

Dependence of sub-micron vaterite container release properties on pH and ionic strength of the surrounding solution

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Abstract. We report on the synthesis and characterization of porous monodisperse vaterite containers with controllable average sizes from 400 nm to 10 μm . Possible release strategies of enclosed substances via recrystallization or by pH-change are presented. As a model experiment, a fluorescent marker was encapsulated and imaged by two-photon microscopy to monitor the dye release. The release process was found to be controllable via the immersion medium's properties. Release times can be further tuned by covering the containers with additional polymer layers, creating a flexible system with promising perspectives for pharmaceutical applications.

Introduction

Calcium carbonate as an inorganic biomaterial has attracted much attention in recent years owing to its advantages such as chemical stability, bioactivity, and biocompatibility [1, 2].

It exists in three anhydrous polymorphic modifications: vaterite, aragonite, and calcite. At ambient temperature and atmospheric pressure, vaterite is a meta-stable phase, while calcite and aragonite are the stable [3]. Sub-micron containers of porous CaCO_3 with high surface area are especially desirable to optimize cellular uptake and drug loading capacity. The fabrication of such particles has been a big challenge [4].

Their size and shape have a strong influence on their stability in solutions and on their recrystallization properties. An efficient way of controlling the size of spherical vaterite particles from 10 down to 0.4 microns has been reported very recently [5]. Apart from shrinking the size, an important issue in this system is the manipulation of the release dynamics. The two most common processes used for cargo release from porous carriers are desorption-adsorption [6–8] and, especially important for small containers, the dissolution of the carrier [9]. Anyway, both processes offer rather limited control of the release rate [6]. In more sophisticated applications, this may indeed become a problem since many target systems for drug delivery need spatial and temporal control of the cargo release.

Here we report on a technique for sub-micron vaterite capsules for a delayed burst release of cargos, exploiting a crystal phase transition, which can be controlled by changing the properties of the surrounding solution.

Materials and methods

Particle synthesis. All chemicals were purchased from Sigma-Aldrich and used without further purification. For the preparation of sub-micron vaterite spheres, the protocol described in [5] was applied. CaCO_3 precipitates, formed as a result of colloidal aggregation during rapid mixing of CaCl_2 and Na_2CO_3 in aqueous solutions, were transformed into ordered spherulites. The chosen concentrations of CaCl_2 and Na_2CO_3 were 0.33 M. Ethylene glycol (EG) was added to this reaction

solution (Na_2CO_3 and CaCl_2 were dissolved each in 2 ml water and 10 ml EG). The solution was stirred at 500 rpm at room temperature for 3 h. The synthesized CaCO_3 particles were carefully washed with ethanol and dried for 1h at 60°C .

Encapsulation and Release. For the encapsulation of Rhodamine 6G (Rh6G), 2 ml of a 1 mg/ml solution of the dye were added to 30mg dried vaterite containers. The uptake happened during 30 min of shaking. After centrifuging with $3200\times g$ for 3min, the remaining free dye molecules were washed off 3 times with ethanol. Finally, the sample was dried again for 3h at 60°C . The dry sub-micron containers could be stored for at least 30 days without any sign of degradation. Storage in ethanol gave equally good result.

Spectrofluorimetry (Cary Eclipse, Varian) was used to measure the Rh6G uptake efficiency. The total weight of particles was kept constant (7 mg) in all experiments. Some loss of capsules during the repeated washing steps may have occurred, but did not exceed a few percent. As a measure of the amount of Rh6G in solution, its fluorescence intensity was recorded at 555 nm. A calibration curve was obtained from fluorescence measurements of known concentrations of Rh6G. Then samples were diluted in water to ensure that measurements are within the linear range of the calibration curve. The total amount of encapsulated molecules was deduced subtracting the measured amount of unloaded and washed-off molecules from the initial amount of 0.015 mg Rh6G which had been added to the system.

To investigate the release process, loaded microcapsules were resuspended in a certain volume of various liquids (pure water, ethanol, cell media, and physiological solution) and allowed to incubate at room temperature in carefully sealed centrifuge tubes. After different incubation times, the samples were centrifuged, then the concentration of dye in the supernatant was measured.

To study the containers' morphology, a field emission scanning electron microscope (XL 30, FEI-Philips) was used. The size distribution of the vaterite containers and the transition between the vaterite and the calcite phase were imaged with magnifications from $5000\times$ to $50000\times$. Statistical image analysis was performed using ImageJ (NIH), based on $N=100$ particles per sample.

The optical studies of the encapsulation and the release processes were performed using a two-photon laser scanning microscope (2PM) (Ultima IV, Prairie Technologies) with a $100\times$ objective (NA 1.0, water immersion, Olympus) and an ultra-short pulsed laser (Mai Tai Deep See HP, Spectra-Physics) as an excitation source at 840nm wavelength. Images were postprocessed using Matlab (Mathworks) and Amira (Visage imaging).

Results and discussion

Vaterite particles with an average size of 400nm were obtained as described above. The recrystallization process from the vaterite to the calcite phase during the immersion in water-based solutions was demonstrated by scanning electron microscopy.

Fig. 1 presents the calcium carbonate particles in different phases during the recrystallization process: The initial state are pure vaterite particles (a), the recrystallization process starts when vaterite particles are put into a water-based solution. The external layer of vaterite starts to solvate and ionize, seeding the formation of calcite crystals from the ions. During this phase, a mix of both phases was observed (b). While after the completion of the recrystallization process only calcite crystals could be found (c).

To investigate the behavior of an incorporated substance during this recrystallization process, a model experiment was performed, encapsulating a fluorescent marker and imaging it by two-photon microscopy and spectrofluorimetry, to monitor the dye release. The symbolic images in Fig. 1 show the dye encapsulation and release scheme based on the recrystallization process described before.

During the 1st days, only a small amount (about 6%) of the encapsulated dye was found in the solution which can be attributed to a desorption-adsorption process. Dye is slowly released and a dynamic equilibrium is obtained. Here the initial particle load influences the dye concentration in the solution. During the 2nd and 3rd day, the recrystallization process sets in and the dye is quickly

released from the containers during the phase transition, increasing the amount of particles in the solution. Up to 55% of the initially encapsulated dye was detected in the solution after the complete recrystallization of all vaterite particles at day 4. The residual dye remained attached to the calcite surface or within inter-particle gaps.

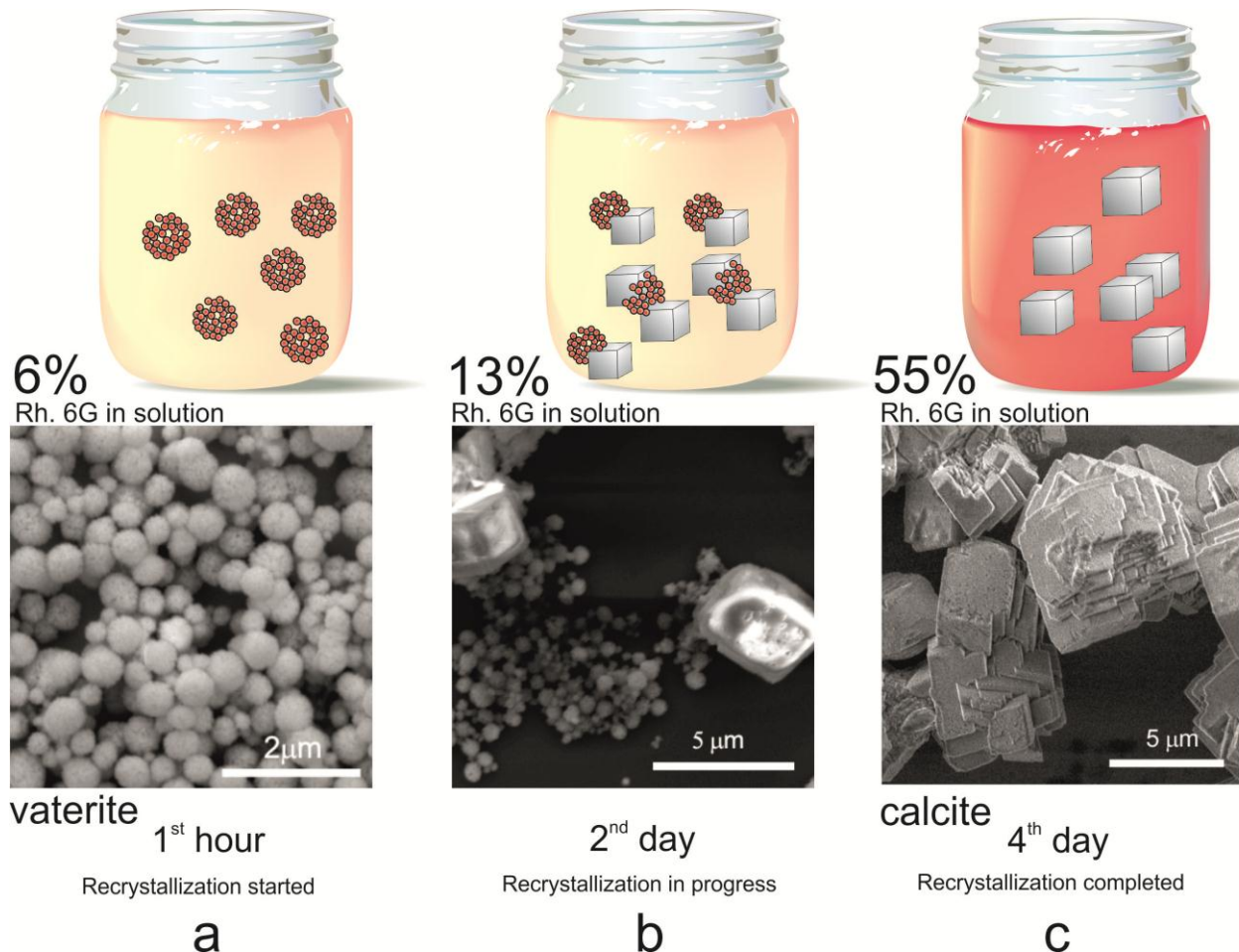


Figure 1 Above) Schematics of the crystal phase transition in water from sub-micron vaterite particles to calcite monocrystals, inducing dye release during the recrystallization. Below) Scanning electron microscopy images of the calcium carbonate crystals in the different phases at different times: a) vaterite particles at the 1st hour of immersion in water based solution, b) calcite and vaterite on the 2nd day of immersion c) calcite crystals on the 4th day after immersion.

The dye release was analyzed by two photon microscopy. Fig. 2 presents a snapshot during the recrystallization process corresponding to the phase in Fig. 1b. The image series show a z-scan through the system with image planes at 2 μm distance. The bright fluorescent spheres at the bottom of the sample are the dye-loaded vaterite containers. Single calcite monocrystals appear as dark shadows. In the higher image planes the vaterite disappears and the bigger calcite crystals give evidence that the fluorescent marker was removed from their centers, their slightly fluorescing surfaces indicate some residual dye. The reconstructed volume image below visualizes these observations again in a 3D view. The non-fluorescing calcite crystals have been artificially highlighted in blue. The appearance of calcite monocrystals and the strong drop in fluorescence were found to be spatially and temporally correlated throughout all experiments, indicating that the dye release is driven by the recrystallization process.

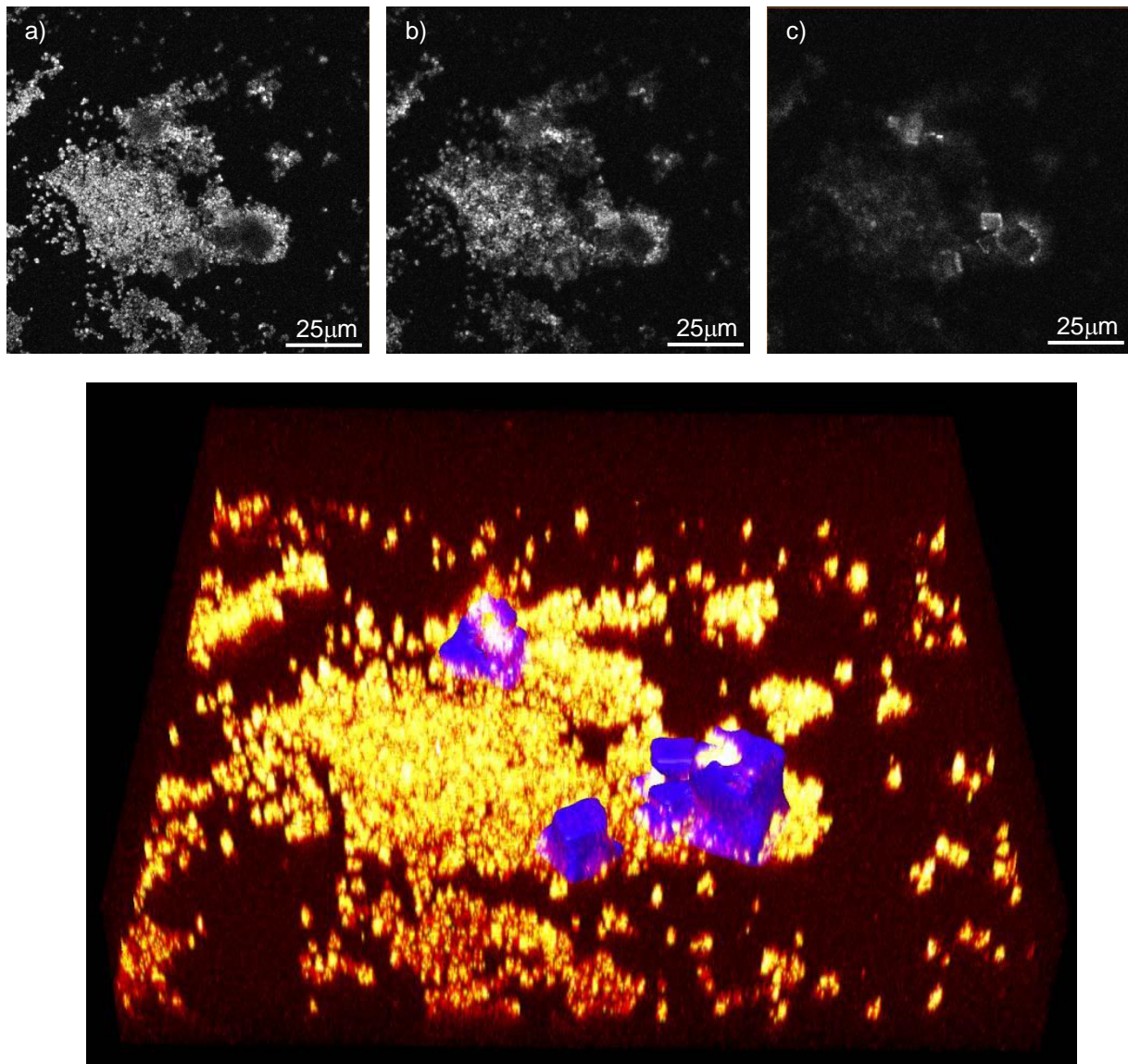


Figure 2) 2PM images of the vaterite and calcite particles during the recrystallization process (corresponding to the situation in Fig. 1b). (a-c) An image stack with the focal plane being shifted upwards in steps of 2 micron. (d) Reconstructed volume image in a 3D view showing the brightly fluorescing vaterite containers (yellow) and the dark calcite crystals (highlighted in blue)

This intrinsic release process has the further advantage to be controllable via the immersion medium (Table 1). After immersion in water for 90 hours, all vaterite particles had recrystallized to calcite. To inhibit recrystallization, containers can be immersed in pure ethanol, in this case only the slow dye desorption process was observed, leaving vaterite crystals intact. An acceleration of the recrystallization process instead was achieved by immersion in sodium chloride solution. For 0.9 % (w/v) of NaCl corresponding to physiological solution, the time for complete recrystallization decreased to 61 hours.

Immersion solution	Immersion time (hours)	Crystalline phase at the end of the immersion time starting from 100% vaterite
Physiological saline (0.9 % w/v of NaCl)	61	100% calcite
Distilled water	90	100% calcite
Pure ethanol	90	100% vaterite

Table 1) Result of the phase transition of vaterite particles during the immersion in different media

Another parameter to control the dye release is the solution's pH. A change from neutral (pH=7) to acid (pH=5) conditions was found to cause a rapid dissolution of the vaterite matrix within seconds, followed by a burst-release of the encapsulated materials.

For applications requiring further active control of the drug release, various coatings can be added to the vaterite containers, as shown in numerous works (for a review see Antipina and Sukhorukov [10]). Depending on the surface modifications, the release can then be triggered by changes of temperature [11, 12] or laser irradiation [13].

The various flexible control mechanisms reported above, together with its simplicity and cost-effectiveness make this system a promising candidate e.g. for drug delivery application.

References

- [1] M. Arruebo, Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology (2011).
- [2] D.V. Volodkin, R. von Klitzing, H. Möhwald, *Angewandte Chemie (Int. Ed.)* 49 (2010) 9258-9261.
- [3] T. Kato, A. Sugawara, N. Hosoda, *Advanced Materials* 14 (2002) 869-877.
- [4] F.C. Meldrum, H. Cölfen, *Chemical Reviews* 108 (2008) 4332-4432.
- [5] B.V. Parakhonskiy, A. Haase, R. Antolini, *Angewandte Chemie (Int. Ed.)* 51 (2012) 1195-1197.
- [6] C. Washington, *International Journal of Pharmaceutics* 58 (1990) 1-12.
- [7] Z. She, M.N. Antipina, J. Li, G.B. Sukhorukov, *Biomacromolecules* 11 (2010) 1241-1247.
- [8] C. Peng, Q. Zhao, C. Gao, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 353 (2010) 132-139.
- [9] J. Panyam, V. Labhsetwar, *Advanced Drug Delivery Reviews* 55 (2003) 329-347.
- [10] M.N. Antipina, G.B. Sukhorukov, *Advanced Drug Delivery Reviews* 63 (2011) 716-729.
- [11] K. Köhler, D.G. Shchukin, H. Möhwald, G.B. Sukhorukov, *The Journal of Physical Chemistry. B* 109 (2005) 18250-18259.
- [12] K. Köhler, H. Möhwald, G.B. Sukhorukov, *The Journal of Physical Chemistry. B* 110 (2006) 24002-24010.
- [13] A.G. Skirtach, C. Dejugnat, D. Braun, A.S. Susa, A.L. Rogach, W.J. Parak, H. Möhwald, G.B. Sukhorukov, *Nano Letters* 5 (2005) 1371-1377.