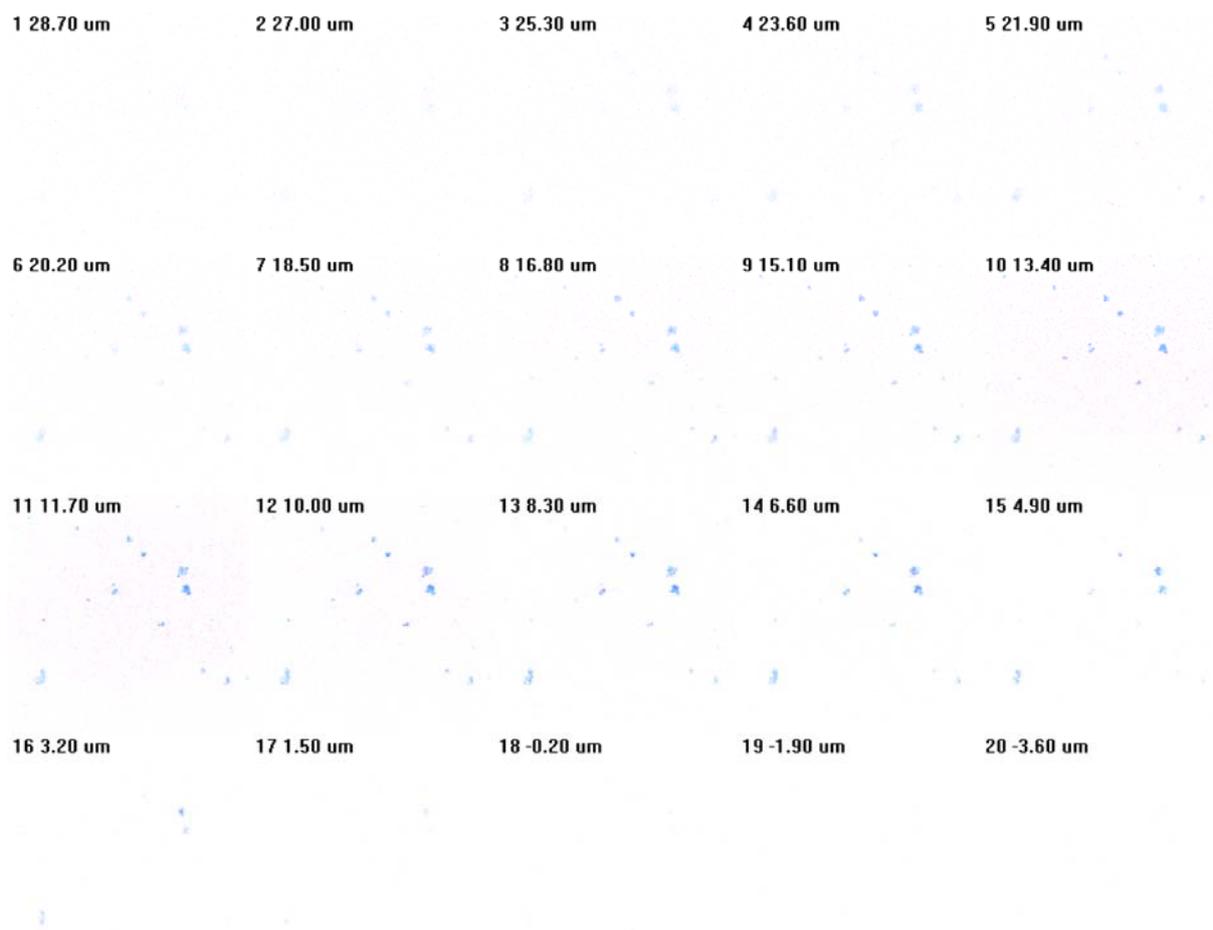


Figure SI 5. Fluorescence images obtained scanning thirty *Xy* sections of a sample of crystals *C* with a confocal microscope for different value of *z* (ranging from 30.60 to -20.60 μm with an interval of 1.80 μm). Inverted colors.

a) L. Kong, O. Tedrow, Y. F. Chan and R. G. Zepp, *Environ. Sci. Technol.* **2009**, *43*, 9155–9160.